Conclusions

Thus, in the attack of secondary amines on TNA, it is thought that the σ complexes on the substitution pathway are formed in a very rapid equilibrium and are much longer lived than that from the primary amine discussed in the previous paper. However, the expected conversion to the substituted picramide product **4** does not occur. There is, instead, a reaction to produce the dimethoxy Meisenheimer complex **3**. The difference between this and the reaction of the primary amine where substitution does occur is possibly due to steric interactions. Thus, in the case of secondary amines, steric interactions between the dialkylamino group and the two ortho nitro groups in the *product* picramide will raise the energy of this species and the transition state for its formation from the intermediate and allow for the slow formation of the dimethoxy complex. Such considerations may well also explain the exceptional stabilities reported for the $OR-NR_1R_2$ complexes from dinitro- and trinitronaphthyl ethers,[9,10] and from hindered picryl ethers with very hindered amines.[11] A complete description of this system was possible only using ^{13}C NMR and flow NMR at low temperatures, although it is important to note that ^1H NMR investigations alone may well be "ambiguous" for reactions involving symmetrical species where only single signals are observed.

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References and Notes

(1) (a) Department of Chemistry, University of Wroclaw, Wroclaw, Poland. (b) Pearson College, Victoria, B.C., Canada.
(2) C. F. Bernasconi, *MTP Int. Rev. Sci.: Org. Chem., Ser. One*, **3**, 33 (1973).
(3) (a) R. Foster and C. A. Fyfe, *Rev. Pure Appl. Chem.*, **16**, 61 (1966); (b) E. Buncel, A. R. Norris, and K. E. Russell, *Q. Rev., Chem. Soc.*, **22**, 123 (1968); (c) M. R. Crampton, *Adv. Phys. Org. Chem.*, **7**, 211 (1969); (d) M. J. Strauss, *Chem. Rev.*, **70**, 667 (1970); (e) C. A. Fyfe in "The Chemistry of the Hydroxyl Group", S. Patai, Ed., Interscience, New York, N.Y., 1971.
(4) J. F. Bunnett and R. E. Zahler, *Chem. Rev.*, **49**, 275 (1951).
(5) C. A. Fyfe, A. Koll, S. W. H. Damji, C. D. Malkiewich, and P. A. Forte, *Can. J. Chem.*, **55**, 1468 (1977).
(6) C. A. Fyfe, A. Koll, and S. W. H. Damji, *J. Am. Chem. Soc.*, preceding paper in this issue.
(7) S. W. H. Damji, Ph.D. Thesis, University of Guelph, 1976.
(8) C. A. Fyfe, M. Cocivera, S. W. H. Damji, T. A. Hostetter, D. Sproat, and J. O'Brien, *J. Magn. Reson.*, **23**, 377 (1976).
(9) S. Sekiguchi, K. Shinoyaki, T. Hirose, K. Matsui, and K. Sekine, *Bull. Chem. Soc. Jpn.*, **49**, 2264 (1974).
(10) S. Sekiguchi, T. Itagaki, T. Hirose, K. Matsui, and K. Sekine, *Tetrahedron*, **29**, 3527 (1973).
(11) L. B. Clapp, H. Lacey, G. G. Beckwith, R. M. Srivastara, and N. Muhammed, *J. Org. Chem.*, **33**, 4262 (1968).

Pyruvamides. 3. Involvement of the Amide Group in Carbinolamine Formation, *gem*-Diamine Formation, and Transimination[1]

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Abstract: Previous work on the addition of nitrogen nucleophiles to the ketone groups of pyruvamides had led to the suggestion that the amide group might be involved as an intramolecular catalyst. The main purpose of the present work was to confirm or to negate this possibility. Rate constants for carbinolamine formation from semicarbazide with pyruvanilide and four ring-substituted pyruvanilides at pH 7 yielded a linear σ correlation with a ρ of 0.28, which is consistent with a normal mechanism involving rate-determining trapping of a zwitterionic intermediate by water. *N*-Methylpyruvanilide reacted one-third as quickly as pyruvanilide did. *gem*-Diamine formation from semicarbazide and pyruvamide imines proceeds to a good equilibrium extent and can be observed directly. Isomerization of the initial semicarbazide-protonated *gem*-diamine to the more stable amine-protonated diamine is responsible for the unusually favorable equilibrium position. An imine for which intramolecular amide group involvement is structurally precluded yielded a pH-rate profile markedly different from that given by a simple pyruvamide imine, but the difference is probably due to factors other than this preclusion. Transimination of pyruvamide and pyruvanilide imines with amines in nonpolar aprotic solvents is slow and is strongly accelerated by small amounts of acid. The rate of the spontaneous reaction is attributable to the amide group behaving simply as an intermolecular proton donor or acid catalyst. *p*-Chloropyruvanilide methylimine reacted four times as rapidly as pyruvanilide methylimine and an N,N-disubstituted imine reacted 100 times more slowly than did the corresponding pyruvamide imine, as would be expected on this basis. A σ correlation for substituents in the amine was linear with $\rho = -0.79$. Reactions in pyridine–water mixtures were no faster than in dry pyridine. We conclude that in no case was the amide group involved as an intramolecular catalyst despite the most favorable geometry of these systems, a finding which bodes ill for the likelihood of intramolecular catalysis by amide groups in biochemical systems generally.

The reaction of pyruvamides with semicarbazide to give carbinolamines, necessary intermediates in the formation of semicarbazones, is rather fast.[2] Of itself this is not unduly surprising. The electron-withdrawing carboxamide group is attached directly to the site of nucleophilic attack, and the effects of such groups in promoting both the rates and the equilibria of formation of carbonyl addition compounds are well documented and entirely reasonable.[3-5] There seemed to be indications, however, that the effects might be larger than would be expected in terms of electronic factors alone. Methyl

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pyruvate,[6] for example, reacts less quickly than pyruvamides do despite the fact that the carboxyalkyl group should be considerably more strongly electron attracting than the amide. The reactivity, the equilibrium constant, and the phosphate buffer catalytic constant for the reaction of *N,N*-dimethylpyruvamide with semicarbazide all are about $^1/_{20}$ of the values for pyruvamides having a proton on the amide nitrogen. Also, not only are carbinolamines derived from such *N*-protopyruvamides with simple amines (benzylamine, propylamine, e.g.) unusually stable, such that they may be isolated and characterized,[2] but they precipitate essentially instantaneously when solutions of pyruvamide and the amine in nonpolar aprotic solvents are mixed. Although this observation of speed is but qualitative ($\ll$0.5 s) it is remarkable that a reaction involving a zwitterionic intermediate, a protonation, and a protolysis should occur so rapidly under such conditions.

The histidine decarboxylase of lactobacillus 30a (histidine carboxy-lyase, EC 4.1.1.22) utilizes N-terminal pyruvamide residues as prosthetic groups.[7] It is one of a small but clearly recognized[8] and growing group of enzymes that make use of α-keto acid entities in much the same ways that the commoner pyridoxal-dependent enzymes use pyridoxal. Simple pyruvamides promote analogous reactions under surprisingly mild conditions.[2] The steps in the enzymic and nonenzymic reaction sequences undoubtedly are very similar, the first being carbinolamine formation between the pyruvamide ketone and the amino group of the amine or amino acid, and initially it was our wish to know more about a seemingly unusually rapid enzyme-model reaction that prompted the present investigation. Amide groups, however, are ubiquitous in protein structures, be it as backbone or as side-chain moieties, and wishful speculations as to their involvement in enzyme mechanism, as intramolecular acid-base catalysts or simply as hydrogen bond donors, seem to be almost as ubiquitous. A study of the involvement of the amide group in pyruvamide carbinolamine formation thus might have quite wide significance. We report here the results of such a study.

Experimental Section

Materials. Pyruvanilide, ring-substituted pyruvanilides, and *N*-methylpyruvanilide were prepared by reacting the appropriate amines with pyridinium hydroxymaleic anhydride[9] except for *p*-nitropyruvanilide, which could not be prepared in this way but was obtained by nitrating pyruvanilide under conventional conditions[10] and also by reacting *p*-nitroaniline with pyruvyl chloride, a reproducible preparation for which appeared[11] during our work. Other pyruvamides were prepared as described previously.[2] Pyruvamide and pyruvanilide alkylimines were prepared by heating the appropriate pyruvamide and alkylamine in benzene for 1–2 h. Refluxing conditions and a longer time (16 h) were necessary in the preparation of *N,N*-dimethylpyruvamide propylimine. Aralkylimines (benzyl, methylbenzyl) could be prepared similarly, although with some loss to tautomerization, but were more conveniently made by adding concentrated aqueous solutions of the amine hydrochlorides to freshly prepared concentrated aqueous solutions of pyruvamide propylimine, whereupon the desired imine would precipitate instantaneously. The cyclic imine-amide 3-methyl-5,6-dihydro-2(1*H*)-pyrazinone (V) was prepared by a slightly modified literature procedure in 65% rather than 9% yield.[12] All of these substances were purified by sublimation (80–100 °C, 0.1 mm) except for liquids (*N*-butylpyruvamide, *N,N*-dimethylpyruvamide, and its propylimine), which were distilled under reduced pressure.

Pyruvamides and imines, and solutions of them in inert solvents, were stored in the dark at −20 °C since they are adversely affected by light within a few days and by storage at ambient temperatures in the dark rather more slowly. Dioxane was purified by refluxing over sodium. Pyridine containing 1% Me_4Si (NMR Specialties Inc.) was stored over type 4A molecular sieve desiccant. It contained no water detectable by Karl Fisher reagent. Semicarbazide hydrochloride (Aldrich) and KCl were recrystallized to low optical density from aqueous ethanol and from water, respectively. Buffer components (Tris, MES, HEPES, CHES[13]) were from Sigma, exhibited acceptably low optical densities, and were used as received.

Stopped-Flow Studies. Procedures and conditions were largely as described previously[2] except that imine solutions were not allowed to stand for 30 min after dilution of the stock solutions in dioxane into water but were introduced into the stopped-flow apparatus and brought into reaction as quickly as possible (<1 min). Ionic strength was 1.0 (KCl). Buffers for semicarbazide-pyruvanilide reactions follow: pH 4, semicarbazide itself; pH 4.5–6.5, 2-*N*-morpholinoethanesulfonic acid (MES, pK_a = 6.15); pH 7–8.5, *N*-2-hydroxyethylpiperazine-*N'*-2-hydroxyethanesulfonic acid (HEPES, pK_a = 7.55); above pH 8.5, cyclohexylaminoethanesulfonic acid (CHES, pK_a = 9.50). Buffer for semicarbazide-pyruvamide imine and semicarbazide-pyrazinone reactions was tris(hydroxymethyl)methylamine (Tris, pK_a = 8.51). Ultraviolet absorbance data generated by the stopped-flow spectrophotometer was recorded in a Biomation Model 805 waveform recorder. Appropriate adjustment of *x* and *y* scale factors was made, following which the data was copied onto a conventional strip chart recorder for examination, analysis, and storage.

NMR Studies. Transimination of pyruvamide imines with aliphatic primary amines is slow enough in benzene, chloroform, and pyridine ($t_{1/2}$ several minutes) to be followed by NMR spectrophotometry. Conversion of an amine into the corresponding pyruvamide imine results in a downfield shift of about 0.3–0.4 ppm for the protons on the carbon atom attached to the amine nitrogen, so that the consumption of the attacking amine and the starting imine, as well as the liberation of the product imine and amine, all may conveniently be observed. Methyl and benzyl imines, and benzyl amines, for which the pertinent peaks are singlets in otherwise empty spectral regions, serve very well indeed. In a typical experiment, 0.5 mL of 1.0 M solution of the imine would be placed in an NMR tube, the instrument adjusted and temperature equilibrated and the spectrum run, and the instrument set just downfield of the desired pair of peaks. The amine (0.5 mmol) would be added, the tube contents thoroughly mixed, and the integral of the spectrum in the narrow region of interest determined at various times after mixing. From time to time the complete spectrum would be checked to ascertain that it did indeed represent the expected four-component mixture. This always was so except in the case of pyridine solutions containing substantial quantities (>30% v/v) of water.

Results

Reactions explored were those of pyruvanilide, various ring-substituted pyruvanilides (I), and *N*-methylpyruvanilide with semicarbazide in water, of a variety of pyruvamide and pyruvanilide imines (II, III, IV) with alkyl- and aralkylamines in aprotic solvents, and of one such imine (IId) and of the cyclic pyruvamide imine 3-methyl-5,6-dihydro-2(1*H*)-pyrazinone (V) with semicarbazide in water.

1. Carbinolamine Formation. Plots of k_{obsd} vs. pH for the reaction of pyruvanilide with semicarbazide within the pH range 4–9 confirmed that the reaction is linearly acid dependent at low pH and breaks cleanly to acid independence at pH ~6 as previously reported.[2] Structure vs. reactivity correlation studies accordingly were conducted at pH 7, pH independence for the various substituted pyruvanilides being confirmed by parallel measurements at pH 6.5 and 7.5 in all cases. With the use of the weakly catalytic zwitterionic buffers in place of the phosphate used previously, a distinct curvature, reflecting catalysis by semicarbazide, was evident in k_{obsd} vs. [semicarbazide] plots. The appropriate rate equation for reversible carbinolamine formation in the pH-independent region, reflecting both buffer and semicarbazide catalysis, thus is

$$k_{obsd} = (k_1[S] + k_{-1})(1 + k_1^B[B]/k_1 + k_1^S[S]/k_1) \quad (1)$$

where [S] and [B] are semicarbazide and buffer concentrations, k_1 and k_{-1} are rate constants for the uncatalyzed forward reaction and its reverse, and k_1^B and k_1^S are third-order rate constants for the buffer-catalyzed and semicarbazide-catalyzed forward reactions, respectively. Plots of k_{obsd} vs.

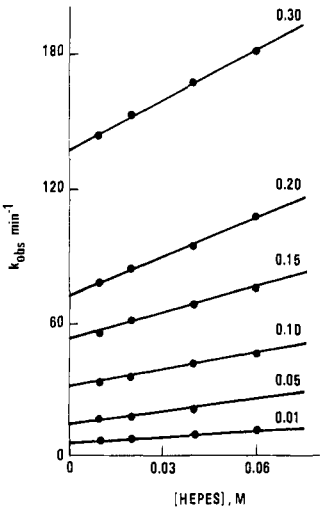


Figure 1. Dependence of rate of reaction of *m*-trifluoromethylpyruvanilide with semicarbazide at 25.0 °C and pH 7.0 upon buffer (HEPES) concentration at various concentrations of semicarbazide (0.01–0.30 M). The lines are calculated from linear least-squares analyses and are computer drawn.

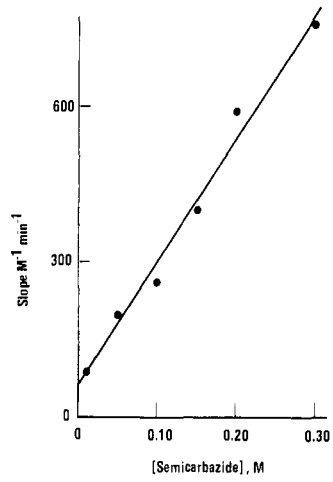
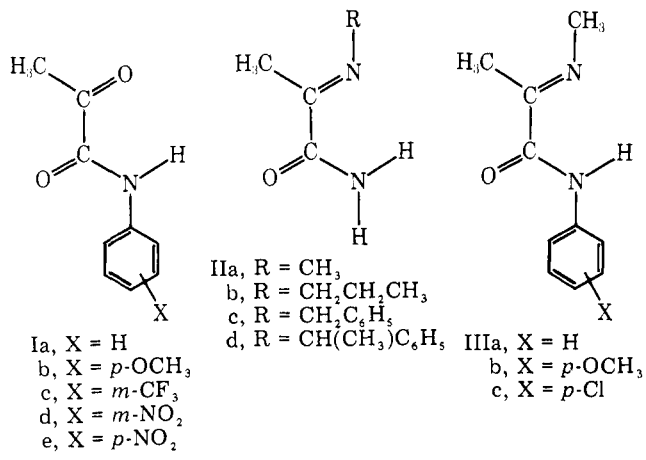
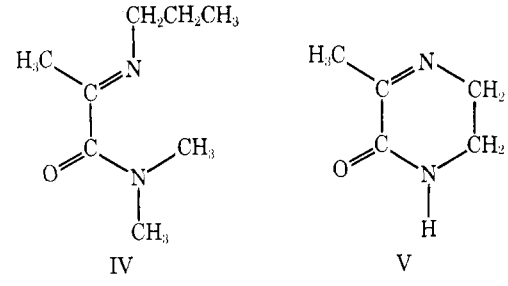


Figure 2. Dependence of slopes of lines in Figure 1 upon semicarbazide concentration. The line is calculated from a linear least-squares analysis and is computer drawn. Its slope is k_1^B and its ordinal intercept is k_{-1}^B for buffer (HEPES) catalysis of the reaction of *m*-trifluoromethylpyruvanilide with semicarbazide at 25 °C and pH 7.





buffer concentration at pH 7 and various semicarbazide concentrations (Figure 1, e.g.) were linear as eq 1 requires and were analyzed for slopes and intercepts using a linear least-squares computer fit. Plots of the slopes thus obtained vs. [semicarbazide] (Figure 2, e.g.) also were linear, were similarly analyzed, and yielded values for k_1^B (slopes) and k_{-1}^B (intercepts) for the various pyruvanilides. Plots of the intercepts of k_{obsd} vs. [buffer] plots against semicarbazide concentrations (Figure 3, e.g.) were not linear. Rearrangement of eq 1 yields

$$I = k_{-1} + [S](k_1 + k_{-1}^S) + [S]^2 k_1^S \qquad (2)$$

(I is the intercept of a k_{obsd} vs. [B] plot) and analysis of the data using a second-order least-squares computer fit yielded theoretical curves (e.g., Figure 3) which fitted the data points most satisfactorily. Correlation coefficients exceeded 0.98 and

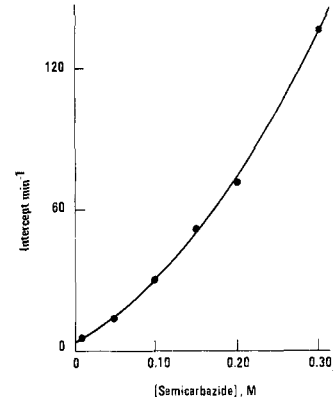


Figure 3. Dependence of intercepts of lines in Figure 1 upon semicarbazide concentration. The line is calculated from a second-order least-squares analysis according to eq 2, is computer drawn, and yields values for k_{-1} (intercept), k_1, and k_1^S for uncatalyzed and semicarbazide-catalyzed reaction of *m*-trifluoromethylpyruvanilide with semicarbazide at 25 °C and pH 7.

the normalized error was less than 1% in all cases. The complete set of rate constants is presented in Table I. It will be noted that k_1 and k_{-1} for pyruvanilide itself are about one-fourth of the values previously reported.[2] The inconsistency appears to be reproducible and seemingly must be attributed to the change from phosphate to the zwitterionic aminoalkanesulfonate buffers even though observed rates are extrapolated to zero buffer in both studies. Phosphate exerts a most powerful buffer catalysis (>100 M^{-1} as opposed to 15 M^{-1} for HEPES) and also, unusually, both $H_2PO_4^-$ and HPO_4^{2-} are effective catalysts, so that the discrepancy may be rooted in a phosphate effect the nature of which remains to be explored. Nevertheless, each set of data seems to be internally self-consistent.

A study of the temperature dependence of the reaction of pyruvanilide with semicarbazide yielded a linear plot of log k_1/T vs. T, from which were obtained $\Delta H^{\ddagger} = 2.6 \pm 0.2$ kcal/mol and $\Delta S^{\ddagger} = -49 \pm 3$ eu. The k_1 values at most of the temperatures did not result rigorously from concentration-dependence studies, but were calculated from the k_{obsd} values using eq 1 and data from Table I. Buffer and semicarbazide concentrations were such that the forward reaction terms contributed at least 80% of k_{obsd}.

Table I. Rate Constants for Carbinolamine Formation from Pyruvanilides and Semicarbazide in Water[a]

	k_1, M^{-1} min^{-1}	k_{-1}, min^{-1}	k_1^B, M^{-2} min^{-1}	k_{-1}^B, M^{-1} min^{-1}	k_1^S, M^{-2} min^{-1}	k_{-1}^S, M^{-1} min^{-1}
pyruvanilide	110 (19)	4.1 (0.73)	1540 (220)	106 (16)	910 (90)	42 (4.2)
p-methoxypyruvanilide	97 (20)	4.2 (0.82)	2010 (28)	73 (3)	890 (90)	35 (3.6)
m-trifluoromethylpyruvanilide	170 (30)	3.7 (1.9)	2360 (150)	62 (25)	890 (95)	21 (2.7)
m-nitropyruvanilide	200 (19)	3.8 (0.95)	2660 (110)	53 (13)	920 (72)	18 (1.5)
p-nitropyruvanilide	170 (22)	5.1 (0.47)	2930 (64)	84 (9)	1230 (28)	54 (4.3)
N-methylpyruvanilide	43 (21)					

[a] k_1, k_{-1}, k_1^S, and k_{-1}^S are forward, reverse, spontaneous, and semicarbazide-catalyzed rate constants, respectively, and are obtained by second-order least-squares analysis of data such as that in Figure 3. k_1^B and k_{-1}^B are forward and reverse HEPES buffer-catalyzed rate constants obtained by linear least-squares analysis of data such as that in Figure 2. Numbers in parentheses are standard deviations based on the least-squares analyses.

Table II. Rate and Equilibrium Constants for Transimination Reactions of Pyruvamide and Pyruvanilide Imines with Aliphatic Amines in Pyridine[a]

item	reaction	k_1, M^{-1} min^{-1}	K_{eq}	
1	benzylamine + pyruvamide methylimine	0.015 (0.005)	0.12	
2	*p*-methoxybenzylamine + pyruvamide methylimine	0.023 (0.006)	0.17	
3	*p*-chlorobenzylamine + pyruvamide methylimine	0.010 (0.002)	0.04	
4	benzylamine + pyruvanilide methylimine	0.024 (0.006)	0.36	
5	benzylamine + *p*-methoxypyruvanilide methylimine	0.023 (0.005)	0.26	
6	benzylamine + *p*-chloropyruvanilide methylimine	0.085 (0.010)	0.42	
item	reaction	rel k_1	K_{eq}	k_1/k_{-1}
7	benzylamine + pyruvamide propylimine	1.00	0.44	0.48
8	methylbenzylamine + pyruvamide propylimine	0.05	0.15	0.17
9	propylamine + pyruvamide benzylimine	2.1	2.3	2.1
10	methylbenzylamine + pyruvamide benzylimine	0.4	0.55	0.68
11	propylamine + pyruvamide methylbenzylimine	0.3	6.7	6.0
12	benzylamine + pyruvamide methylbenzylimine	0.6	1.8	1.5
13	benzylamine + *N,N*-dimethylpyruvamide propylimine	0.01	0.42	

[a] Items 1-6 at 25 °C, initial concentration 1.0 M in each reactant; numbers in parentheses are average magnitudes of departure of data points from the calculated line. Items 7-13 at 35 °C, initial concentration 0.5 M in each reactant. K_{eq} are from final concentrations of reactants; k_1/k_{-1} for comparison are from initial slopes of complementary reactions.

2. Transimination in Aprotic Solvents. A typical set of data, that for the consumption of benzylamine by reaction with pyruvanilide methylimine in pyridine, is shown in Figure 4. Concomitant disappearance of the methylimine and appearance of the conjugate imine and amine always were observed. Similar results were obtained in benzene (30% faster), in chloroform (60% faster), and in pyridine containing up to 25% of water. For the transimination reaction

$$I + A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} A' + I' \qquad (3)$$

(I is imine, A is amine)

the use of 1 M starting concentrations for both reactants reduces the second-order rate equation to

$$[A_t] = [A_0][A_{eq}](1 + e^{kt})/([A_0](e^{kt} - 1) + 2[A_{eq}]) \qquad (4)$$

where $k = 2k_1[A_{eq}]/(1 - [A_{eq}])$. Newton's method[14] was applied for this equation to the measured $[A_t]$ and t values using the known $[A_0]$ and the apparent $[A_{eq}]$ at about 5 half-lives as the starting point for the iterative approximation, and best values for $[A_0]$, $[A_{eq}]$, and k_1 were extracted. There being no good way to generate standard deviations of all these parameters, reliability was judged by comparing calculated and known $[A_0]$ values and by qualitative observation of the agreement of raw $[A_t]$ vs. t data with the calculated line (see Figure 4). Rate and equilibrium data thus obtained for the reaction of pyruvamide methylimine with three ring-substituted benzylamines and for benzylamine with three ring-substituted pyruvanilide methylimines are presented as the first six items in Table II. Data from a study of the effect of added acetic acid on the reaction of pyruvanilide methylimine with benzylamine was treated in the same manner and yielded the information in Figure 5. In another study, designed to explore the effects of steric rather than electronic factors and to demonstrate that the same equilibrium position is reached from either direction, rate constants were obtained simply from initial slopes of curves drawn by eye and equilibrium positions similarly were judged from asymptotic convergence of infinity

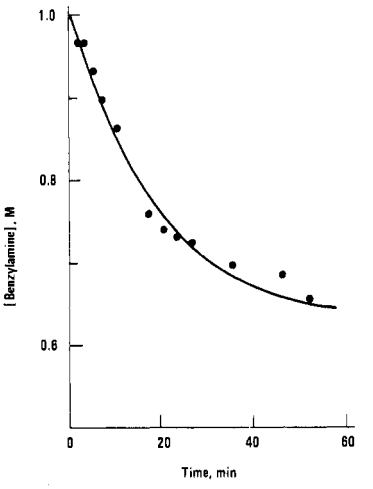


Figure 4. Consumption of benzylamine in a solution in pyridine initially 1 M in both benzylamine and pyridine at 25 °C. The line is calculated as described in the text and is computer drawn.

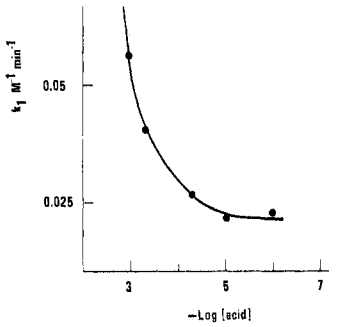


Figure 5. Dependence of k_1 for reaction of benzylamine with pyruvanilide methylimine in pyridine at 25 °C upon concentration of added acid.

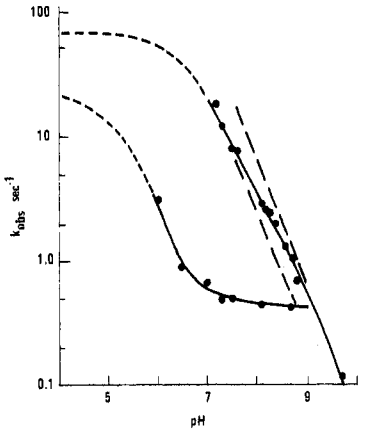


Figure 6. Dependence of rate of *gem*-diamine formation from pyrazinone V (upper line) and imine IId (lower line) with semicarbazide upon pH. Values of k_{obsd} are at 0.1 M semicarbazide and are extrapolated to zero buffer (Tris) concentration. Extensions to low pH (dotted lines) calculated using estimated pK_a values in Table III.

values for complementary reactions. These equilibrium constants and the relative rate constants are given as items 7–13 of Table II.

3. Reaction of Imines with Semicarbazide in Water. *gem*-Diamine Formation. When solutions of semicarbazide and of the imines chosen for study were mixed, there occurred a rather rapid drop in optical density ($t_{1/2}$ = 30–500 ms) succeeded by further spectroscopic changes. Within rather restricted limits of semicarbazide concentration and pH such as are indicated by the range of data points in Figure 6 and which varied quite markedly from one imine to another, the initial changes were cleanly exponential and gave good semilogarithmic plots in the more basic solutions and good to usable Guggenheim plots under neutral to weakly acidic conditions. Plots of k_{obsd} for this phenomenon are presented as a function of pH in Figure 6 and of semicarbazide concentration in Figure 7. That the phenomenon is indeed *gem*-diamine formation is indicated by the following considerations: (a) Control experiments in the absence of semicarbazide, as well as the linear dependence of k_{obsd} on semicarbazide concentration (Figure 7), serve to discount hydration and reaction with buffer (Tris) as causes for the drop in optical density. Hydration invariably is seen as a second exponential phase in reactions at the lower pH limits. (b) Semicarbazone formation is even slower than hydration (although much faster than from the corresponding ketones) and results in an increased optical density. (c) The imines undergo hydrolysis quite slowly in unbuffered, degassed, distilled water, so that the optical density change is not due to carbinolamine formation from ketone formed in the imine solution prior to admixture with the buffered semicarbazide.

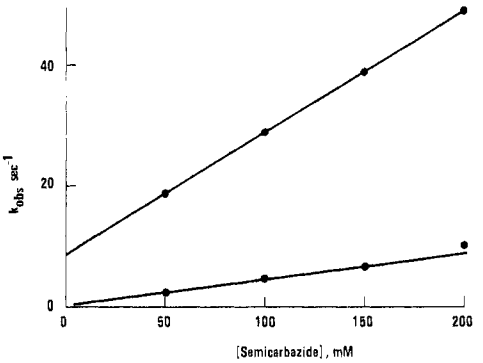
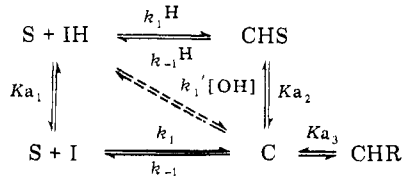


Figure 7. Dependence of rate of *gem*-diamine formation from pyrazinone V (upper line) and imine IId (lower line) with semicarbazide upon semicarbazide concentration at pH 8.1. All k_{obsd} values are extrapolated to zero buffer (Tris) concentration.

This exclusion, most important because k_{obsd} for the acyclic imine in the pH-independent region is quite similar to that for the reaction of pyruvamide itself,[2] is corroborated by the absence of an intercept in the k_{obsd} vs. semicarbazide plot for the imine (Figure 7, lower line) and by the observation that other pyruvamide imines (benzyl, propyl) exhibit markedly different reactivities. (d) This latter observation serves to exclude the possibility that we are looking at the reaction of a Tris imine formed by transimination with the buffer within the dead time of the instrument (3 ms).

gem-Diamine formation, like transimination,[15] is a simple reaction which is rendered kinetically complex and ambiguous by the various protonations and protolyses which attend the coordination step. A model which appears to be sufficient for our purposes is shown as eq 5 and the rate and equilibrium



(5)

expressions to which it gives rise are eq 6 and 7, respectively.

$$k_{obsd} = k_1^H[H][S]/(Ka_1 + [H]) + k_{-1}^H[H]Ka_3/Ka_2(Ka_3 + [H]) + k_1[S]Ka_1/(Ka_1 + [H]) + k_{-1}Ka_3/(Ka_3 + [H]) \quad (6)$$

$$K_{S.I_t}^{C_t} = K_1^H Ka_2(Ka_3 + [H])/Ka_3(Ka_1 + [H]) = K_1 Ka_1(Ka_3 + [H])/Ka_3(Ka_1 + [H]) \quad (7)$$

S is semicarbazide; I is imine, >C=N—R; IH is protonated imine, >C=N$^+$HR; C is neutral *gem*-diamine, $NH_2CONHNH(C<)NHR$; CHS is semicarbazide-protonated *gem*-diamine monocation, $NH_2CONHN^+H_2(C<)$-NHR; CHR is amine-protonated *gem*-diamine monocation, $NH_2CONHNH(C<)N^+H_2R$; $K_1^H = k_1^H/k_{-1}^H = K_{S.IH}^{CHS}$; $K_1 = k_1/k_{-1} = K_{S.I}^{C}$; $K_{S.I_t}^{C_t}$ is the apparent equilibrium constant between total *gem*-diamine and total imine at any pH, ([C] + [CHS] + [CHR])/[S](1 + IH). The denominator term (Ka_3 + [H]) which occurs in eq 7 and in the back-reaction terms of eq 6 actually represents the three-parameter expression (Ka_3[H]/Ka_2 + Ka_3 + [H]), but Ka_2 is very large compared with both Ka_3 and [H] so that the Ka_3[H]/Ka_2 term is always negligible compared with one or both of the subsequent terms.

Reaction of the cyclic imine 3-methyl-5,6-dihydro-2(1*H*)-pyrazinone (V) with semicarbazide (Figure 6, upper line) is acid dependent throughout the pH range we were able

Table III. Estimated Acid-Dissociation, Rate, and Equilibrium Constants for Species and Steps in *gem*-Diamine Formation (Equation 5)[a]

	pyrazinone V	imine IId
pK_{a_1}	6.6	5.5
pK_{a_2}	1.2	1.2
pK_{a_3}	8.5	7.0
k_1^H, M^{-1} min^{-1}	4×10^4	1.5×10^4
k_{-1}^H, min^{-1}	1.4×10^9	
k_1, M^{-1} min^{-1}	<60	250
k_{-1}, min^{-1}		<3
k_1', M^{-2} min^{-1}	$<1.5 \times 10^9$	2×10^{11}
$K_{S.IH}^{CHS}$ (= K_1^H in eq 7)	3×10^{-5}	
$K_{S.I}^{C}$ (= K_1 in eq 7)	7	>80
$K_{S.IH}^{CHR}$ (see eq 7)	6×10^2	

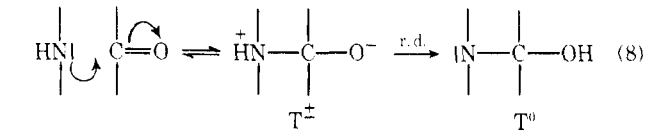
[a] k_1 and k_1' for imine IId and the limits for k_{-1} and K_1 for this imine and for k_1 and k_1' for pyrazinone V are obtained directly from the experimental data. $K_{S.IH}^{CHS} = [CHS]/[S][IH]$, $K_{S.I}^{C} = [C]/[S][I]$, and $K_{S.IH}^{CHR} = [CHR]/[S][IH]$, all at equilibrium. pK_a values were estimated as follows. Pyrazinone V, pK_{a_1}, piperidine (11.2), α CONH for CH_2 (−2.2 + 0.1), α CH_3 (+0.1), imine from related amine (−2.4) gives 6.6; pK_{a_2}, semicarbazide (3.6), *N*-ethyl (0.3), α CONH (−2.2), α NH (−0.5) gives 1.2; pK_{a_3}, piperidine (11.2), α CONH for CH_2 (−2.2 + 0.1), α CH_3 (+0.1), α NH (−0.5) gives 8.5. Imine IId: pK_{a_1}, *N*-ethylbenzylamine (9.6), α CONH (−2.2), α CH_3 (+0.1), imine from related amine (−2.4) gives 5.1; pK_{a_2}, as for pyrazinone V, 1.2; pK_{a_3}, *N*-ethylbenzylamine (9.6), α CONH (−2.2), α NH (−0.5), α CH_3 (+0.1) gives 7.0. The adjustment of −2.4 for imine from related amine is based largely on the data of W. P. Jencks and E. H. Cordes, *J. Am. Chem. Soc.*, **85**, 2843 (1963). Other adjustments are average values based on data from D. D. Perrin, "Dissociation Constants of Organic Bases in Aqueous Solution", Butterworths, London, 1965; "Handbook of Biochemistry and Molecular Biology", Vol. 1, 3rd ed., Gerald D. Farman, Ed., CRC Press, Cleveland, Ohio; "Handbook of Chemistry and Physics", 49th ed., Robert C. Weast, Ed., CRC Press, Cleveland, Ohio.

to study. The third and fourth terms of eq 6 may therefore be neglected. The first term, which describes the contribution of the forward reaction of the protonated imine to k_{obsd}, requires a break to acid independence below the p*K* of the imine (pK_{a_1}). At pH below 7, unfortunately, *gem*-diamine formation becomes too fast and side reactions interfere too much for this requirement to be verified. An estimate (Table III) of 6.6 for the p*K* was used to extend the profile (dotted line) yielding a limiting k_{obsd} of about 67 s^{-1} and an estimated rate constant, k_1^H, of 4×10^4 M^{-1} min^{-1} for reaction of the protonated cyclic imine with semicarbazide. The rate equation requires k_{obsd} to vary linearly with semicarbazide concentration at constant pH and permits an intercept corresponding to the contribution of the back reaction. The upper line of Figure 7 demonstrates both the linearity and the intercept for the cyclic imine at pH 8.1. Its slope, 20.5 M^{-1} s^{-1}, multiplied by $(Ka_1 + [H])/[H]$, also gives k_1^H as 4×10^4 M^{-1} min^{-1}. Both computations involve the estimated K_{a_1}, of course, but that from Figure 7 involves a substantial allowance for the contribution of the back reaction to k_{obsd} while that from extension of the profile in Figure 6 does not, so that the agreement is not in any way the inevitable consequence of circular argument. The intercept of the k_{obsd} vs. [S] plot and the estimated values of pK_{a_2} and pK_{a_3} in Table III yield a value of 1.4×10^9 min^{-1} for the back reaction rate constant k_{-1}^H. The form of the back reaction term in eq 6 requires that it should contribute to k_{obsd} in a pH-dependent manner above pK_{a_3} but should contribute only a pH-independent term at pH significantly below this value. Since the forward reaction remains pH dependent down to pK_{a_1}, which certainly must be lower than pK_{a_3}, this requires the contribution from the back reaction to disappear as the pH is lowered from about $pK_{a_3} + 1$ to $pK_{a_3} - 1$. Careful inspection of the pH-rate profile reveals distinctly the required break from linearity and unit slope, centered at about pH 8.4, close to the estimated pK_{a_3} (8.5). The ratio between the back and forward terms of the rate equation reduces to $k_{-1}^H K_{a_1}/k_1^H K_{a_2}[S]$ at high pH. Table I data yield a value of 1.4 for this ratio at [S] = 0.1 M. The separation between the parallel tangents to the pH-rate profile (Figure 6, upper line, broken lines at about pH 8) agrees well with this ratio.[16]

In contrast to the behavior of the cyclic imine, the pH-rate profile for the reaction of pyruvamide methylbenzylimine (IId) is cleanly biphasic (Figure 6, lower line). Equations 5, 6, and 7 are presumed to apply by virtue of their apparent validity for the cyclic imine. For the acid-dependent region, pH 6–6.5, an estimated pK_a of 5.1 for the imine combined with a k_{obsd} of 2.2 s^{-1} at pH 6.1 yields the dotted continuation of the profile to low pH and an estimated value of 1.5×10^4 M^{-1} min^{-1} for k_1^H. At pH 7–8 k_{obsd} becomes pH independent. Its linear dependence on semicarbazide concentration at pH 8.1 (Figure 7, lower line) shows an intercept which is less than 1% of the slope. Equation 6 thus reduces to its third term alone and the second-order rate constant for reaction of semicarbazide with the unprotonated imine, k_1, is given directly by $k_{obsd}/[S]$ as 250 M^{-1} min^{-1}. This number is the only definite value in Table III which does not depend on estimated pK_a values. It is kinetically ambiguous, however. The pH-independent reaction could well represent rate-determining hydroxide ion catalyzed attack of semicarbazide on the protonated imine rather than uncatalyzed attack on the imine free base, and there is evidence[15,17] that, with poor nucleophiles at least, the third-order process often is the preferred one. The rate constant k_1' for such a process would be equal to $k_1 Ka_1/Kw$, 2×10^{11} M^{-2} min^{-1} in the present instance, a value which is more than 100 times greater than the maximum possible corresponding value for the cyclic imine ($<1.5 \times 10^9$), whereas the reactivities toward uncatalyzed attack are threefold apart in the opposite direction (1.5×10^4 and 4×10^4). Nevertheless, this pathway cannot be firmly excluded. Neither, for that matter, can those requiring rate-determining trapping subsequent to rapid coordination.

Discussion

The mechanism for carbinolamine formation from simple carbonyl compounds has been rather thoroughly explored.[3,4,5,18] The characteristics of the reactant pair pyruvamide–semicarbazide meet extremely well the criteria established by Sayer et al.[5] for rate-determining trapping of a rapidly formed carbinolamine zwitterion or coordination complex, $T^{\pm}$, in the pH region 3–8 (eq 8). *gem*-Diamine formation from imines, which leads ultimately to transimination just as carbinolamine formation leads to imine formation, undoubtedly proceeds along similar lines (eq 9). The amide



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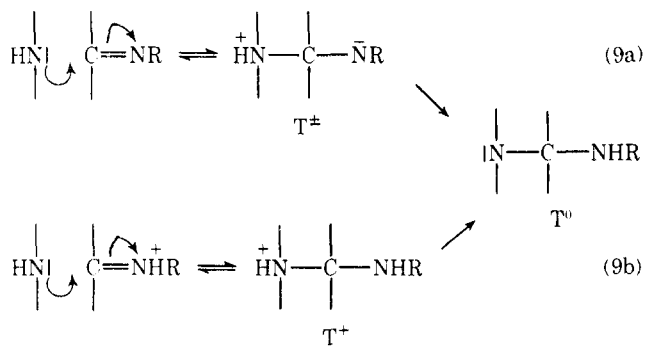
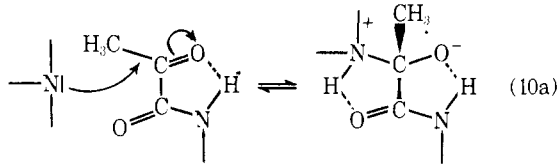


(9a)

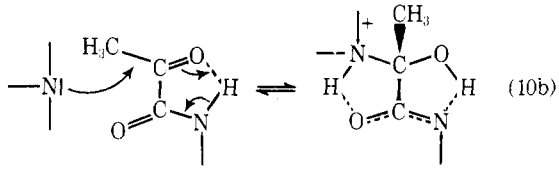
(9b)

proton of pyruvamides is known to hydrogen bond to the ketone group[2,19] and we suggested in an earlier communication that this intramolecular interaction might be at the root of certain noticeable discrepancies in the rates of reaction of these ketones with nitrogen nucleophiles. The interaction might be no more than hydrogen bonding as in eq 10a, leading to a slight en-



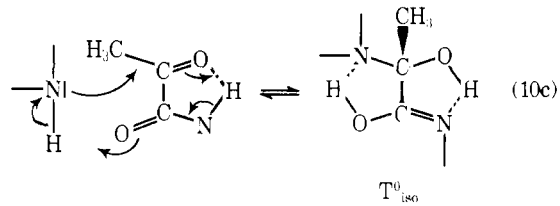
(10a)

hancement of the electrophilicity of the ketone and a modest stabilization of the zwitterion, or, as in eq 10b, it might take



(10b)

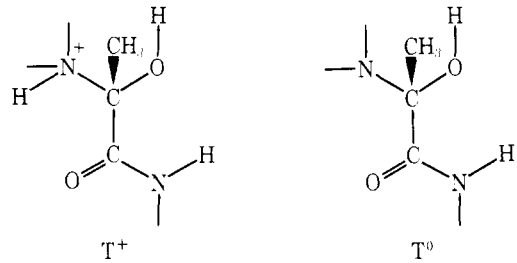
the form of an intramolecular general acid catalysis so that the alternative zwitterion having the anionic charge transferred to the amide group would be formed, or, by subsequent or concerted proton transfer from the developing ammonium ion to the amide oxygen as in eq 10c, the intermediate might take



(10c)

T^0_{iso}

the form of a carbinolamine isoamide, T^0_{iso}. Protonation or proton switch then would lead from any of these intermediates to the product carbinolamine T^+ or T^0. The same possibilities



T^+ T^0

can be envisaged for assistance to *gem*-diamine formation from free imines (=NR in place of ketone =O in eq 10a-c) but not from protonated imines. Amide is likely to have only a feeble intrinsic catalytic capability[18,20] but it is not easy to envisage a system better fitted structurally and geometrically than this one for the intramolecular amplification of this potential. Intramolecular proton donation by the β NH of acidic acylhydrazines (pK_a = 9.7-13) is thought to be responsible for a 10-100-fold enhancement of their rate of reaction with benzaldehydes.[5] These NH groups probably are some orders of magnitude more acidic than are the amides we are considering,[21] but the orientational requirements of the pyruvamide reaction are certain to be considerably less.

Our studies designed to test these possibilities have been (1) a comparative examination of *gem*-diamine formation in water from a simple pyruvamide imine and one (pyrazinone V) in which the amide proton is prevented from contacting the imine nitrogen; (2) a structure-reactivity correlation study for carbinolamine formation from pyruvanilides, the acidity of the amide proton being changed by varying the substituents in the aromatic ring, and a determination of the effect of removing the amide proton entirely; (3) a determination of the activation entropy for carbinolamine formation for comparison with known activation entropies for comparable reactions of simple carbonyl compounds; (4) structure-reactivity correlations for transimination of pyruvamide and pyruvanilide imines in nonpolar aprotic solvents, the basicity of the attacking nucleophile and the acidity of the amide proton being changed by varying the substituents in the aromatic rings; a determination of the effects of added acid; and again, a determination of the effect of removing the amide proton entirely.

The addition of amines to protonated imines (eq 9b) and quaternary immonium ions is fast.[15,17] Trapping of the initial coordination product thus is as likely as the coordination step to be rate determining, and trapping has indeed been shown to be rate determining in the addition of hydroxylamine and *N*-methylhydroxylamine to a quaternary benzalpyrrolidinium salt.[15] From the buffer effects reported, one can deduce that at zero buffer concentration trapping was only about five times slower than coordination, so that the preference is not a strong one. Semicarbazide is a 300-fold weaker base than hydroxylamine and should be a poorer nucleophile, but protonated pyruvamide imines should be better electrophiles than benzaldimmonium ions, so that one cannot predict a priori whether trapping or coordination should be rate determining in our case. Despite the limited range of experimental data obtainable and the tentative nature of the pK_a estimates in Table III, we feel that the consistency of calculations based on eq 5, 6, and 7 with the observed profiles (Results, item 3) justifies the conclusion that coordination is rate determining and that the rate, equilibrium, and acidity constants given in Table III are useful, albeit approximate, numbers. Unprotonated imines are much less reactive than imine salts, and in situations where the rate-determining step even in the reaction of the protonated imine might be in doubt one is reasonably certain that coordination rather than trapping would be rate determining for the neutral species. The amide proton of the pyrazinone V certainly cannot hydrogen bond intramolecularly to the imine nitrogen, and the striking difference between the two pH vs. rate profiles presented in Figure 6, and particularly the existence of the acid-independent pathway for the acyclic imine IId above pH 7, might well be taken as strong evidence in favor of a measure of intramolecular assistance in the latter case. We shall see, however, that such a conclusion probably would be wrong and certainly is unjustified. Consider first the acid-dependent regions of the two profiles. While the two lines are separated by 100-fold in reactivity, three-quarters of the separation is attributable to the difference between the pK_a values for the imines (1.5 pH units) and the resulting 30-fold difference in the concentration of the protonated species at a given pH. The pK_a values in Table III may lack absolute reliability but the difference between them is essentially the difference between the known pK_a values of the corresponding amines (piperidine, 11.2; ethylbenzylamine, 9.6) and is likely to be quite reliable. The remaining threefold lower reactivity of the protonated acyclic amine is reasonably attributable to steric effects of the bulky methylbenzyl group, so that the acid-dependent profile for an unhindered aliphatic imine might well be close to that exhibited by the cyclic compound. Such limited data as we have been able to obtain for the propyl- and methylimines is at least not inconsistent with this conclusion. In the pH-independent region for the acyclic imine there are three possibilities for the rate-determining step. While we feel that the evidence favors coordination of semicarbazide to the unprotonated imine in this role, the other possibilities cannot be discounted and we shall consider all of them. The electrophilicity of a free imine should vary inversely with its basicity, so that pH independence for an imine of higher pK_a would appear only at a higher pH and lower k_{obsd}. Even using a logarithmic correlation coefficient (α_{elec}) between reactivity and

pK_a as low as 0.4, calculations based on the data of Figure 6 require that an acyclic pyruvamide imine having a pK_a of 6.6 would not show pH independence within the pH and k_{obsd} limits explored. The profile for such an imine thus would be very similar to that exhibited by the cyclic imine. Conversely, in order to have indicated significant intramolecular assistance, the profile for imine IId would have had to exhibit pH independence at a much lower pH and with a much higher k_{obsd} than is observed. Accordingly, if reaction of semicarbazide with the free imine is rate determining in *gem*-diamine formation, the profiles do not indicate any intramolecular assistance by the amide group. A second possibility for rate determination in the pH-independent region, rate-determining hydroxide ion catalyzed addition to protonated amine, is quickly disposed of. There is no significant likelihood that an amide proton, even in an ideal juxtaposition, could augment the reactivity of a *protonated* imine. Finally, there is the possibility of rate-determining water-mediated proton switch following rapid addition to imine free base (eq 9a). This would require a truly remarkable facilitation of the latter step which, in contrast to the corresponding reaction of both ketones and protonated imines, normally is too slow to be observed. A similar measure of stabilization of the N^+–N^- zwitterion also would be needed. The only likely possibility is that of coordination concerted with proton transfer from the amide proton and to the amide oxygen (eq 10c, =NR in place of ketone =O) leading to the *gem*-diamine isoamide, T^0_{iso}, as intermediate. Such a mechanism might rationalize the notably similar rate constants, about 200 M^{-1} min^{-1}, for both the ketones and their imines in the pH-independent region, since reversion of isoamide to amide would then be rate determining in both cases. We note, however, that the rate constant for addition of semicarbazide to *p*-nitrobenzaldehyde at pH 5, where proton switch is known to be rate determining, also is close to 200 M^{-1} min^{-1}, so that the coincidence may indeed be just that. We conclude that the pyrazinone–imine comparison is ambiguous as regards this final mechanistic possibility, which we feel to be a less likely one in any event, and hence we regard it as indicating negatively or at best dubiously on the question of intramolecular assistance by the amide group to *gem*-diamine formation.

To the best of our knowledge, direct observation of spectroscopic changes consistent with *gem*-diamine formation during transimination reactions has not previously been reported. The intermediacy has been called into question, indeed,[22] even though stable naturally occurring alicyclic *gem*-diamines such as the alkaloid calycanthine[23] have been known for many years. The equilibrium constants which may be deduced from the rate and acidity data in Table III for the various imine–*gem*-diamine equilibria to which eq 5 gives rise reveal that the stabilities of *gem*-diamines derived from semicarbazide, and hence their capability of being observed, derive substantially simply from the great acidity of semicarbazidium ions ($K_{a_2} \gg K_{a_1}$ and K_{a_3}). For pyrazinone V, for example, $K_{S.IH}^{CHS}$, which is the equilibrium constant for formation of the semicarbazide-protonated diamine cation CHS from semicarbazide and protonated imine, is $k_1^H/k_{-1}^H = 3 \times 10^{-5}$ M^{-1}, a value which is hardly likely to give rise to an observable optical density change. The equilibrium actually observed in acid solution (pH < pK_a and pK_{a_3}), however, is that with the isomeric amine-protonated *gem*-diamine cation CHR, $K_{S.IH}^{CHR} = K_{S.IH}^{CHS}K_{a_2}/K_{a_3}$, 6×10^2 M^{-1}. Even in basic solution (pH > pK_{a_1} and pK_{a_3}), the constant between neutral imine and neutral *gem*-diamine would be $K_{S.I}^C = K_{S.IH}^{CHS}K_{a_2}/K_{a_1}$, 7 M^{-1}, and the value between pH = pK_{a_1} and pH = pK_{a_3} would be pH dependent according to eq 7 and would lie between 7 and 600 M^{-1}. Such equilibria give readily observable concentrations of products. In contrast, attack of a simple amine ($K_{a_2} \sim K_{a_3} < K_{a_1}$) would give an equilibrium constant in ac6i solution which would be simply the coordination equilibrium constant and the constant in basic solution would be even smaller. At any pH below the pK_a of the attacking amine, moreover, these equilibria would be displaced unfavorably as a result of protonation of the amine by a factor which certainly would counterbalance any favorable displacement resulting from greater inherent nucleophilicity of the stronger base (β assumed ≥ 1). The equilibrium constant for *gem*-diamine formation from semicarbazide and acyclic imine IId in basic solution ($K_{S.I}^C$, pH 8.1 > pK_{a_3}) is even greater than that for the pyrazinone. The difference may be no more than tenfold, which would be attributable, other things being equal, to the difference in the pK_a values of the imines alone. Other product-stabilizing factors may include the freedom of the acyclic diamine to adopt conformations which do not conform to Deslongchamps' rules[24] and to hydrogen bonding of amine nitrogen to amide NH, a factor which seems to contribute about 1.5 kcal to the stabilities of pyruvamide carbinolamines.[2] The lack of intercept in Figure 7 for *gem*-diamine formation from the acyclic imine thus does not in any way reflect a substantial difference in mode of reaction.

Carbinolamine formation from aldehydes and ketones is strongly assisted by electron-withdrawing substituents. Hammett correlations for the reaction of methoxyamine with ring-substituted benzaldehydes,[5] for example, are linear with σ values and yield coefficients (ρ) of 1.7 for the rate of coordination of amine with aldehyde (formation of $T^{\pm}$), 2.0 for the equilibrium constant for this coordination, and 1.4 for the rate of the two-step process (formation of T^0) under conditions such that water-mediated trapping of the coordination product is rate determining. Such a level of substituent sensitiveness is consistent with the attachment of the aromatic ring directly to the center of nucleophilic attack. In pyruvanilides (Ia–e) the ring is insulated from the ketone carbon by the amide moiety, and any electronic effect transmitted along bonds necessarily would be more weakly felt. The work of Peters and Johnson,[25] which compares the pK_as of substituted benzylamines (ρ = 1.23) and glycinanilides (ρ = 0.23), indicates that the amide group is about 20% efficient in transmitting substituent effects in the direction of interest to us. A ρ of about 0.26 thus might be anticipated for pyruvanilide carbinolamine formation. In contrast, the acidity of the amide proton should be very sensitive to substituent effects, and direct interactions between this proton and the ketone group, such as eq 10a–c envisage, should give rise to substantial ρ values, certainly no less than unity and likely much larger depending on the nature of the intramolecular assistance. A plot of the logarithm of the rate constants (k_1, Table I) for carbinolamine formation between semicarbazide and various ring-substituted pyruvanilides against the σ constants for the substituents is presented in Figure 8. The slope is 0.28, a value consistent with a simple electronic substituent effect on the ketone carbon and entirely inconsistent with any special effect of the acidity of the amide proton. Equally as noteworthy as the low ρ is the linearity with simple σ values. *p*-Nitroanilinium ion is about 5000 times more acidic than anilinium ion is, the nitro group being conjugated with the amine nitrogen, and a similarly disproportionate acidity for *p*-nitropyruvanilide would have led to a large positive deviation from a σ plot had the affected proton been involved in a kinetically significant manner. *N*-Methylpyruvanilide is a little less reactive than pyruvanilide just as *N,N*-dimethylpyruvamide is less reactive than *N*-propylpyruvamide. The difference is slight enough (2.5-fold) to allow the elimination of the remote possibility that the catalytic effectiveness of the amide groups of *all* of the pyruvanilides examined in the σ–ρ correlation was so great that increase in acidity was without effect. The value of −49 eu for the activation entropy of the reaction of semicarbazide with pyruvanilide is similar to that of −43 eu obtained by Chaturvedi and Cordes[26] for its reaction with *p*-hydroxybenzaldehyde, the

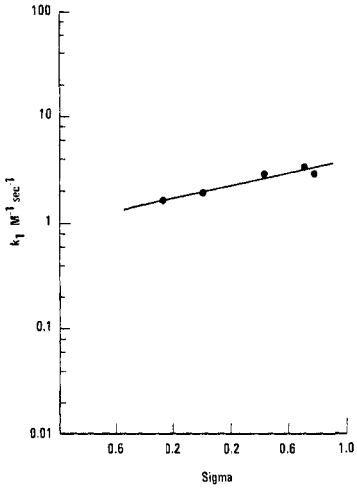
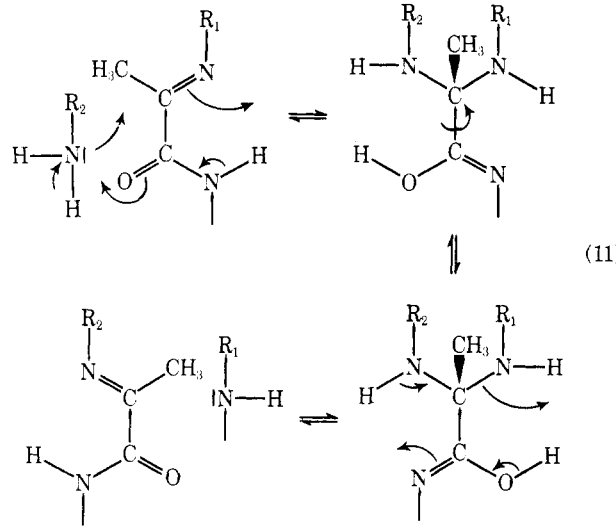


Figure 8. Hammett correlation for carbinolamine formation from semicarbazide and ring-substituted pyruvanilides. The line is calculated from a least-squares analysis and is computer drawn.



(11)

magnitude of which was reasoned to reflect the orientational requirements for water-mediated proton switch. We conclude from these studies as from that of the cyclic and acyclic imines that, in water at least, the amide group plays no unusual role in facilitating nucleophilic addition to pyruvamides.

The study of the transimination of pyruvamide imines in aprotic solvents was deliberately designed to afford the greatest possible opportunity for intramolecular assistance by the amide group to show itself. *gem*-Diamine formation, certainly a necessary and rate-determining intermediate step, requires coordination and trapping steps (eq 9) both of which would be difficult. The diamine zwitterion $T^{\pm}$ would be unstable by virtue both of the low polarity of the solvent and the lack of solvation of the ionic charges. The anionic moiety of the diamine zwitterion, moreover, should be about ten orders of magnitude more basic and correspondingly more difficultly accessible than the oxy anion of a carbinolamine zwitterion. Trapping of the zwitterion, be it by protonation or by proton switch, is likely to be no less difficult. In the absence of water, amide is the strongest acid present and the likeliest bifunctional switch mediator, and its effectiveness in an intermolecular fashion at 1 M concentration is unlikely to compare in any way with that of H_3O^+ at 10^{-7} M or water at 55 M concentrations in the aqueous solution studies. Each of these unfavorable factors is capable of intramolecular amelioration by the amide group, no matter whether coordination or trapping is rate determining. The amide group is known to be hydrogen bonded to the imine nitrogen in these solvents so that the geometry is certain to be perfect, and the intermediacy of a *gem*-diamine isoamide (eq 10c) becomes distinctly plausible, the total obviation of ionic charges likely being enough to offset the energetic cost (~11 kcal[27]) of amide–isoamide tautomerization. Were this intermediate to undergo a 180° bond rotation, moreover, it could decoordinate directly to products with no need whatever for intermolecular proton transfers. Three studies were effected to test these possibilities: substituent and steric effects in the attacking amine; substituent and steric effects in the imine attacked; and the effect of added acid. The results are presented in Table II and Figure 5. Unassisted transimination was quite slow, about 10^4-fold slower than the reaction of comparable imines with semicarbazide in water and at least the same amount slower than the preparative metathesis with amine salts in water (Experimental Section) in which precipitation of the product imine is essentially instantaneous. Items 7–12 of Table II confirm that the same equilibrium position is reached equally well from either direction, albeit not at the same rate, and indicate that the reaction is somewhat more sensitive to steric effects in the attacking amine than in the imine attacked. The imine of *N,N*-dimethylpyruvamide reacts very slowly indeed, the half-time being hours as opposed to minutes, but a typical equilibrium position is reached eventually. A σ–ρ correlation for the attacking amine, constructed using items 1–3 of Table II, is linear and indicates a simple dependence of reactivity on basicity with a ρ of −0.79, while the relative reactivity of substituted imines (items 4–6 of Table II) might be taken to suggest a nonlinear or biphasic dependence of reactivity on electrophilicity, with a rather substantial ρ of +2.2 between pyruvanilide and *p*-chloropyruvanilide. These considerations are minor, however, beside the interpretation of the effect of added acetic acid which is shown in Figure 5. Enhancement of reaction rate above the uncatalyzed level is linear with concentration of added acid above 5×10^4 M and almost certainly represents addition to the protonated imine. Benzylamine is by far the strongest base present in the system (pK_a = 9.3) and at 1 M benzylamine concentration virtually all of any added acid will be present as benzylammonium ion. The pK_a of the imine may be estimated by the procedures used in Table III, methylethylamine having a pK_a of 10.6, to be 6.0. Assuming that pK_a values may continue, even in pyridine, to reflect the *relative* basicities of neutral nitrogen bases undergoing protonation, the ratio of protonated to unprotonated imine at 10^{-3} M added acid would be 5×10^{-7}. Division of the k_1 *enhancement* (0.032 M^{-1} min^{-1}) by this ratio yields a second-order rate constant, k_1^H, for reaction of benzylamine with protonated imine of 6.4×10^4 M^{-1} min^{-1}. In the unlikely event that the acid-assisted reaction represents benzylammonium-catalyzed addition to unprotonated imine, the third-order constant would be 32 M^{-2} min^{-1}. Whatever the mechanism of the acid-assisted reaction, the rate at 5×10^{-4} M added acid is double that for the spontaneous reaction and at 5×10^{-5} M added acid the enhancement disappears, which is to say that the unassisted reaction proceeds at a rate equal to that for an acid-assisted process at a benzylammonium ion concentration of 5×10^{-4} M. The pK_a of the amide proton of pyruvanilide can be estimated rather reliably not to be greater than 16.3;[21] it might be as much as three or four pH units lower but it is very unlikely to be higher. Assuming again that relative acidities and basicities in pyridine may be reflected by pK_a values, then in a mixture 1 M in amide and amine the concentration of ammonium ion would be $(K_{a_{amide}}/K_{a_{amine}})^{1/2}$, i.e., $(10^{-16.3}/10^{-9.3})^{1/2}$ or 3×10^{-4} M, just about enough to maintain the observed rate. Even this is in the nature of a lower limit, since the amide pK_a probably is lower than 16.3; but then again, relative acidities and basicities may be only roughly reflected by pK_a values. It seems reasonable to conclude, however, that

spontaneous transimination in aprotic solvents occurs at a rate which is just about that to be expected for an ordinary acid-assisted intermolecular process. The fourfold greater reactivity of *p*-chloropyruvanilide methylimine probably reflects merely the greater acidity of its amide group and the correspondingly higher concentration of benzylammonium ion. Pyruvamide methylimine and *p*-methoxypyruvanilide methylimine both are only slightly less reactive than pyruvanilide methylimine even though both should be distinctly weaker acids. It is possible that the similarity reflects the ultimate intervention of a modicum of intramolecular assistance, but other explanations can be envisaged and a propinquity effect of this magnitude is of vanishing interest in any event. Apropos of the lower reactivity of *N,N*-dimethylpyruvamide propylimine, one notes that, in the absence of amide protons, the strongest acid remaining is the pyruvamide methyl group with a likely pK_a of about 20. In order to be certain that spontaneous transimination does not result from a remarkable catalytic effect of traces of water even in carefully dried pyridine, a parallel series of reactions was carried out in pyridine–water mixtures. Rates of reaction were unchanged even at water concentrations as high as 25% v/v! Water, $pK_a = 15.7$, should generate 5×10^{-4} M benzylammonium ion at 1 M and four times as much at 16 M (30%). As with amide groups, however, it is reasonable to suggest that the effective acidity of water in pyridine may be significantly lower than its theoretical pK_a. At water concentrations of 50% v/v and higher new peaks appeared in the NMR spectrum the nature of which we have chosen not to investigate at this time.

We conclude that the amide group of pyruvamides does not provide any assistance of an intramolecular general acid, general base or concerted general acid–base nature to carbinolamine formation, *gem*-diamine formation, or transimination in water or in nonpolar aprotic solvents. The high reactivity of the pyruvamide ketone and ketimine groups toward nitrogen nucleophiles results solely from the inductive effect of the amide group attached to the ketone carbon. The diminished reactivities of pyruvate esters and *N,N'*-dialkylpyruvamides which in part stimulated this investigation must be attributed to steric factors and to a nice balance of rate determination between formation and trapping of zwitterionic intermediates. We continue to believe that the carbinolamines which precipitate so quickly when pyruvamides and amines are mixed in aprotic solvents are carbinolamine isoamides and that *gem*-diamine isoamides are present in transimination reaction mixtures in proportions (<5%) too small to be seen. Work pertinent to these contentions continues. Categorically, however, these substances are not kinetically significant. Finally, we would observe that the total absence of intramolecular involvement of the amide group in a system and under conditions so ideally designed to permit its intervention casts considerable doubt on its ability to function in any unusual way as an acid–base catalyst in biochemical systems.

References and Notes

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(2) P. R. Young, L. G. Howell, and T. C. Owen, *J. Am. Chem. Soc.*, **97,** 6544 (1975); T. C. Owen and P. R. Young, *FEBS Lett.*, **43,** 308 (1974).
(3) E. H. Cordes and W. P. Jencks, *J. Am. Chem. Soc.*, **84,** 4319 (1962); B. M. Anderson and W. P. Jencks, *ibid.*, **82,** 1773 (1960).
(4) S. Rosenberg, S. M. Silver, J. M. Sayer, and W. P. Jencks, *J. Am. Chem. Soc.*, **96,** 7986 (1974).
(5) J. M. Sayer, B. Pinsky, A. Schonbrunn, and W. Washtien, *J. Am. Chem. Soc.*, **96,** 7998 (1974).
(6) T. Pino and E. H. Cordes, *J. Org. Chem.*, **36,** 1668 (1971). The reaction of pyruvate esters and pyruvamides with semicarbazide fits very well the criteria laid down by Sayer et al.[5] for imine formation by two concurrent mechanisms and for the occurrence of two negative breaks in pH vs. rate profiles. The published profile for methyl pyruvate (Pino and Cordes) does indeed show two such breaks (pH 2.3 and 5.3), although it was not interpreted as doing so at the time of publication, of course (a third break at pH 6 undoubtedly reflects a change from acid catalyzed to uncatalyzed or base catalyzed in the dehydration step and is not of interest here). On this interpretation, the break at pH 5.3 reflects a change from acid trapping of $T^{\pm}$ ($k^{H} = K_n k_3$) to water-mediated proton switch ($k = K_n k_4$) as the rate-determining step and corresponds mechanistically to the break observed at pH 5.5 for pyruvamides[2] and pH 6 for pyruvanilides (present work) in the same reaction. For methyl pyruvate one may deduce $K_n k_4 \sim 10\ M^{-1}\ min^{-1}$ and $K_n k_3 \sim 10^6\ M^{-2}\ min^{-1}$. Present work yields $K_n k_4 = 110\ M^{-1}\ min^{-1}$ and $K_n k_3 \sim 1.5 \times 10^7\ M^{-2}\ min^{-1}$ for pyruvanilide. Values of 240 and $\sim 2 \times 10^7$ for *N*-propylpyruvamide and 430 for pyruvanilide from previously published data[2] probably are four times too big (see text). Thus in both the uncatalyzed and the acid-catalyzed reactions, pyruvamides are some 5–15 times more reactive than methyl pyruvate is.
(7) W. D. Riley and E. E. Snell, *Biochemistry*, **7,** 3520 (1968); P. A. Recsei and E. E. Snell, *ibid.*, **9,** 1492 (1970).
(8) E. E. Snell, *Trends Biol. Sci.*, **2**(6), 131 (1977).
(9) A. Wohl and L. H. Lips, *Ber.*, **40,** 2312 (1907).
(10) Procedure was that for nitration of acetanilide given by A. I. Vogel, "Practical Organic Chemistry", 3rd ed., Wiley, New York, N.Y., 1962, p 581.
(11) H. C. J. Ottenheijm and J. H. M. DeMan, *Synthesis*, 163 (1975).
(12) A. A. Carr, Jr., C. H. Tilford, and W. L. Kuhn, U.S. Patent 3 056 784; *Chem. Abstr.*, **58,** 5700*g* (1963). In a modified procedure developed by Michael W. Hardee, ice-cold solutions of ethylenediamine (1.25 mL) and ethyl pyruvate (2.0 mL), each in absolute ethanol (25 mL), are mixed and refrigerated for 48 h, the solvent is removed at room temperature under reduced pressure, and the crude solid product (2.02 g, 98%) is purified in 1-g portions by sublimation (85 °C, 0.25 Torr).
(13) W. Gueffroy, "Buffers", Calbiochem Inc., La Jolla, Calif., 1975.
(14) Y. Bard, "Nonlinear Parameter Estimation", Academic Press, New York, N.Y., 1974; B. Schneid, "Theory and Problems in Numerical Analysis", McGraw-Hill, New York, N.Y., 1968.
(15) J. L. Hogg, D. A. Jencks, and W. P. Jencks, *J. Am. Chem. Soc.*, **99,** 4772 (1977).
(16) At pH 8.4 the lower broken line gives 1.0 s^{-1} and the upper one gives 2.4 s^{-1} for k_{obsd}.
(17) K. Koehler, W. Sandstrom, and E. H. Cordes, *J. Am. Chem. Soc.*, **86,** 2413 (1964).
(18) W. P. Jencks, *Prog. Phys. Org. Chem.*, **2,** 63 (1964); *Chem. Rev.*, **72,** 705 (1972).
(19) G. Fischer, G. Oehme, and A. Schellenberger, *Tetrahedron*, **27,** 5683 (1971); R. G. Lawton, personal communication.
(20) W. P. Jencks, *J. Am. Chem. Soc.*, **94,** 4731 (1972).
(21) There is a dearth of good data for the acidities (as opposed to the basicities) of amides. The best available estimates seem to be those of Homer and Johnson (R. B. Homer and C. D. Johnson in "The Chemistry of Amides", J. Zabicky, Ed., S. Patai, series Ed., Wiley-Interscience, New York, N.Y., 1970), calculated from the Hines' equilibrium data (J. Hine and M. Hine, *J. Am. Chem. Soc.*, **74,** 5266 (1952)) using Bowden's relationship (K. Bowden, *Chem. Rev.*, **66,** 115 (1966)). They give 17.6 as pK_a for acetanilide. Pyruvic acid has pK_a 2.25 units lower than acetic acid, from which we estimate a minimum ΔpK of −1.3 units for the more weakly electron-attracting ketimine group, which would give 16.3 as the *highest* plausible value for the pK_a of pyruvanilide methylimine. Comparison of the range of Homer and Johnson's estimates with the pK values for the corresponding carboxylic acids indicates that the ΔpK effects of both electron-attracting and electron-withdrawing groups on amides are double those on acids, so that the pertinent adjustment might well be 2.6–3.0 units. Moreover, as Homer and Johnson point out, their values may all be 1.3 units too high. Thus, the lowest likely value for the pK_a of pyruvanilide methylimine would be 12–13.
(22) E. H. Abbott and A. E. Martell, *J. Am. Chem. Soc.*, **93,** 5852 (1971).
(23) R. H. F. Manske, Ed., "The Alkaloids", Vol. II and VII, Academic Press, New York, N.Y., 1952 and 1960.
(24) P. Deslongchamps, *Tetrahedron*, **31,** 2463 (1975).
(25) C. Peters and C. Johnson, *J. Org. Chem.*, **40,** 1517 (1975).
(26) B. Chaturvedi and E. H. Cordes, *J. Am. Chem. Soc.*, **89,** 4631 (1967).
(27) A. R. Fersht, *J. Am. Chem. Soc.*, **93,** 3504 (1971).