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Association Phenomena

IV. Catalysis of the Ionization of Dihydroxyacetone Phosphate by Monofunctional, Difunctional, and Trifunctional Amines[1]

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The rate of the ionization of dihydroxyacetone phosphate (DHAP) has been measured in the presence of (a) simple monofunctional primary, secondary, and tertiary amines, (b) monoprotonated diamines, and (c) monoprotonated *N*-[*N*′-(arylalkyl)aminoalkyl]guanidines of the general structure $Ar(CH_2)_mNH(CH_2)_nN{=}C(NH_2)_2$ in which the aryl groups are phenyl, 2-pyridyl, and 4-pyridyl, the values of *m* are 1, 2, and 3, and the values of *n* are 2 and 3. Evidence for bifunctional catalysis by several diamines has been obtained, but evidence is marginal for trifunctional catalysis by the guanidinium compounds in which the guanidinium moiety interacts electrostatically with the phosphate of DHAP, the secondary amino group forms an iminium bond with the carbonyl group of DHAP, and the 2-pyridyl moiety abstracts the α-proton of DHAP to generate the carbanion. Although the guanidinium compounds are uniformly better catalysts than their simpler analogs, only compound **7** catalyzes the ionization of DHAP at a rate slightly faster than would be predicted on the basis of its pK_a value.

Aldolase, the enzyme that catalyzes the condensation of dihydroxyacetone phosphate (DHAP) with glyceraldehyde-3-phosphate to yield fructose-1,6-diphosphate, is known to contain three moieties that are involved in the catalytic process, viz. a positively charged region that binds the phosphate portion of DHAP by electrostatic interaction,[2] the ε-amino group of a lysine residue that forms a Schiff base with the carbonyl group of DHAP,[3] and a base that abstracts the α-hydrogen of DHAP to form the carbanion-

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[1] This paper is dedicated to Professor William S. Johnson, whose dexterous and ebullient instruction of one of its authors (CDG) when he was a graduate student at the University of Wisconsin in the 1940s showed that author how to do research and planted the bioorganic seeds that are sprouting 30 years later in the 1970s.

[2] Inorganic phosphate (*1*) and organic phosphates (*2*) have been shown to bind to aldolase. A number of other anions also bind to aldolase and inhibit its activity (*3*), the extent of inhibition being proportional to the ionic strength and to the charge on the anion (*3*). The strength of the binding between DHAP and aldolase is at a maximum at a pH where DHAP exists primarily in the dianionic form. All of these data suggest an electrostatic interaction between DHAP and a positively charged site on aldolase. That guanidine groups constitute this site is indicated by the loss in activity when aldolase is treated with reagents that selectively modify the arginine residues (*4*).

[3] Treatment of a mixture of aldolase and ^{14}C-labeled DHAP with sodium borohydride followed by complete hydrolysis yields a single radioactive fragment, identified as β-glyceryllysine (*5*). Amino acid sequencing studies of aldolase have shown that the lysine-227 residue is the one involved in the Schiff base formation. (*6*).

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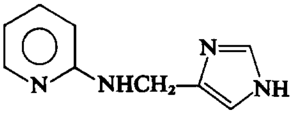
like intermediate that initiates the aldol condensation.[4] Several years ago a polyfunctional compound (**1**) was synthesized containing moieties which it was hoped might serve these several purposes.



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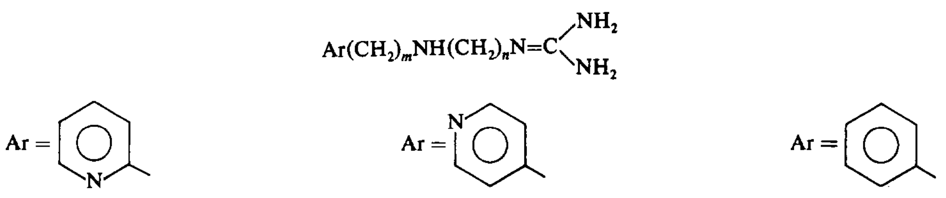
Compound **1**, however, failed to exert any catalytic effect whatsoever, some of the reasons for which were revealed in a subsequent study (*11*). The present investigation, building on this earlier work, describes another attempt to construct an "enzyme model" for aldolase.

Synthesis of N-[N'-(Arylakyl)-aminoalkyl]guanidines

Among the several difficulties with **1** as an aldolase model are the lowered basicities of the imidazole nitrogens (intended as the phosphate binding moiety) and the pyridine nitrogen (intended as the α-proton abstracting moiety) resulting from their close proximity in the molecule. To ameliorate this condition several *N*-[*N'*-(arylalkyl)-aminoalkyl]guanidines have been synthesized in which the three nitrogen-containing functional groups are separated by one or more methylene groups, as shown in Fig. 1.



$$\mathrm{Ar(CH_2)}_m\mathrm{NH(CH_2)}_n\mathrm{N{=}C}\begin{array}{l}\diagup\mathrm{NH_2}\\ \diagdown\mathrm{NH_2}\end{array}$$

2 ($m = 1, n = 2$)
3 ($m = 2, n = 2$)
4 ($m = 3, n = 2$)
5 ($m = 1, n = 3$)
6 ($m = 2, n = 3$)
7 ($m = 3, n = 3$)

8 ($m = 3, n = 2$)
9 ($m = 3, n = 3$)

10 ($m = 1, n = 2$)
11 ($m = 2, n = 2$)
12 ($m = 3, n = 2$)
13 ($m = 1, n = 3$)
14 ($m = 2, n = 3$)
15 ($m = 3, n = 3$)

FIG. 1. *N*-[*N'*-(Arylakyl)-aminoalkyl]guanidines.

Concomitantly, the stereoelectronic difficulties associated with **1** as a catalyst are reduced.[5] In the present series a guanidine rather than an imidazole moiety has been incorporated as the intended phosphate-binding site; guanidine is a very much stronger

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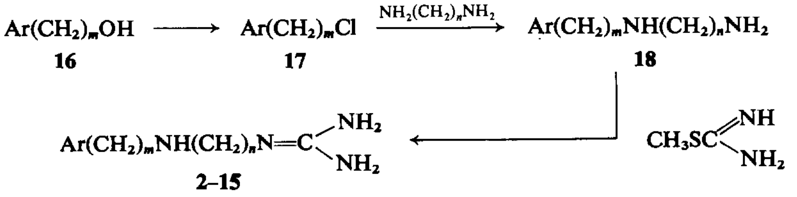
[4] The presence of carbanion-like intermediates in the aldolase-catalyzed reaction is indicated by the reaction of DHAP-aldolase with carbanion-specific reagents such as tetranitromethane (*7*) and hexacyanoferrate (III) (*8*). Selective modification of the histidine residues of aldolase leaves its ability to bind DHAP essentially unchanged but reduces the rate at which DHAP exchanges its C-3 hydrogens with the solvent (*9*). The histidine residue responsible for this effect has been shown to be near the carboxyl-terminus of aldolase (*10*), and it presumably is the α-hydrogen abstracting moiety in aldolase.

[5] In studies of the bifunctional amine-catalyzed dedeuteration of isobutyraldehyde-2-d and acetone-d_6 Hine and co-workers (*12*) have shown the importance of stereoelectronic considerations. In these systems the optimum catalysis was achieved when the α-hydrogen and the nitrogen moiety are separated by six intervening atoms in the chain (i.e., an eight-membered pseudocyclic intermediate).

base than imidazole (therefore, remains essentially completely protonated at pH 7–8) and has been shown to be effective in binding phosphates not only in enzyme systems (*4*) but also in simple model systems (*13*). The synthesis of the compounds shown in Fig. 1 involved the conversion of the arylalkanols (**16**) to the arylalkyl chlorides (**17**), condensation of **17** with an alkanediamine to yield the *N*-(arylalkyl)-alkanediamines (**18**), and treatment of **18** with *S*-methylisothiourea to yield the *N*-[*N'*-(arylakyl)-aminoalkyl]-guanidines (**2–15**):



In most cases the products were purified as dinitrates or trinitrates, although with **5** and **7** the sulfates proved to be more satisfactory. The salts are stable, water-soluble, nonhygroscopic compounds that give positive Sakaguchi tests (*14*) for the guanidine moiety and have elemental analyses, ir spectra, and nmr spectra compatible with the designated structures.

pK_a Measurements of the N-[N'-(Arylalkyl)-aminoalkyl]guanidines

The pK_a values of compounds **2–15** were measured titrimetrically (*15*) at 35.00 ± 0.05 °C, and the values at zero ionic strength were calculated by means of the Davies equation (*16*), as listed in Table 1.

TABLE 1

pK_a VALUES OF *N*-[*N'*-(ARYLALKL)-AMINOALKYL]GUANIDINES

	pK_a of secondary nitrogen		pK_a of pyridine nitrogen
Compound	$I = 0.100$	$I = 0$[a]	$I = 0$[a]
2	6.594 ± 0.004	6.265 ± 0.003	<2[b]
3	7.425 ± 0.006	7.075 ± 0.003	2.829 ± 0.006
4	7.901 ± 0.010	7.566 ± 0.007	4.020 ± 0.009
5	7.684 ± 0.008	7.312 ± 0.004	<2[b]
6	8.431 ± 0.008	8.098 ± 0.005	3.007 ± 0.015
7	9.028 ± 0.004	8.684 ± 0.001	4.222 ± 0.009
8	7.863 ± 0.008	7.510 ± 0.004	4.744 ± 0.006
9	8.926 ± 0.005	8.585 ± 0.009	4.792 ± 0.001
10	7.284 ± 0.004	6.939 ± 0.005	
11	7.609 ± 0.003	7.274 ± 0.007	
12	8.054 ± 0.003	7.693 ± 0.004	
13	8.356 ± 0.004	8.026 ± 0.008	
14	8.679 ± 0.006	8.347 ± 0.007	
15	0.106 ± 0.003	8.767 ± 0.008	

[a] pK_a values measured at ionic strengths of 0.005–0.021 and corrected to zero ionic strength.

[b] pK_a value is too low to be determined accurately by the titrimetric method.

The base-weakening effect of the aryl group (benzene or pyridine) and the guanidinium group on the secondary nitrogen is a function of proximity, falling off with increasing numbers of intervening bonds. For the benzene compounds **10–15** the pK_a values conform closely to the expression $pK_a = 0.374x + 1.078y + 2.947$ where x and y are the numbers of bonds separating the benzene ring and the guanidinium group, respectively, from the secondary amine moiety. For the pyridine compounds **2–7** the analogous expression $pK_a = 0.668x + 1.063y + 1.776$ holds with reasonably good accuracy.

Factors Affecting the Rate of Ionization of Dihydroxyacetone Phosphate

The rate of ionization of DHAP was measured by the tetranitromethane (TNM) method of Riordan (*7, 17*), using a [TNM]/[DHAP] ratio that was sufficiently high that the reaction is pseudo-zero order with respect to DHAP. In most instances the reactions were carried out at a pH equal to the apparent pK_a of the amine that was employed as the catalyst so that the amine itself acted as a buffer. Although tetranitromethane reacts rapidly with carbanions, other nucleophiles also have the potentiality for inducing the formation of nitroformate, which is the chromophore that is determined spectrophotometrically. Thus, measurements for the rate of formation of nitroformate from TNM and the catalyst alone were made, and these values were then subtracted from the overall rates (in the presence of DHAP) to give the value for the rate of ionization of DHAP. In the majority of cases, these corrections were small.

To determine the order of the reaction with respect to DHAP, the rate constants for the pseudo-zero order reactions were measured with two representative systems (**3** and **12**) as a function of DHAP concentration. A plot of the data gives a straight line with zero intercept in both instances and indicates a first-order dependence with respect to DHAP. Thus, rate constants for the pseudo-first-order ionization of DHAP can be obtained from any particular value for the pseudo-zero-order process by dividing by the DHAP concentration. From the slopes of the lines it was determined that the rate constants for the pseudo-first-order reactions of **3** and **12** are 3.93×10^{-5} and 1.06×10^{-4} $\sec^{-1}$, respectively.

To determine the order of the reaction with respect to the amine catalysts, the rates of reaction were measured at four different catalyst concentrations. A plot of the rate constants for the pseudo-first-order reactions against the total amine concentration gave straight lines, indicating a first-order dependence on the amine. To determine whether the monoprotonated amine, the diprotonated amine, or both act as catalysts, the rates at various total amine concentrations were studied as a function of the [amine-H^+]/[amine-$2H^{2+}$] ratio. A plot of these data gives a straight line, the slope of the line increasing with increasing [amine-H^+]/[amine-$2H^{2+}$] ratio. Employing a technique suggested by Jencks (*18*), the slopes of these lines were plotted against the fraction of amine-H^+. The left-hand intercept value of zero indicates that there is no significant catalysis by the amine-$2H^{2+}$ species, and the right-hand intercept value provides a measure of the rate constant for the amine-H^+ species. The straight line relationship indicates that the rate constants for the second-order reactions can be obtained simply by determining the slope of the plot of the rate constants for the pseudo-first-order reactions vs amine-H^+. The rate constants for the second-order reactions involving the catalysts can also be obtained from the slope of the plot of catalyst concentration vs the rates of the pseudo-first-order reactions, the left-hand intercept in these instances giving a measure of the hydroxide-catalyzed reaction. A comparison of the "intercept" method and the "slope" method for determining the rate constants for **3**, for example, gave values of 5.30×10^{-3} and 5.26×10^{-3} M^{-1} $\sec^{-1}$, respectively. Similar results were obtained with **11**, from which it was concluded that all of the compounds **2–15** behave in a comparable fashion.

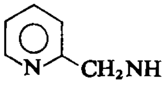
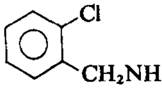
To determine the concentration of tetranitromethane necessary to establish pseudo-first-order conditions for the ionization of DHAP, rates at various TNM concentrations were measured. It was determined that TNM concentrations of 1.67×10^{-3} M or greater are sufficient for 10^{-3} M solutions of DHAP if the rate constant for the zero-order ionization does not exceed 7×10^{-8} M^{-1} $\sec^{-1}$. In those instances in which rate constants larger than this were anticipated, the concentration of TNM was increased accordingly.

Catalysis of Ionization of Dihydroxyacetone Phosphate by Simple Amines

Several primary amines with pK_a values ranging from ca. 5 to 9 were tested; the results are listed in Table 2. A plot of log k_2 vs pK_a values (Fig. 2) shows that if a straight line is drawn through the points for the two monoamines, the points for the three diamines all fall above this line. This suggests that in these cases ionization of

TABLE 2

RATE CONSTANTS FOR THE PSEUDO-FIRST-ORDER AND SECOND-ORDER IONIZATION OF DIHYDROXYACETONE PHOSPHATE CATALYZED BY PRIMARY AMINES

			$k_{\psi 1} \times 10^5$ ($\sec^{-1}$)				
Catalyst[a,b]		pK_a'[c]	[cat][d] = 0.002 M	[cat][d] = 0.004 M	[cat][d] = 0.006 M	[cat][d] = 0.008 M	$k_2 \times 10^3$ (M^{-1} $\sec^{-1}$)[e]
$NCCH_2NH_2$	(**19**)	5.33	—[f]	—[f]	—[f]	—[f]	<0.20[g]
$NH_3^+CH_2CH_2NH_2$	(**20**)	7.25[h]	1.27	1.52	1.84	2.19	1.54 ± 0.08
$NCCH_2CH_2NH_2$	(**21**)	7.63	1.85	1.99	2.07	2.15	0.49 ± 0.05
	(**22**)	8.48	1.55	2.16	2.81	3.50	3.25 ± 0.06
$NH_3^+CH_2CH_2CH_2NH_2$	(**23**)	8.80[h]	3.00	5.45	7.07	8.59	9.2 ± 0.8
	(**24**)	8.66	0.81	1.66	2.06	2.60	2.9 ± 0.3

[a] Amine present as buffer and catalyst.

[b] Buffer ratio = 1.0.

[c] Apparent pK_a of amine moiety at ionic strength = 0.100.

[d] [cat] = Concentration of unprotonated amine.

[e] k_2 values determined from least squares determination of the slope of a plot of $k_{\psi 1}$ vs [cat]; error limits given are standard deviations of the slope.

[f] No observable catalysis was detected.

[g] It is estimated that 0.20×10^{-3} M^{-1} $\sec^{-1}$ is the lowest value for k_2 that could be reliably detected by the present methods.

[h] Symmetry-corrected pK_a'.

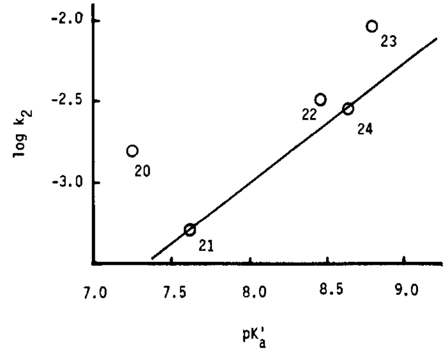


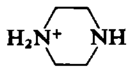
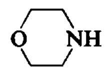
FIG. 2. Plot of log of rate constants vs pK_a' of catalyst for the ionization of dihydroxyacetone phosphate in the presence of primary amines.

DHAP involves Schiff base formation and intramolecular proton removal in a fashion analogous to that proposed by Hine *et al.* (*19*) for the dedeuteration of acetone-d_6 by bifunctional amines.

Several secondary amines with pK_a values ranging from ca. 5 to 9 were tested; the results are shown in Table 3. A plot of log k_2 vs pK_a values, shown in Fig. 3, indicates

TABLE 3

RATE CONSTANTS FOR THE PSEUDO-FIRST-ORDER AND SECOND-ORDER IONIZATION OF DIHYDROXYACETONE PHOSPHATE CATALYZED BY SECONDARY AMINES

			$k_{\phi 1} \times 10^5$ (sec^{-1})				
Catalyst[a,b]		pK_a'[c]	[cat][d] = 0.002 *M*	[cat][d] = 0.004 *M*	[cat][d] = 0.006 *M*	[cat][d] = 0.008*M*	$k_2 \times 10^3$ (M^{-1} sec^{-1})[e]
$(NCCH_2CH_2)_2NH$	(**25**)	5.29	—[f]	—[f]	—[f]	—[f]	<0.20[g]
	(**26**)	5.85[h]	0.15	0.22	0.24	0.30	0.24 ± 0.003
$CH_3NH_2^+CH_2CH_2NHCH_3$	(**27**)	7.26[h]	3.09	5.25	7.16	9.16	10.1 ± 0.2
$NCCH_2CH_2NHCH_3$	(**28**)	7.84	1.94	2.35	2.93	3.22	2.2 ± 0.2
	(**29**)	8.36	2.86	4.49	5.71	7.03	6.9 ± 0.3
$(HOCH_2CH_2)_2NH$	(**30**)	8.75	3.95	7.14	11.5	15.0	18.8 ± 0.8

[a] Amine present as buffer and catalyst.

[b] Buffer ratio = 1.0.

[c] Apparent pK_a of amine moiety at ionic strength = 0.100.

[d] [cat] = Concentration of unprotonated amine.

[e] k_2 values determined from least squares determination of the slope of a plot of $k_{\phi 1}$ vs [cat]; error limits given are standard deviations of the slope.

[f] No observable catalysis was detected.

[g] It is estimated that 0.20×10^{-3} M^{-1} sec^{-1} is the lowest value for k_2 that could be reliably detected using the present methods.

[h] Symmetry corrected pK_a'.

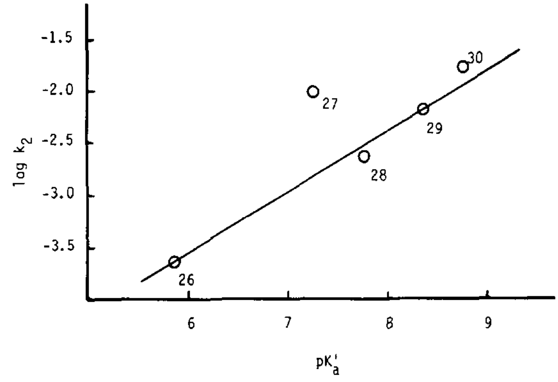
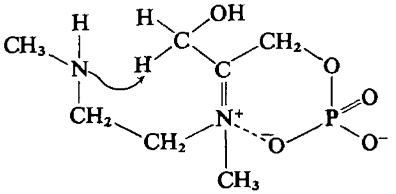


FIG. 3. Plot of log of rate constants vs pK_a' of catalyst for the ionization of dihydroxyacetone phosphate in the presence of secondary amines.

that the points for the monoamines describe an approximately straight line with the point for the diamine falling above this line. *N,N'*-Dimethylethylene-diamine, in fact, is eight times more effective as a catalyst than its pK_a would predict by this comparison, again suggesting that ionization of DHAP involves iminium ion formation and intramolecular proton removal, e.g., via a structure such as **31**.



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The tertiary amines tested were limited to pyridines, the results of which are shown in Table 4. Attempts to include two aliphatic tertiary amines, viz., *N,N'*-dimethylpiperazine and triethanolamine, were unsuccessful because of the unexpectedly high rate of reaction of these amines with TNM in the absence of DHAP coupled with the nonlinear production of nitroformate in the presence of DHAP. Four of the six pyridines tested showed measurable catalytic activity, and a plot of log k_2 vs pK_a values gave the straight line shown in Fig. 4.

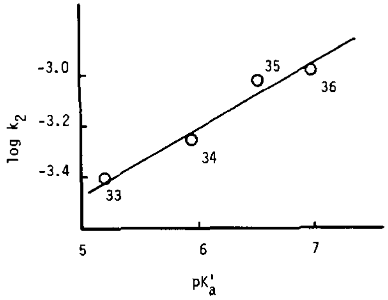
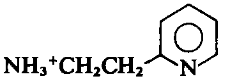
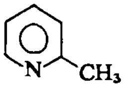
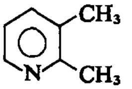
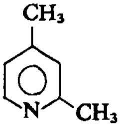
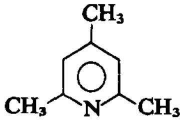


FIG. 4. Plot of log of rate constants vs pK_a' of catalyst for the ionization of dihydroxyacetone phosphate in the presence of pyridines.

TABLE 4

RATE CONSTANTS FOR THE PSEUDO-FIRST-ORDER AND SECOND-ORDER IONIZATION OF DIHYDROXYACETONE PHOSPHATE CATALYZED BY PYRIDINES

			$k_{\psi 1} \times 10^5$ (sec^{-1})				
Catalyst[a,b]		pK_a'[c]	[cat][d] = 0.002 M	[cat][d] = 0.004 M	[cat][d] = 0.006 M	[cat][d] = 0.008 M	$k_2 \times 10^3$ (M^{-1} sec^{-1})[e]
	(32)	4.05	—[f]	—[f]	—[f]	—[f]	<0.20[g]
	(33)	5.20	0.15	0.27	0.36	0.39	0.41 ± 0.07
	(34)	5.95	1.16	1.35	1.46	1.50	0.6 ± 0.1
	(35)	6.54	1.25	1.50	1.68	1.85	0.99 ± 0.07
	(36)	6.98	1.31	1.52	1.68	1.93	1.01 ± 0.06
	(37)	7.40	1.34	1.37	1.28	1.28	<0.20[g]

[a] Amine present as buffer and catalyst.

[b] Buffer ratio = 1.0.

[c] Apparent pK_a of amine moiety at ionic strength = 0.100.

[d] [cat] = Concentration of unprotonated amine.

[e] k_2 values determined from least squares determination of the slope of a plot of $k_{\psi 1}$ vs [cat]; error limits given are standard deviations of the slope.

[f] No observable catalysis was detected.

[g] It is estimated that 0.20 × 10^{-3} M^{-1} sec^{-1} is the lowest value for k_2 that could be reliably detected using the present methods.

A comparison of the Brønsted plots in Figs. 2–4 indicate that the order of catalytic activity with respect to the ionization of DHAP is tertiary amines > secondary amines > primary amines. A similar order has been observed by Hine and co-workers (*20*) in the dedeuteration of isobutyraldehyde-2-d and acetone-d_6. If Schiff base formation were a major contributor to the catalysis, tertiary amines would not be expected to be effective; if steric factors inherent in the amine itself were important, secondary amines would be expected to be less effective than primary amines. As has been intimated by several investigators (*21*), solvation effects are probably responsible; primary amines, being most tightly solvated as a result of their smaller size and greater hydrogen bonding capacity, are less effective nucleophiles than secondary and tertiary amines, which are progressively less tightly solvated.

Catalysis of Ionization of Dihydroxyacetone Phosphate by N-[N′-(Arylakyl)-aminoalkyl]guanidines

The rate constants for the pseudo-first-order reactions of the polyfunctional compounds **2**–**15** were determined at 35°C at a pH equal to the pK_a of the secondary amine function and at four different concentrations of catalyst. The results are summarized in Table 5, and a plot of the log k_2 vs pK_a values is shown in Fig. 5. Also included in Fig. 5 is the Brønsted plot of the simple monofunctional secondary amines (see Fig. 3), which shows that the polyfunctional amines are more effective catalysts than their monofunctional counterparts. To determine if the guanidinium moiety contributes to this enhancement in catalytic effectiveness, the rate of ionization of DHAP was measured in the presence of hexylguanidinium nitrate. Over a concentration range of 0.004–0.016 *M* in hexylguanidinium nitrate, no change in the rate constant for the pseudo-first-order ionization of 10^{-3} *M* DHAP was observed, indicating that the guanidinium moiety per se has no catalytic action. Similarly, the catalysis of the

TABLE 5

RATE CONSTANTS FOR THE PSEUDO-FIRST-ORDER AND SECOND-ORDER IONIZATION OF DIHYDROXYACETONE PHOSPHATE CATALYZED BY *N*-[*N*′-(ARYLALKYL)AMINO]GUANIDINES

		$k_{\psi 1} \times 10^5$ (sec^{-1})				
Catalyst[a,b]	pK_a' [c]	[cat][d] = 0.002 *M*	[cat][d] = 0.004 *M*	[cat][d] = 0.006 *M*	[cat][d] = 0.008 *M*	$k_2 \times 10^3$ (M^{-1} sec^{-1})[e]
2	6.59	0.46	0.67	0.89	1.02	0.95 ± 0.07
3	7.43	1.86	2.87	4.06	4.97	5.3 ± 0.2
4	7.90	3.67	6.40	9.91	12.0	13.7 ± 0.4
5	7.68	2.57	3,99	5.51	7.04	7.5 ± 0.1
6	8.43	6.05	11.7	17.1	22.4	27.2 ± 0.3
7	9.03	11.9	29.0	46.3	65	89 ± 2
8	7.86	3.28	5.41	8.33	10.4	12.1 ± 0.6
9	8.93	9.16	18.6	27.8	38.0	47.9 ± 0.8
10	7.28	1.27	1.86	2.38	3.01	2.88 ± 0.07
11	7.61	2.25	3.61	5.00	6.37	6.88 ± 0.02
12	8.05	4.35	7.69	11.0	14.5	16.6 ± 0.2
13	8.36	5.23	9.79	14.4	19.2	23.2 ± 0.2
14	8.68	7.73	14.8	21.4	29.1	35.4 ± 0.7
15	9.11	10.8	22.0	34.2	43.3	55 ± 2

[a] Amine present as buffer and catalyst.

[b] Buffer ratio = 1.0.

[c] Apparent pK_a of secondary amine moiety at ionic strength = 0.100.

[d] [cat] = Concentration of unprotonated amine.

[e] k_2 values determined from least squares determination of the slope of a plot of $k_{\psi 1}$ vs [cat]; error limits given are standard deviations of the slope.

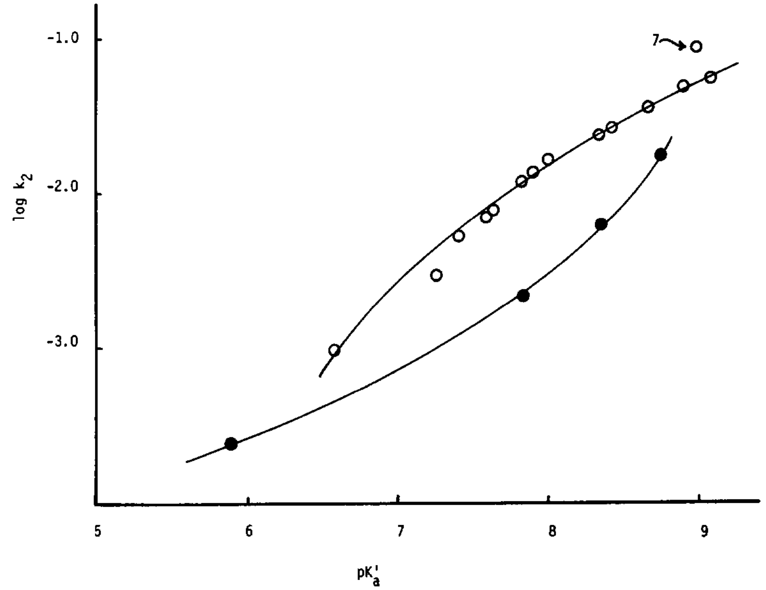
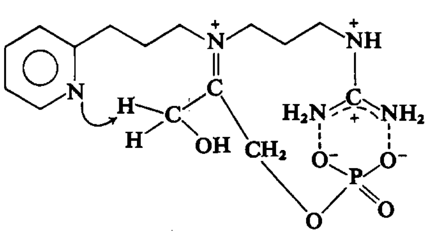


FIG. 5. Plot of log of rate constants vs pK_a' of catalyst for the ionization of dihydroxyacetone phosphate in the presence of monofunctional secondary amines (●) and trifunctional N-[N'-(arylalkyl)amino]guanidines (○).

ionization of DHAP by diethanolamine and by morpholine was shown to be unaffected by the addition of equimolar amounts of hexylguanidinium nitrate. Thus, it appears that it is the combination of a guanidinium moiety and a secondary amine function in the same molecule that results in a more effective catalyst. That the increased effectiveness of compounds **2–7** arises from a cooperativity between the guanidinium and secondary amine moieties and not from the presence of the pyridine moiety is indicated by the fact that all of the compounds **2–15**, with the possible exception of **7**, give rates that fall very close to the Brønsted line. Only compound **7** shows a hint of trifunctional catalysis of the type that was sought in this investigation, as illustrated by structure **38**; the rate of



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ionization of DHAP in the presence of **7** is slightly greater than would be predicted from its pK_a value. That **7** is not more effective as a trifunctional catalyst must be due, in part, to the low basicity of the pyridine nitrogen, the putative α-hydrogen abstracting moiety. With a pK_a of 4.22, it is 10 times less basic than pyridine itself (pK_a = 5.20), the weakest of the simple amines whose catalytic effect was measurable by the techniques used in the present investigation. The synthesis of analogs of **7** containing more basic moieties than pyridine is, therefore, contemplated. Also contemplated is the investigation of trifunctional catalysis under conditions involving a less aqueous environment in the region of the catalyst, conditions which should enhance the electrostatic attraction between the guanidinium and phosphate moieties.

EXPERIMENTAL[6]

Synthesis of N-[N′-(Arylaklyl)-aminoalkyl]guanidines (**2**–**15**)

General procedures. Compounds **2**–**15** were prepared by treating the appropriate *N*-(arylalkyl)diamine with a 50% excess of *S*-methylisothiourea hemisulfate in aqueous solution. A 0.010-mol sample of the diamine was placed in a 200-ml three-necked round-bottomed flask containing a gas inlet and a reflux condenser fitted at the top with a gas outlet leading to a trap containing 200 ml of Clorox (5% sodium hypochlorite). A solution containing 2.08 g (0.015 mol) of *S*-methylisothiourea hemisulfate in 50 ml of distilled water was added, stirring (magnetic) and heating were initiated, and a vacuum was applied to the trap to pull a gentle stream of air through the reaction mixture to entrain methyl mercaptan and carry it into the trap. Heating and stirring were continued for 2 hr, the reaction mixture was then allowed to cool to room temperature, and the product was isolated and purified as the sulfate, dihydrochloride, dinitrate, or trinitrate salt. To prepare the sulfates, the reaction mixture was treated with 2 *N* sulfuric acid until the pH of the solution was ca. 5–6, and acetone was then added to the point of slight turbidity. The solution was cooled slowly to −20°C, and the precipitate that formed was removed by filtration. The filtrate was warmed and again treated with acetone until slightly turbid. This solution was cooled and filtered, and the combined solid was recrystallized from aqueous acetone. To prepare the dihydrochlorides, the reaction mixture was treated with a saturated solution of barium chloride until all the sulfate ion was converted to barium sulfate. The pH of the solution was adjusted to 5–6 by the addition of 1 *N* hydrochloric acid, and the barium sulfate was removed by centrifugation. The clear supernatant was evaporated, the residue was dried for 10 hr in a vacuum desiccator, and the solid was recrystallized from absolute ethanol. To prepare the dinitrates and trinitrates, the sulfate ion was precipitated with barium nitrate, and 1 *N* nitric acid was added to pH 5–6 for the dinitrates and pH 1–2 for the trinitrates. The barium sulfate was removed by centrifugation, and the clear supernatant was evaporated. The residue was dried by adding and then evaporating three 30-ml portions of anhydrous methanol and three 20-ml portions of benzene. The residue was dried 10–15 hr in a vacuum desiccator and then recrystallized from the appropriate solvent.

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[6] Melting points were determined on a Thomas–Hoover Uni-Melt apparatus and are uncorrected. Infrared spectra were obtained on a Perkin–Elmer Model 137 Infracord spectrometer. Nuclear magnetic resonance spectra were obtained on a Varian Model A-60A spectrometer at 37°C using tetramethylsilane as an internal standard with nonaqueous solvents and sodium 2,2-dimethyl-2-silapentane-5-sulfonate as the internal standard in D_2O solutions; chemical shifts are reported as δ values, coupling constants are given in hertz, and the abbreviations "dist, s, d, t, quart, quint, and m" refer to "distorted, singlet, doublet, triplet, quartet, quintet, and multiplet," respectively. Visible spectra were obtained on a Cary Model 11 spectrometer. The measurements of pH were made at 35°C with a Corning Model 12 research meter and a Corning Series 500 silver/silver chloride (ceramic junction) combination electrode. Elemental analyses were carried out by Mikroanalytisches Laboratorium, Vienna, Austria, by Galbraith Laboratories, Knoxville, Tenn., and by Industrial Testing Laboratories, St. Louis, Mo.

2-(2-Pyridylmethylamino)ethylguanidine (**2**). 2-(Chloromethyl)pyridine hydrochloride was obtained in 80% yield as light tan needles, mp 125–127.5°C [lit (*22*), 128–129°C], by treatment of 2-pyridylmethanol with hydrochloric acid followed by thionyl chloride. A total of 8 g (0.049 mol) of this material was added in 1-g portions at 10-min intervals to 30 g (0.50 mol) of ethylenediamine in a 250-ml round-bottomed flask immersed in a cooling bath maintained at 10°C. Stirring at 10°C was continued for 14 hr, and the reaction mixture was then poured into 100 g of crushed ice and worked up to give 5.84 g (79%) of a pale yellow oil, bp 121–123°C/0.15 mm. Treatment of this material with *S*-methylisothiourea followed by nitric acid, as described above, gave a 54% yield of product after one recrystallization from methanol, mp 163–164.5°C. Three additional recrystallizations from methanol gave the dinitrate of **2** as short, prismatic needles: mp 163–164.5°C; ir (KBr), 3400 (NH stretch), 1680 (C=N stretch), 1620 (NH bend), 1380 cm^{-1} (NO_3^-); nmr (D_2O), δ 8.50 (dist. d, 1, C-6 Py), 7.82 (dist t, 1, C-4 Py), 7.52–7.25 (m, 2, C-3, 5 Py), 4.47 (s, 2, $PhCH_2N$), 3.85–3.30 (m, 4, $NCH_2CH_2N{=}$).

Anal. Calcd for $C_9H_{17}N_7O_6$: C, 33.86; H, 5.37; N, 30.71. Found: C, 33.79; H, 5.38; N, 30.61.

2-[2-(2-Pyridyl)ethylamino]ethylguanidine (**3**). 2-(2-Chloroethyl)pyridine (*23*) was obtained in 90% yield from 2-(2-hydroxyethyl)pyridine and thionyl chloride and isolated as a colorless oil, bp 75–77·5°C/6 mm [lit (*23*), 85–90°C/10 mm]. Treatment of this material with a five-fold excess of ethylenediamine at room temperature yielded 74% of 2-[2-(pyridyl)ethylamino]ethylamine as a colorless oil, bp 97–102°C/0.1 mm [lit (*24*), 102–104°C/0.13 mm]. Subsequent reaction with *S*-methylisothiourea followed by nitric acid, as described above, gave 17% of product after two recrystallizations from absolute ethanol; mp 117–120°C. Four additional recrystallizations from absolute ethanol gave the dinitrate of **3**: mp 121.5–122.5°C; ir (KBr), 3330, 2750 (NH stretch), 1670 (C=N stretch), 1600 (NH bend), 1360 cm^{-1} (NO_3^-); nmr (D_2O), δ 8.41 (dist d, 1, C-6 Py), 7.75 (dist t, 1, C-4 Py), 7.40–7.15 (m, 2, C-3, 5 Py), 3.85–3.10 (m, 8, $\mathrm{PyC}\mathit{H}_2\mathrm{CH_2NHC}\mathit{H}_2\mathrm{C}\mathit{H}_2\mathrm{N}{=}$).

Anal. Calcd for $C_{10}H_{19}N_7O_6$: C, 36.04; H, 5.75; N, 29.42. Found: C, 36.24; H, 5.76; N, 29.70.

2-[3-(2-Pyridyl)propylamino]ethylguanidine (**4**). 3-(2-Pyridyl)propanol, was prepared from 2-picoline and methyllithium followed by ethylene oxide and obtained in 52% yield as a colorless oil, bp 92–94°C/0.1 mm [lit (*25*), 106°C/3 mm]. Treatment of this material with concentrated hydrochloric acid by a method similar to that described by Eisch and co-workers (*26*), converted it to 2-(3-chloropropyl)pyridine hydrochloride in 60% yield, mp 108–110°C [lit (*27*), 95–96°C]. Treatment with a 10-fold excess of ethylenediamine, as described above in the preparation of **2**, at 5°C for 24 hr gave a 45% yield of 2-[3-(2-pyridyl)propylamino]ethylamine as a reddish-brown oil: bp 120–126°C; nmr ($CDCl_3$), δ 8.33 (dist d, 1, C-6 Py), 7.40 (dist t, 1, C-4 Py), 7.08–6.76 (m, 2, C-3, 5 Py), 2.94–2.46 (m, 8, $\mathrm{PyC}\mathit{H}_2\mathrm{CH_2C}\mathit{H}_2\mathrm{NHC}\mathit{H}_2\mathrm{C}\mathit{H}_2\mathrm{NH_2}$), 1.85 (dist quint, 2, $\mathrm{PyCH_2C}\mathit{H}_2\mathrm{CH_2N}$), 1.28 (s, NH and NH_2; disappears on shaking with D_2O). Treatment with *S*-methylisothiourea followed by nitric acid, as described above, gave 43% of product after recrystallization from 95% ethanol, mp 155–158°C. Further recrystallization from 95% ethanol and then from a 1:1 mixture of absolute ethanol and methanol gave the trinitrate of **4**: mp 159–160°C; ir (KBr), 3350 (NH stretch), 1670 (C=N stretch), 1630 (NH stretch), 1380 cm^{-1} (NO_3^-); nmr (D_2O), δ 8.70 (dist d, 1, C-6 Py), 8.48 (dist d, 1, C-4 Py), 8.05–7.77 (m, 2, C-3, 5 Py), 3.87–3.12 (m, 8, $\mathrm{PyC}\mathit{H}_2\mathrm{CH_2C}\mathit{H}_2\mathrm{NHC}\mathit{H}_2\mathrm{C}\mathit{H}_2\mathrm{N{=}}$), 2.33 (dist quint, 2, Py $\mathrm{CH_2C}\mathit{H}_2\mathrm{CH_2N}$).

Anal. Calcd for $C_{11}H_{22}N_8O_9$: C, 32.20; H, 5.40; N, 27.31. Found: C, 32.25; H, 5.45; N, 27.23.

3-[(2-Pyridyl)methylamino]propylguanidine (**5**). 2-(Chloromethyl)pyridine hydrochloride was treated with 1,3-propanediamine in the fashion described above in the preparation of **2** to yield 3-(2-pyridylmethylamino)propylamine as a pale yellow oil, bp 107–109°C/0.03 mm [lit (*28*), 95°C/0.05 mm]. Treatment with *S*-methylisothiourea followed by sulfuric acid, as described above, gave 75% of product after recrystallization from a 9:1 mixture of water and acetone, mp 308–311°C. Two additional recrystallizations yielded the sulfate of **5** as white, jagged needles: mp 310–311°C (dec, sealed tube); ir (KBr), 3400 (NH stretch), 1660 (C=N stretch), 1640 (NH bend), 1110 cm^{-1} (SO_4^{2-}), nmr (D_2O),[7] δ 8.50 (dist d, 1, C-6 Py), 7.83 (dist t, 1, C-4 Py), 7.52–7.25 (m, 2, C-3, 5 Py), 4.39 (s, 2, $\mathrm{PyCH_2N}$), 3.45–3.10 (m, 4, $\mathrm{NC}\mathit{H}_2\mathrm{CH_2C}\mathit{H}_2\mathrm{N{=}}$), 2.06 (dist quint, 2, $\mathrm{NCH_2C}\mathit{H}_2\mathrm{CH_2N{=}}$).

Anal. Calcd for $C_{10}H_{19}N_5SO_4$: C, 39.33; H, 6.27; N, 22.93. Found: C, 39.21; H, 6.17; N, 22.89.

3-[2-(2-Pyridyl)ethylamino]propylguanidine (**6**). 2-(2-Chloroethyl)-pyridine (see above) was converted to 3-[2-(2-pyridyl)ethylamino]propylamine by treatment with a five-fold excess of 1,3-propanediamine and obtained in 66% yield as a colorless oil, bp 113–117°C/0.10 mm (lit (*28*), 107°C/0.05 mm). Reaction with *S*-methylisothiourea followed by nitric acid, as described above, gave 52% of an amorphous white solid, mp 155–157.5°C, after trituration of the crude product with four 40-ml portions of acetone followed by crystallization from methanol. Additional recrystallizations from methanol and a 1:1 mixture of absolute ethanol and methanol yielded the trinitrate of **6** as white, granular crystals: mp 159–160°C; ir (KBr), 3350 (NH stretch), 1680 (C=N stretch), 1640 (NH bend), 1375 cm^{-1} (NO_3^-); nmr (D_2O), δ 8.72 (dist d, 1, C-6 Py), 8.50 (d of d, 1, C-4 Py), 8.08–7.80 (m, 2, C-3, 5 Py), 3.65 (s, 4, $\mathrm{PyC}\mathit{H}_2\mathrm{C}\mathit{H}_2\mathrm{N}$), 3.37 and 3.32 (pair of t, J = 7 Hz, 4, $\mathrm{NC}\mathit{H}_2\mathrm{CH_2C}\mathit{H}_2\mathrm{N{=}}$), 2.08 (quint, J = 7 Hz, 2, $\mathrm{NCH_2C}\mathit{H}_2\mathrm{CH_2N{=}}$).

Anal. Calcd for $C_{11}H_{22}N_8O_9$: C, 32.20; H, 5.40: N, 27.31. Found: C, 32.25; H, 5.34; N, 27.14.

3-[3-(2-Pyridyl)propylamino]propylguanidine (**7**). Treatment of 2-(3-chloropropyl)pyridine hydrochloride (see above) with a 10-fold excess of 1,3-propanediamine, as described above in the preparation of **2**, at −20°C for 48 hr yielded 51% of 3-[3-(2-pyridyl)propylamino]propylamine as a reddish-brown oil: bp 135–138°C/0.1 mm; nmr ($CDCl_3$), δ 8.30 (dist d, 1, C-6 Py), 7.40 (dist t, 1, C-4 Py), 7.09–6.78 (m, 2, C-3, 5 Py), 2.95–2.47 (m, 8, $\mathrm{PyC}\mathit{H}_2\mathrm{CH_2C}\mathit{H}_2\mathrm{NHC}\mathit{H}_2\mathrm{CH_2C}\mathit{H}_2\mathrm{NH_2}$), 1.85 (dist quint, 2, $\mathrm{PyCH_2C}\mathit{H}_2\mathrm{CH_2N}$, 1.55 (quint, J = 6.5 Hz, 2, $\mathrm{NCH_2C}\mathit{H}_2\mathrm{CH_2NH_2}$), 1.15 (s, 3, NH and NH_2; disappears on shaking with D_2O). Reaction with *S*-methylisothiourea followed by sulfuric acid, as described above, yielded 55% of

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[7] The sulfate salt is not sufficiently soluble in D_2O to obtain an nmr spectrum. Therefore, it was converted to a D_2O solution of the dinitrate salt by mixing equimolar amounts of the sulfate and barium nitrate in D_2O. After warming and shaking the mixture for 5 min, the barium sulfate was removed by centrifugation.

product, mp 248–251°C, which was recrystallized twice from a 2:1 mixture of water and acetone to give the sulfate of **7** as colorless irridescent platelets: mp 251.5–253°C (sealed tube); ir (KBr), 3400 (NH stretch), 1670 (C=N stretch), 1630 (NH bend), 1120 cm^{-1} (SO_4^{2-}); nmr (D_2O),[7] δ 8.45 (dist d, 1, C-6 Py), 7.88 (dist t, 1, C-4 Py), 7.50–7.24 (m, 2, C-3, 5 Py), 3.52–2.80 (m, 8, $\text{PyC}H_2\text{CH}_2\text{C}H_2\text{NHC}H_2\text{CH}_2\text{C}H_2\text{N=}$), 2.17 (dist quint, 4, $\text{PyCH}_2\text{C}H_2\text{CH}_2\text{NHCH}_2\text{C}H_2\text{CH}_2\text{N=}$).

Anal. Calcd for $C_{12}H_{23}N_5SO_4$: C, 43.23; H, 6.95; N, 21.01. Found: C, 43.05; H, 6.85; N, 20.73.

2-[3-(4-Pyridyl)propylamino]ethylguanidine (**8**). 4-(3-Chloropropyl)pyridine was treated with a five-fold excess of ethylenediamine to yield 61% of 2-[3-(4-pyridyl)propylamino]ethylamine as a colorless oil: bp 115–118°C/0.3 mm; ir (neat), 3300 (NH stretch), 2950 (CH stretch), 1600 (NH_2 and NH bend), 1410, 1130 (CN stretch), 800 cm^{-1} (pyridine CH bend); nmr ($CDCl_3$), δ 8.52 (dist d, 2, C-2, 6 Py), 7.15 (dist d, 2, C-3, 5 Py), 2.90–2.48 (m, 8, $\text{PyC}H_2\text{CH}_2\text{C}H_2\text{NHC}H_2\text{C}H_2\text{NH}_2$), 1.78 (dist quint, 2, $\text{PyCH}_2\text{C}H_2\text{CH}_2\text{N}$), 1.21 (s, 3, NH and NH_2; disappears on shaking with D_2O); trinitrate salt, mp 180–181.5°C (*Anal.* Calcd for $C_{10}H_{20}N_6O_9$: C, 32.61; H, 5.47; N, 22.82. Found: C, 32.46; H, 5.43; N, 22.63). Reaction with *S*-methylisothiourea followed by nitric acid, as described above, yielded 63% of product after recrystallization from a 5:1 mixture of methanol and water, mp 186.5–188.5°C. Two additional recrystallizations from this same mixture followed by one from 95% ethanol gave the trinitrate of **8** as colorless, thin, jagged needles: mp 189.5–191°C; ir (KBr), 3400 (NH stretch), 1670 (C=N stretch), 1630 (NH bend), 1380 cm^{-1} (NO_3^-); nmr (D_2O), δ 8.67 (d, $J = 6.5$ Hz, 2, C-2, 6 Py), 7.89 (d, $J = 6.5$ Hz, 2, C-3, 5 Py), 3.83–2.97 (m, 8, $\text{PhC}H_2\text{CH}_2\text{C}H_2\text{NHC}H_2\text{C}H_2\text{N=}$), 2.24 (dist quint, 2, $\text{PyCH}_2\text{C}H_2\text{CH}_2\text{NH}$).

Anal. Calcd. for $C_{11}H_{22}N_8O_9$: C, 32.20; H, 5.40; N, 27.31. Found: C, 32.05; H, 5.37; N, 26.81.

3-[3-(4-Pyridyl)propylamino]propylguanidine (**9**). 4-(3-Chloropropyl)pyridine (*29*) was treated with a five-fold excess of 1,3-propanediamine to yield 76% of 3-[3-(4-pyridyl)propylamino]propylamine as a colorless oil: bp 141–146°C/0.03 mm; ir (neat), 3320 (NH stretch), 2940 (CH stretch), 1600 (NH bend) 1420, 1130 (CN stretch), 800 cm^{-1} (pyridine CH stretch); nmr ($CDCl_3$), δ 8.53 (dist d, 2, C-2, 6 Py), 7.13 (dist d, 2, C-3, 5 Py), 2.90–2.50 (m, 8, $\text{PyC}H_2\text{CH}_2\text{C}H_2\text{NHC}H_2\text{CH}_2\text{C}H_2\text{NH}_2$), 1.80 (dist quint, 2, $\text{PyCH}_2\text{C}H_2\text{CH}_2\text{N}$), 1.60 (quint, $J = 7$ Hz, 2, $\text{NCH}_2\text{C}H_2\text{CH}_2\text{N}$), 1.10 (s, 3, NH and NH_2; disappears on shaking with D_2O); trinitrate salt, mp 124–126°C (*Anal.* Calcd for $C_{11}H_{22}N_6O_9$: C, 34.56; H, 5.80; N, 21.98. Found: C, 34.48; H, 5.73; N, 21.83). Treatment with *S*-methylisothiourea followed by nitric acid, as described above, gave 60% of product after recrystallization from methanol, mp 156.5–157.5°C. Additional recrystallization from methanol and from a 1:1 mixture of methanol and ethanol produced the trinitrate of **9** as granular crystals: mp 159–160.5°C; ir (KBr), 3400 (NH stretch), 1670 (C=N stretch), 1645 (NH bend), 1380 cm^{-1} (NO_3^-); nmr (D_2O), δ 8.85 (d, $J = 6.5$ Hz, 2, C-2, 6 Py), 7.92 (d, $J = 6.5$ Hz, 2, C-3, 5 Py), 3.15 (dist quint, 8, $\text{PyC}H_2\text{CH}_2\text{C}H_2\text{NHC}H_2\text{CH}_2\text{C}H_2\text{N=}$), 2.08 (dist quint, 4, $\text{PyCH}_2\text{C}H_2\text{CH}_2\text{NH-CH}_2\text{C}H_2\text{CH}_2\text{N=}$).

Anal. Calcd for $C_{12}H_{24}N_8O_9$: C, 33.96; H, 5.70; N, 26.40. Found: C, 33.71; H, 5.68; N, 26.28.

2-(Benzylamino)ethylguanidine (**10**). 2-(Benzylamino)ethylamine was obtained in 72% yield as a colorless liquid: bp 80–83°C/0.5 mm [lit (*30*), 102°C/5 mm); mp of dihydrochloride 260–264°C (dec) [lit (*31*), 260°C (dec)]. Treatment with *S*-methylisothiourea yielded a crude product which was recrystallized from a 2 : 1 mixture of absolute ethanol and anhydrous methanol to afford 47% of the dihydrochloride of **10** as fine, white needles: mp after two additional recrystallizations from absolute ethanol 228.5–229.5°C; ir (KBr), 3300 (NH stretch), 1690 (C=N stretch), 1630 (NH bend), 770, 710 cm^{-1} (CH bend); nmr (D_2O), δ 7.55 (s, 5, ArH), 4.31 (s, 2, $\mathrm{ArC}H_2$), 3.62 (dist t, 2, $\mathrm{NC}H_2\mathrm{CH_2N}{=}$), 3.33 (dist t, 2, $\mathrm{NCH_2C}H_2\mathrm{N}{=}$).

Anal. Calcd for $C_{10}H_{18}N_4Cl_2$: C, 45.29; H, 6.84; N, 21.13. Found: C, 45.59; H, 6.73; N, 21.27.

2-(2-Phenylethylamino)ethylguanidine (**11**). 2-(2-Phenylethylamino)ethylamine was obtained in 70% yield as a colorless liquid: bp 86.5–94°C/0.2 mm [lit (*30*), 136–140°C/12 mm); mp of dinitrate 164.5–166°C. Treatment with *S*-methylisothiourea followed by nitric acid, as described above, yielded a crude product which was recrystallized from a 4 : 1 mixture of anhydrous methanol and anhydrous ether to afford 83% of the dinitrate of **11** as an amorphous powder, mp 126–131°C. Two more recrystallizations from a 1 : 1 mixture of absolute ethanol and anhydrous methanol, followed by drying at 120°C (0.1 mm) for 14 hr gave irridescent, jagged plates: mp 145.5–146.5°C; ir (KBr), 3300 (NH stretch), 1660 (C=N stretch), 1620 (NH bend), 1375 cm^{-1} (NO_3^-); nmr (D_2O), δ 7.36 (s, 5, ArH), 3.70–2.80 (m, 8, $\mathrm{Ar}C\mathrm{H_2}C\mathrm{H_2NH}C\mathrm{H_2}C\mathrm{H_2N}{=}$). The dipicrate of **3** was obtained as fine, bright yellow needles after recrystallization from acetone–ethanol, mp 208–209.5°C.

Anal. Calcd for $C_{23}H_{24}N_{10}O_{14}$: C, 41.57; H, 3.64; N, 21.08. Found: C, 41.13; H, 3.53; N, 20.84.

2-(3-Phenylpropylamino)ethylguanidine (**12**). 2-(3-Phenylpropylamino)ethylamine was obtained in 70% yield as a colorless liquid: bp 95–102°C/0.35 mm. The dinitrate was obtained as small, irridescent plates: mp 170.5–171.5°C; ir (KBr), 3400 (NH_3^+ stretch), 2950 (CH stretch), 2800 (NH_2^+ stretch), 1600 (NH bend), 1380 cm^{-1} (NO_3^-).

Anal. Calcd for $C_{11}H_{20}N_4O_8$: C, 43.42; H, 6.62; N, 18.41. Found: C, 43.42; H, 6.62; N, 18.41.

Treatment with *S*-methylisothiourea followed by nitric acid, as described above, yielded a crude product which was triturated with acetone, filtered, and dried to afford 80% of a white, amorphous powder, mp 128.5–130.5°C. Two recrystallizations from absolute methanol gave the dinitrate of **12** as fine, white needles: mp 131.5–132.5°C; ir (KBr), 3300, 3140, 2700 (NH stretch), 1680 (C=N stretch), 1630 (NH bend), 1370 cm^{-1} (NO_3^-); nmr (D_2O), δ 7.26 (s, 5, ArH), 3.50 (dist t, 2, $\mathrm{CH_2N}{=}$), 3.28–2.83 (m, 4, $CH_2\mathrm{NH}CH_2$), 2.60 (t, $J = 7$ Hz, 2, $\mathrm{ArC}H_2$), 1.88 (quint, $J = 7$ Hz, 2, $\mathrm{ArCH_2C}H_2\mathrm{CH_2N}$).

Anal. Calcd for $C_{12}H_{22}N_6O_6$: C, 41.62; H, 6.40; N, 24.26. Found: C, 41.67; H, 6.35; N, 24.32.

3-(Benzylamino)propylguanidine (**13**). 3-(Benzylamino)propylamine was obtained in 76% yield as a colorless oil: bp 91–92°C/0.5 mm [lit (*32*), 98–102°C/1 mm]. Treatment with *S*-methylisothiourea followed by nitric acid, as described above, yielded 88% of product after one recrystallization from absolute ethanol, mp 140.5–143°C. Three additional recrystallizations from absolute ethanol yielded the dinitrate of **13** as short, jagged needles: mp 143.5–144.5°C; ir (KBr), 3350 (NH stretch), 1660 (C=N stretch), 1380 cm^{-1} (NO_3^-); nmr (D_2O), δ 7.42 (s, 5, ArH), 4.15 (s, 2, ArCH_2N), 3.30–2.90 (m, 4, N$CH_2$$CH_2$$CH_2$N=), 1.88 (quint, J = 7 Hz, 2, N$CH_2$$CH_2$$CH_2$N=).

Anal. Calcd for $C_{11}H_{20}N_6O_6$: C, 39.76; H, 6.07; N, 25.29. Found: C, 39.67; H, 6.07; N, 25.22.

3-(2-Phenylethylamino)propylguanidine (**14**). 3-(2-Phenylethylamino)propylamine was obtained in 70% yield as a colorless oil, bp 91–92°C/0.5 mm [lit (*32*), 98–102°C/1 mm]. Treatment with *S*-methylisothiourea followed by nitric acid, as described above, yielded a crude product which was recrystallized from 1:1 methanol–diethyl ether to give 66% of the dinitrate of **14**: mp 175.5–177°C. Five additional recrystallizations yielded jagged needles: mp 179.5–180°C; ir (KBr), 3400 (NH stretch), 1670 (C=N stretch), 1640 (NH bend, 1380 cm^{-1} (NO_3^-); nmr (D_2O), δ 7.25 (s, 5, ArH), 3.46–2.82 (m, 8, Ar$CH_2$$CH_2NHCH_2$$CH_2$$CH_2$N=), 1.92 (quint, J = 7.5 Hz, 2, N$CH_2$$CH_2$$CH_2$N=).

Anal. Calcd for $C_{12}H_{22}N_6O_6$: C, 41.62: H, 6.40; N, 24.26. Found: C, 41.46; H, 6.39; N, 24.17.

3-(3-Phenylpropylamino)propylguanidine (**15**). 3-(3-Phenylpropylamino)-propylamine was obtained in 75% yield as a colorless oil, bp 107–108°C/0.03 mm [lit (*33*), 120–126°C/1.5 mm]. Treatment with *S*-methylisothiourea followed by nitric acid as described above, yielded a crude product which was triturated with five 30-ml portions of acetone and five 30-ml portions of anhydrous ether. Recrystallization from methanol gave 1.89 g (52%) of product, mp 158–159.5°C. Four additional recrystallizations from a 1:1 mixture of absolute ethanol and methanol gave the dinitrate of **15** as short needles: mp 161–161.5°C; ir (KBr), 3300, 2750 (NH stretch), 1670 (C=N stretch), 1640 (NH bend), 1380 cm^{-1} (NO_3^-): nmr (D_2O), δ 7.27 (s, 5, ArH), 3.18 (t, J = 7 Hz, 2, CH_2N=), 3.12–2.80 (m, 4, CH_2NHCH_2), 2.60 (dist t, 2, ArCH_2, 1.86 (dist quint, 4, Ar$CH_2$$CH_2$$CH_2NHCH_2$$CH_2$$CH_2$N=).

Anal. Calcd for $C_{13}H_{24}N_6O_6$: C, 43.33; H, 6.71: N, 23.32. Found: C, 43.11; H, 6.68; N, 23.29.

Dihydroxyacetone Phosphate

Synthesis of DHAP. Employing an acetylation recipe developed in this laboratory by Dr. R. S. Buriks, a mixture of 45.0 g (0.5 mol) of dihydroxyacetone, 150 g of anhydrous sodium carbonate, and 150 g of anhydrous sodium sulfate in 2 liters of acetone was stirred for 30 min at room temperature, heated to reflux with a water bath held at 70°C, and treated, dropwise over a period of 3 hr, with a solution of 44 g (0.56 mol) of acetyl chloride in 100 ml of acetone. Heating and stirring were continued for 1 hr, the water bath was then removed, and stirring was continued an additional 2 hr. The solution was filtered, the solid was washed with two 250-ml portions of acetone, and the combined acetone solution was treated with 20 ml of water and stirred for 15 min. The solution was then dried by stirring for 1 hr with 500 g of anhydrous sodium sulfate and then overnight with 20 g of powdered calcium hydroxide. The solution was filtered, the solids were washed with two 250-ml portions of dry acetone, and the combined filtrate was evaporated. The residue was extracted with five 200-ml portions of benzene, and the benzene solution was dried over magnesium sulfate, filtered, and evaporated to give a crude product that was distilled to afford 19.90 g (30%) of dihydroxyacetone monoacetate as a colorless oil, bp 82–85°C/0.10 mm [lit (*34*), 70–73°C/0.01 mm]. This was immediately converted to cyclohexylammonium dihydroxyacetone phosphate dimethyl ketal, according to published procedures (*34*), and obtained as fine, white needles, MP 180–181.5°C (dec) [lit (*34*), 183–185°C (dec)]. A solution of 100 mg (0.23 mmol) of this material in 8 ml of distilled water was treated with 2 g of Dowex 50X4 resin (200–400 mesh) that had been washed repeatedly with 5-ml portions of 1 *N* hydrochloric acid followed by 5-ml portions of distilled water until the washings were no longer acidic to pH test paper. The mixture was swirled for 60 sec, the resin was removed by filtration and washed twice with 1-ml portions of distilled water, and the filtrate was heated for 4 hr at 38–40°C to hydrolyze the ketal. The resulting solution was freeze-concentrated to a volume of 5 ml to give a stock solution of dihydroxyacetone phosphate (ca 0.05 *M*) which could be stored at −20°C for 1 month with less than 1% decomposition.

Assay of DHAP. The concentration of DHAP in the stock solution was determined by enzymatic assay (*35*) using α-glycerophosphate dehydrogenase and dihydronicotinamide adenine dinucleotide (NADH). The amount of dihydroxyacetone (DHA) present in the DHAP stock solution was determined using a modification of the enzymatic method of Wieland and Witt (*36*) which involves phosphorylation of dihydroxyacetone to DHAP (glycokinase + ATP) and determination of DHAP by the method indicated above. It was demonstrated that this assay for DHA can be carried out in the presence of a large excess of DHAP if an appropriate excess of NADH is employed. By means of this assay it was determined that the stock solutions of DHAP, prepared as described above, contained ca. 0.7% DHA. Attempts to remove this small amount of DHA by the method of Hall and Knowles (*37*) were unsuccessful, the eluate from the Dowex 1 × 8 (Cl^- form, 200–400 mesh) anion exchange column having the same 0.7% DHA as the original. The DHA in DHAP has little effect on the kinetic measurements, however, for the rates of ionization of DHA and DHAP are comparable.[8]

pK_a of DHAP. A 2.96-ml portion of a 0.0338 *M* solution of DHAP and 0.80 ml of 1.00 *M* sodium nitrate were added to 6.09 ml of carbonate-free distilled water contained in a 20-ml pH measurement cell in a constant temperature bath held at 35°C. The solution was stirred by passing a stream of purified (*15*) nitrogen through it, and a standardized solution of 1.00 *N* sodium hydroxide was added through a calibrated 50-μl syringe in 2-μl portions. After each addition the pH reading was allowed to stabilize (ca. 2 min) and then recorded. The addition of sodium hydroxide was continued to ca.

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[8] Rate of ionization measurements for DHA and DHAP indicate that with pyridine as the catalyst DHA ionizes approximately two times more rapidly than DHAP. Experiments with tris(hydroxymethyl)methanamine (Tris) in the present investigation showed that the second-order rate constants for Tris are almost identical for DHA and DHAP. The hydroxide ion-catalyzed ionizations appear to proceed at different rates, however, with DHAP being more responsive than DHA, as the following data indicate:

	$k_{\phi 1} \times 10^5$ (sec^{-1})				
Substrate	0.02*	0.03*	0.04*	0.05*	$k_2 \times 10^3$ (M^{-1} sec^{-1})
DHA	0.87	1.10	1.38	1.48	0.28 ± 0.04
DHAP	2.63	2.71	2.95	3.23	0.27 ± 0.04

Asterisks denote values of $[\mathrm{cat}]_{total}$. Reactions were carried out with a [Tris]/[Tris–HNO_3] ratio of 3.0.

pH 8.5. From a plot of the pH vs amount of sodium hydroxide added it was determined from the inflection points that $pK_1' = 2.35 \pm 0.02$ and $pK_2' = 5.98 \pm 0.02$. Application of the usual ionic strength corrections affords thermodynamic pK_a values of 2.46 and 6.31 for pK_1 and pK_2, respectively.

Kinetic Measurements

The rates of ionization of dihydroxyacetone phosphate in water at 35°C were measured in the presence of excess tetranitromethane according to the method of Christen and Riordan (*7, 17*). Measurements were made at total catalyst concentrations of 0.004–0.016 *M* and, in most cases, at a pH identical with the apparent pK_a of the catalyst. Tetranitromethane concentrations were $1.67\text{–}6.67 \times 10^{-3}$ *M*, the dihydroxyacetone phosphate concentration was 10^{-3} *M*, and the total ionic strength was 0.100 (with sodium nitrate). The initial rates of ionization were determined spectrometrically by following the appearance of nitroformate at 350 nm, which is equal to the rate of carbanion formation from DHAP.

In a typical determination the following solutions were added to a 3.5-ml spectrometer cell: (a) a volume of 0.200 *M* catalyst stock solution which, upon dilution to 3.00 ml, gave the desired catalyst concentration, (b) enough 0.1000 *N* sodium hydroxide to neutralize both the catalyst and the DHAP to the desired pH, (c) enough carbonate-free distilled water to bring the final volume to 3.00 ml. The cell was placed in the thermostatted cell holder in the spectrometer and allowed to come to thermal equilibrium (ca. 15 min). A 30-μl volume of 0.2 *M* tetranitromethane was then added, followed by a quantity of DHAP stock solution such that dilution to 3.00 ml gave a DHAP concentration of 0.001 *M*. The final solution was quickly mixed, and the absorbance was recorded as a function of time (in minutes) over a period of 5–15 min. The contribution to the observed rate by the action of species other than DHAP or TNM was measured in the absence of DHAP and subtracted from the total rate to give the rate of ionization of DHAP. The corrected slope values were divided by the extinction coefficient for nitroformate at 350 nm [14,400 (*39*)] and by 60 sec/min to give pseudo-zero-order rate constants in units of moles per liter · second. Dividing this value by the concentration of DHAP (e.g., 10^{-3} *M*) gave the pseudo-first-order rate constants, in units of seconds^{-1}, listed in Tables 2–6.

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