Kinetic and Mechanistic Studies of the *N*-Bromosuccinimide-Promoted Oxidative Decarboxylation of Glycine, DL-Alanine, and DL-Valine

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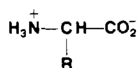
The kinetics of the oxidative decarboxylation of glycine (**1a**), DL-alanine (**1b**), and DL-valine (**1c**) promoted by *N*-bromosuccinimide (NBS) have been studied as a function of pH. The pH–rate profile for **1a** is bell-shaped while those for **1b** and **1c** exhibit an inverted-bell shape. At pH 3.7, **1a** exhibits Michaelis–Menten kinetic behavior but shows substrate inhibition at pH 5.0. Both **1b** and **1c** display zero-order dependence on substrate concentration at pH 3.7 but exhibit Michaelis–Menten behavior at pH 5.0. The oxidation of **1a** is characterized by a solvent deuterium isotope effect of $k(H_2O)/k(D_2O) = 2.0$ and a secondary deuterium isotope effect (glycine-d_5) of $k_H/k_D = 1.35$. Proton inventories with glycine under two different sets of conditions both exhibit significant downward curvature with negative curvature parameters. A mechanism involving the formation of an acyl hypobromite of **1a**, its slow decomposition to an imine, and subsequent rapid conversion of imine to products is proposed. Both **1b** and **1c** undergo oxidation by a mechanism involving the slow abstraction of the α hydrogen as hydride ion from the substrate as well as its acyl hypobromite to give the imine.

Oxidative decarboxylation of α-amino acids is one of the well-documented biochemical processes. There are several analogous nonenzymatic chemical processes. The exact mechanism of the chemical process of oxidative decarboxylation of α-amino acids is not well understood, however, and remains an area for further experimentation.

Both primary and secondary amino acids may be converted to aldehydes by using halogens and inorganic hypohalites. The mechanism of these degradations remains unclear in part because of the difficulty caused by the observation of different oxidation products at different pH values.

Better control of pH is possible by using *N*-bromosuccinimide (NBS) as the source of positive bromine. Thus, several groups have studied the oxidative degradation of amino acids and their derivatives, including peptides, using NBS.[1-4] The mechanism is still unclear, however. We chose to investigate the kinetics of the oxidative decarboxylation of the simple amino acids, glycine (**1a**), DL-alanine (**1b**), and DL-valine (**1c**) in buffered aqueous media for these reasons.



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a R = H
b R = CH_3
c R = $CH(CH_3)_2$

Results

The solutions of the amino acids **1a–c** and NBS were scanned in both the ultraviolet and visible regions of the spectrum. None of the separate solutions showed any significant absorption in these regions but a mixture of the amino acid and NBS exhibits an absorption maximum at 240 nm. This absorbance is found to decrease with time. This clearly shows that the reaction involves the formation of an intermediate which then decays to products.

When the reaction between, for example, **1a** and NBS in an aqueous solution buffered to pH 3.7 is examined at 240 nm, the absorbance first increases rapidly to a maximum and then decreases to its final value. The time to reach the maximum at 240 nm is between 0 and 50 s depending on the concentration of the amino acid. In the case of **1b** or **1c** the time to reach the absorption maximum was too fast to measure by conventional techniques at all concentrations. Thus, the absorbance at 240 nm follows the characteristic form for the appearance and decay of an intermediate. Except for some very early points, the data appear to correspond to a simple first-order decay.

Table I. Observed First-Order Rate Constants for the Reaction of Alanine or Valine with NBS[a] in Water Buffered to pH 5.0 with Acetic Acid–Sodium Acetate at 25.00 ± 0.05 °C[b]

	10^5k_1, s^{-1}	
10^3[substrate], M	alanine	valine
4.5	734 ± 9	113 ± 3
9	760 ± 3	122 ± 7
18	790 ± 3	141 ± 4
36	913 ± 10	157 ± 3
72	1126 ± 7	176 ± 7
90	1277 ± 48	191 ± 6

[a] [NBS] = 6.0×10^{-4} M. [b] Ionic strength was maintained at 0.5 M with $NaClO_4$.

When the reaction is monitored at 240 nm for the disappearance of the intermediate at pH 3.7, it is observed that the rate of the reaction between NBS and **1a** exhibits a Michaelis–Menten-type dependence on the concentration of **1a** (Figure 1) and that the reaction between NBS and **1b** or **1c** exhibits no dependence on substrate concentration (see Experimental Section).

At pH 5, the rate of the reaction between NBS and **1a** increases initially and then drops off as the substrate concentration is increased further as shown in Figure 2. However, the rate of the reaction between NBS and **1b** or **1c** exhibits a small increase with the increase in substrate concentration (Table I).

The reaction between **1a** and NBS exhibits a bell-shaped pH–rate profile with a maximum at about pH 6.0. The reaction with **1b** or **1c**, in dramatic contrast, shows an *inverted* bell-shaped profile with a rate minimum at about pH 6–6.5 (Figure 3).

The reaction between glycine-d_5 and NBS is 1.35 times slower than that between undeuterated glycine and NBS at pH 3.7 in aqueous solution under the limiting conditions. The reaction between glycine and NBS is twice as fast in protium oxide as in deuterium oxide at pH 3.7 (Table II).

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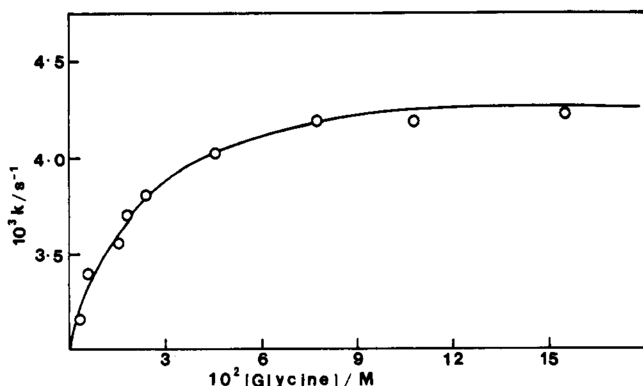

Figure 1. Plot of the first-order rate constants for the reaction between glycine and NBS vs. the concentration of glycine at pH 3.7 (acetic acid–sodium acetate buffer) at 25.00 ± 0.05 °C. The ionic strength was maintained at 0.5 M with sodium perchlorate and the NBS concentration was 6 × 10^{-4} M.

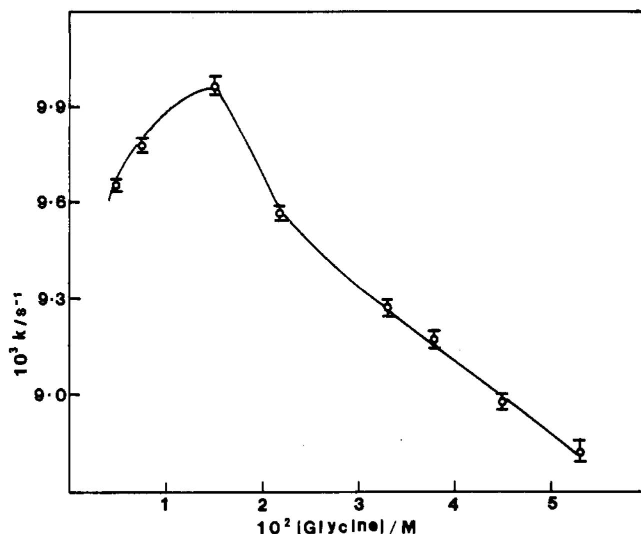

Figure 2. Plot of the first-order rate constants for the reaction between glycine and NBS vs. the concentration of glycine at pH 5.0 (acetic acid–sodium acetate buffer) at 25.00 ● 0.05 °C. The ionic strength was maintained at 0.5 M with sodium perchlorate and the NBS concentration was 6 × 10^{-4} M.

Table II. Observed First-Order Rate Constants for the Oxidative Decarboxylation of Glycine[a] by NBS[b] in Mixtures of H_2O–D_2O of Atom Fraction Deuterium *n* Buffered to pH(D) 3.7 with Acetic Acid-H(D) and Sodium Acetate at 25.00 ± 0.05 °C

atom fraction of deuterium (*n*)	no. of runs	10_4k_n, s^{-1} (obsd)
0.000	5	216 ± 2[d]
0.249	5	175 ± 2
0.499	5	146 ± 2
0.748	5	123 ± 3
0.998	5	108 ± 1

[a] Glycine (8.0 × 10^{-3} M) was used in both H_2O and D_2O solutions. [b] [NBS] = 6.0 × 10^{-4} M. [c] Ionic strength was maintained at 0.5 M with $NaClO_4$. [d] Error limits are standard deviations.

However, when glycine-d_5 is used in deuterium oxide the normal solvent isotope effect is increased to 2.87 (Table III).

The rate of the reaction between glycine and NBS has been studied in protium oxide, deuterium oxide, and in mixtures of the two solvents of different atom fractions of deuterium *n*, and the observed rate constants are collected in Table II. These proton inventory studies produce a deep downward bowing in a plot of rate constant k_n vs. *n* as shown in Figure 4. The curvature parameter, γ,[5] is calculated to be −0.91 ± 0.16.

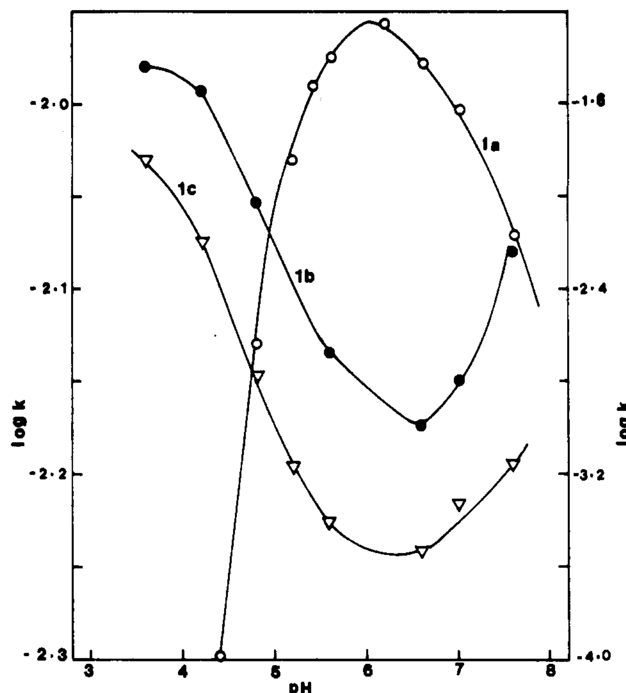

Figure 3. pH–rate profiles for the reaction of glycine (open circles), alanine (filled circles), or valine (triangles) with NBS at 25.00 ± 0.05 °C. The left scale is for glycine and the right scale for alanine and valine. The pH was maintained by mixing the appropriate volumes of stock solutions of 0.1 M citric acid and 0.2 M disodium hydrogen phosphate. The ionic strength was maintained at 0.5 M with sodium perchlorate.

Table III. Observed First-Order Rate Constants for the Oxidative Decarboxylation of Glycine[a] by NBS[b] in Mixtures of H_2O–D_2O of Atom Fraction Deuterium *n* Buffered to pH(D) 3.7 with Acetic Acid-H(D) and Sodium Acetate

atom fraction of deuterium (*n*)	no. of runs	10^4k_n, s^{-1} (obsd)	10^4k_n, s^{-1} (calcd)[e]
0.000	5	213 ± 3[d]	213
0.249	5	154 ± 2	157
0.499	5	116 ± 1	119
0.748	5	92 ± 1	93
0.998	5	74 ± 1	73

[a] Glycine was used in H_2O and glycine-d_5 was used in D_2O. Mixtures of these were used for other atom fractions of deuterium *n*. [glycine] = [glycine-d_5] = 6.0 × 10^{-4} M. [b] [NBS] = 6.0 × 10^{-4} M. [c] Ionic strength was maintained at 0.5 M with $NaClO_4$. [d] Error limits are standard deviations. [e] Calculated using ϕ_j = 1.418 and a secondary isotope effect of 1.45 in eq 13.

The same type of proton inventory studies have been carried out by using glycine in protium oxide and glycine-d_5 in deuterium oxide (Table III). Again, the plot of k_n vs. *n* produces a deep downward bowing (figure not shown) with a curvature parameter of −0.63 ± 0.21.

The reaction between glycine ethyl ester and NBS was found to be about 10 times slower than that between glycine and NBS under identical conditions. When betaine hydrochloride is used as a substrate no absorption is observed. The variation of NBS concentration at a constant concentration of glycine under pseudo-first-order conditions was found to have no effect on the rate of the reaction (Experimental Section). This shows that the reaction is first order in NBS concentration. The variation of ionic

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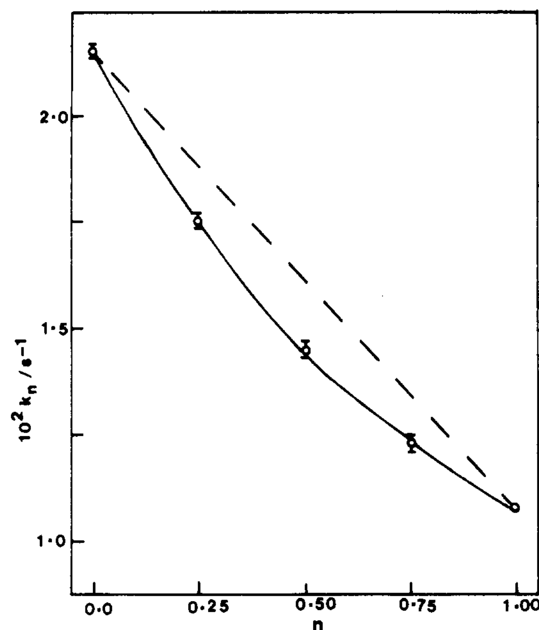
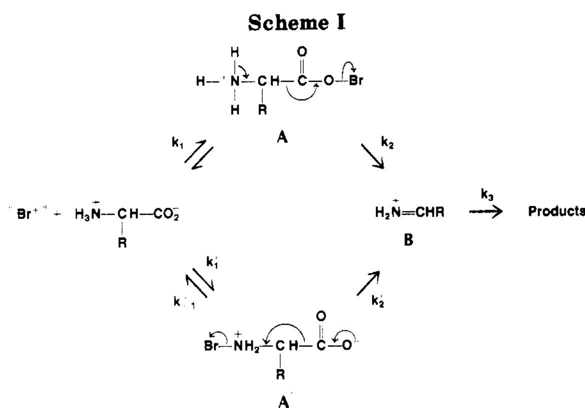


Figure 4. Proton inventory plot for the oxidative decarboxylation of glycine by NBS at pH 3.7 or the equivalent pD at 25.00 ● 0.05 °C. Acetic acid-(H or D)–sodium acetate buffers were used to maintain pH(D). The ionic strength was maintained at 0.5 M with sodium perchlorate. The solid line was calculated on the basis of eq 7 with ϕ_j = 1.418. The data are from Table II. Where error bars are omitted, the circles encompass them. The dashed line is included merely to emphasize the nonlinearity of the plot.

Scheme I



strength was also found to have no effect on the rate of the reaction between glycine and NBS (Experimental Section).

Discussion

It is first necessary to identify the intermediate indicated by the absorbance at 240 nm in order to begin to unravel the mechanism of the oxidative decarboxylation of amino acids. This absorption seems to indicate the presence of a carbon–nitrogen π-bond as in an imine. Two possible mechanistic paths for the reaction that could generate such a species are shown in Scheme I.[6]

If the absorption at 240 mm is due to the imine intermediate, then the observed first-order decrease in absorbance corresponds to the first-order decay of this intermediate (i.e., the k_3 step). This step does not involve the reduction of the positive bromine species. However, when the reaction between glycine and NBS is followed by monitoring the disappearance of the oxidizing species (i.e., the positive bromine species) by iodimetry, the observed first-order rate constant is found to be identical with that observed by monitoring the absorbance change at 240 mm within experimental error. This clearly indicates that the disappearance of A or A′ (or both) and the disappearance of the Schiff base (B) occur at the same rate. This is possible only if the reaction is an irreversible consecutive reaction as shown in eq 1. Use of the conservation

$$\mathrm{A} \xrightarrow{k_2} \mathrm{B} \xrightarrow{k_3} \text{products} \quad (1)$$

$$[\mathrm{A}] = [\mathrm{A}_0]e^{-k_2t} \quad (2)$$

$$[\mathrm{B}] = [\mathrm{A}_0]k_2\{e^{-k_2t} - e^{-k_3t}\}/\{k_3 - k_2\} \quad (3)$$

equations yields eq 2 and 3. In this system, B is a transient intermediate such as a Schiff base. If $k_3 \ll k_2$, then the disappearance of the Schiff base B is the slow, rate-limiting step. If this is the case, then the rate constant obtained by iodimetrically monitoring the disappearance of the positive bromine cannot be equal to that obtained by monitoring the disappearance of the imine at 240 nm. On the other hand, if $k_3 \gg k_2$ (i.e., if the disappearance of the bromo intermediate A or A′ or both is the slow step), then the concentration of the imine B would reach a steady-state concentration with a rate constant k_3 and decay slowly with a rate constant k_2. In other words, the intermediate appears to be formed with its decomposition rate constant and decompose with its formation rate constant. This is readily understood for the initial rate treatment.[7] This rationalizes the equivalence of the rate constants obtained by the two different methods. Imines of this type with hydrogen attached to the nitrogen can be observed spectrophotometrically,[8] but they cannot be isolated[9] as they undergo further decomposition.

The reaction between glycine (or glycine-d_5) and NBS exhibits a secondary isotope effect of k_H/k_D = 1.35 and a kinetic solvent deuterium isotope effect of $k(H_2O)/k(D_2O)$ = 2.0. The use of glycine in protium oxide and glycine-d_5 in deuterium oxide gives an observed isotope effect of 2.87, which is approximately equal to the product of the secondary isotope effect and the kinetic solvent deuterium isotope effect. This suggests that the observed secondary effect of 1.35 is due to isotopic substitution at the α-carbon. The observed secondary effect is almost equal to that of 1.36 calculated theoretically[10] from the vibrational frequency change in the rate-limiting conversion of sp^3 carbon to sp^2 carbon. This indicates that the breakdown of A or A′ to imine (B), and not the conversion of imine to product, is rate limiting.

The presence of alkyl groups at the α-carbon has only a very minor effect on the amino and carboxylic groups as evidenced by their pK_a values; for most amino acids the p$K_{a(1)}$ = 2.1 ± 0.3 and p$K_{a(2)}$ = 9.6 ± 0.7. Hence, the absence of an initial time lag for the attainment of the absorption maximum at 240 nm in the case of alanine and valine suggests that the formation of the imine intermediate is faster with these amino acids than with glycine. This again suggests that the formation of the imine intermediate involves the cleavage of the bond between the α-carbon and the carboxylic carbon in the fashion indicated by path A rather than path A′ of Scheme I. The slight electron-releasing effect of the alkyl groups should favor the cleavage mode in path A over that in path A′ by stabilizing any

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partial positive charge that develops on the α-carbon in the transition state due to the possible nonconcertedness of bond making and bond breaking. The nonconcerted nature of the reaction may be attributed to the positive charge on the nitrogen which allows carbon–carbon bond cleavage to precede carbon–nitrogen bond formation to some extent. This suggests that the oxidizing species attacks the carboxylate group rather than the amino group in the amino acid. The absence of any reaction between NBS and betaine may seem to contradict this conclusion, but it does not. Clearly the A′ path is impossible with betaine and the lack of reaction in the path A case is probably due to the difficulty inherent in the breakdown of the resultant bromo intermediate to the reactive imine intermediate.

Most of the our studies were carried out under acidic conditions under which the amino group of 1 would exist essentially in the protonated form. The oxidizing halogen species is an electrophile so the interaction between this species and the protonated amine is unlikely as well.

At pH values above the isoelectric point, the protonated amino group ionizes and attack of the oxidizing electrophile may be more likely. If this is so then there should be an enhancement of the rate at these pH values but such an enhancement is not observed with glycine. This suggests that there is no electrophilic attack on the amino nitrogen or if there is such attack that it is not productive.

Esterification of the carboxyl group should significantly affect the electrophilic attack on the ester oxygen while having little effect on attack at the amino group. However, esterification will have a large influence on the decomposition of the bromo intermediate formed in the latter case. The fact that the ethyl ester of glycine reacts with NBS about 10 times slower than does glycine also suggests that the electrophilic attack by positive bromine occurs at the carboxyl group rather than at the amino group. Since this is so, one would expect addition of acetate ion to compete for the electrophilic bromine and to decrease the rate of the reaction. However, added acetate actually increases the rate of the reaction. Acetate can react with the brominating agent to form acetyl hypobromite, an effective brominating agent itself. However, if this agent is formed it would readily react with the amino acid in a step comparable to the k_1 step which is prior to the rate-limiting step. Therefore, such a mechanism would not result in a rate increase. Acetate, which has been shown by us to act as a general base catalyst, is probably acting to abstract the proton from the nitrogen. Furthermore, this observation suggests that the rate-determining step involves catalysis by acetate ion. Such catalysis under acidic conditions is possible only if the breakdown of the bromo intermediate (A in Scheme I) is the rate-limiting step.

The negative value of the curvature parameter, γ, obtained in the proton inventory studies with protium oxide–deuterium oxide mixtures suggests the possibility of parallel transition states or the involvement of reactant-state fractionation factors or both.[5] The possibility of parallel paths as in Scheme I has already been excluded. Detailed analysis of the proton inventory data and closer scrutiny of the model again leads to the exclusion of parallel paths in the oxidative decarboxylation of glycine.

The general form of the equation which describes the dependence of the rate constant, k_n, on the atom fraction of deuterium, n, in a solvent mixture consisting of protium oxide and deuterium oxide is shown in eq 4.[5,11-16] The

$$k_n = k_0 \frac{\prod_i^{\mathrm{TS}}(1 - n + n\phi_i)}{\prod_j^{\mathrm{RS}}(1 - n + n\phi_j)} \quad (4)$$

parameters ϕ_i and ϕ_j are isotopic fractionation factors for isotopically exchangeable hydrogenic sites in the transition state (TS) and reactant state (RS), respectively. These isotopic fractionation factors measure the deuterium to protium preference for the site in question relative to the deuterium to protium preference for an exchangeable solvent site.

It can be shown that reactions with parallel transition states with the second transition state containing an equal or greater number of isotopically contributing sites than the first will produce proton inventories containing a greater amount of curvature than those with only one transition state. For reactions proceeding by parallel paths there are two possibilities to consider. Equation 5 is the

$$k_n = k_0 \frac{k_{0,1}(1 - n + n\phi_1)^y + k_{0,2}(1 - n + n\phi_2)^z}{(k_{0,1} + k_{0,2})\prod_j^{\mathrm{RS}}(1 - n + n\phi_j)} \quad (5)$$

$$k_n = k_0 \frac{k_{0,1}(1 - n + n\phi_1)^y + k_{0,2}(1 - n + n\phi_2)^z}{k_{0,1} + k_{0,2}} \quad (6)$$

form of eq 4 for a system involving two parallel paths in which reactant-state fractionation is important while eq 6 is the form of the equation if no reactant-state fractionation factors are important. In these equations, $k_{0,1}$ and $k_{0,2}$ are the rate constants for the reaction through transition states 1 and 2, respectively, in pure protium oxide, y and z are the number of protons involved in transition states 1 and 2, respectively, and k_0 is the sum of the two rate constants. Neither eq 5 nor 6 can account for the observed linearity of the plot of $(k_0/k_n)^{1/2}$ vs. n.

The linear plot (of $k_0/k_n)^{1/2}$ vs. n suggests the involvement of reactant-state fractionation factors. As far as the substrate is concerned, only the protons on the protonated amino group, with fractionation factors of 0.97,[13] are of importance. In Scheme I, only one proton on the protonated amino group is changed in going to transition state A′. This change is insufficient to account for the deep curvature in the proton inventory plot, and, thus, the curvature and negative γ value must be due to the oxidizing species.

Most investigations of NBS oxidations of organic substrates have assumed that the molecular NBS acts only through its positive polar end.[17-19] However, since the nitrogen of NBS will be protonated at the experimental pH in the present study, production of Br^+ and its subsequent solvation is likely. Thus, H_2O^+Br is a possible effective oxidizing species. This species can be produced in situ either by nucleophilic attack of water on the pro-

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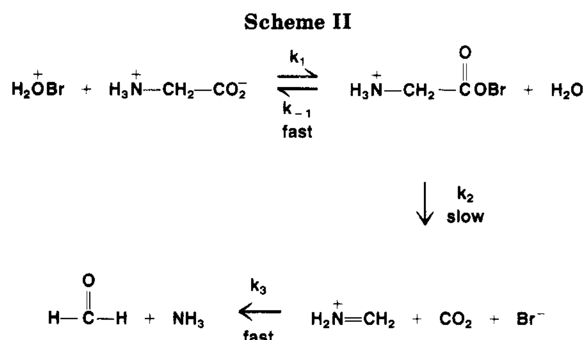
Scheme II



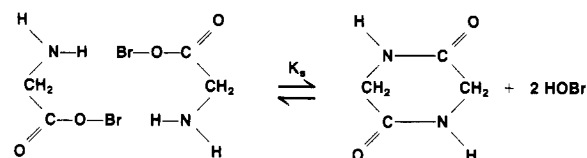


Figure 5. Dimerization of the proposed intermediate to give 2,5-diketopiperazine.

tonated NBS or by the production of HOBr produced by the nucleophilic attack of water on NBS. The fractionation factors for the two protons in the H_2O^+Br species may be responsible for the isotope effect and the observed curvature in the proton inventory plot.

If we assume, based on the earlier arguments, that the reaction proceeds only through transition state A in Scheme I and that it is the reactant-state fractionation factors of the two protons in H_2O^+Br that are solely responsible for the curvature in the proton inventory, then eq 4 takes on the form shown in eq 7. This equation shows

$$k_n = k_0(1 - n + n\phi_j)^{-2} \quad (7)$$

that a plot of $(k_0/k_n)^{1/2}$ vs. n must be linear. In fact, such a plot made from the data of Table II is linear with a slope $(\phi_j - 1)$ of 0.418. This gives a fractionation factor of 1.418 for the H_2O^+Br protons. Gold and Lowe[20] had earlier assumed a value very near this for the fractionation factor for the protons of the water molecule associated with boric acid [i.e., $H_2O^+B(OH)_3^-$]. Thus, the observed value of 1.418 seems reasonable for the fractionation factor of H_2O^+Br protons since the equilibrium dissociation is similar to that for the boric acid system as is seen in eq 8 and 9.

$$H_2O + H_2O^+B(OH)_3^- \rightleftharpoons B(OH)_4^- + H_3O^+ \quad (8)$$

$$H_2O + H_2O^+Br \rightleftharpoons HOBr + H_3O^+ \quad (9)$$

Although the value of 1.418 for the fractionation factor for the protons of the solvated Br^+ ion is rather large, this value may be explained in the following way. In the course of solvation of Br^+ by water it is not possible for the cation to delocalize the electron density to other areas. In the terminology of Gutman[21] this means there will be no spillover effect. However, there will be a decrease in fractional positive charge at the cation and there will be a pileup effect at the donor atom of the solvent. Because of the ability of the oxygen atom to delocalize electrons toward the bromo cation with the resultant decrease in freedom of O–Br bond rotation, the protons attached to the oxygen should have a fractionation factor indicating a "tighter than bulk solvent" bonding situation for the protons. Such fractionation factors have been reported for *gem*-diol protons.[22]

Thus, the reaction between glycine and NBS involves the rapid formation of the bromo intermediate by the interaction of the oxidizing species, H_2O^+Br, and the carboxylate group of glycine followed by the slow decomposition of the bromo intermediate in the rate-limiting step as shown in Scheme II.

The rate law corresponding to Scheme II, shown in eq 10, accounts for the observed dependence of the rate on

$$\text{rate} = k_2[\text{HOBr}][\text{glycine}]/\{(k_{-1}/k_1) + [\text{glycine}]\} \quad (10)$$

glycine concentration at pH 3.7. The dependence of rate on glycine concentration at pH 5.0 may be due to the formation of an inactive complex by the interaction of two bromo ester intermediate molecules which, like glycine ethyl ester, dimerize to 2,5-diketopiperazine (Figure 5). If this is the case, then eq 10 will become

$$\text{rate} = k_2[\text{HOBr}][\text{glycine}]/\{(k_{-1}/k_1) + [\text{glycine}] + K_s[\text{glycine}]^2\} \quad (11)$$

At low concentrations of glycine eq 11 will convert to eq 12. Thus, as the concentration of glycine increases the

$$\text{rate} = k_1k_2[\text{HOBr}][\text{glycine}]/k_{-1} \quad (12)$$

rate would increase. We still must explain why the rate reaches a maximum value as a function of glycine concentration, however. This drop off in rate at still higher concentrations of glycine is likely due to the increased liklihood of formation of the inactive 2,5-diketopiperazine.

Any proposed mechanism for the oxidative decarboxylation of glycine must account for the observed complex variation of rate with pH shown in Figure 3. The breakdown of the bromo ester intermediate is a base-catalyzed reaction since the base can remove the acidic proton on the amino nitrogen. In contrast, the hydrolysis of the imine into ammonia and aldehyde is an acid-catalyzed reaction since only the cationic imine can undergo hydrolysis.[23] As the pH is increased the rate of the base-catalyzed breakdown of the bromo ester continues to increase, but the rate of the collapse of the imine decreases. This occurs until the pH increases above the pk_a of the Schiff base, which is about 6.7.[24] Above this pH there occurs a change in rate-limiting step so that k_3 becomes rate limiting. Thus, any increase in pH above the pK_a of the imine increases the concentration of the unprotonated imine which decreases the rate.

The observed secondary deuterium isotope effect of 1.35 is the ratio of the rate constants for the reaction between glycine and NBS and that between glycine-d_5 and NBS in protium oxide. Since the pk_a of the amino group in glycine is 9.6, the N–^{2}H protons in glycine-d_5 undergo rapid exchange with the solvent protium oxide. This means that the observed secondary deuterium effect is due to the α-deuteria. The observed solvent deuterium isotope effect of 2.0 (Table II) is the ratio of the rates of the reaction between glycine and NBS in protium oxide and deuterium oxide. Here again, the NH protons undergo exchange with the solvent but any exchange results in a decrease in the atom fraction of deuterium in the solvent which leads to a small decrease in the solvent isotope effect. If we assume that any small β-deuterium isotope effect approximately compensates the decrease in the solvent isotope effect, then

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we can safely assume that the observed effect of 2.0 is the real solvent isotope effect. In any case, the difference is likely to be negligible.

An isotope effect of 2.87 is observed (Table III) when glycine and glycine-d_5 are used as substrates in protium oxide and deuterium oxide, respectively. In this case, there is no isotopic exchange so the observed effect of 2.87 should be the product of the α-deuterium effect due to the bonding frequency changes of the C–L (L = H or D) positions, the β-deuterium effect due to the hyperconjugative effect of the adjacent N–L (L = H or D) positions and the kinetic solvent deuterium isotope effect. Since the solvent isotope effect is 2.0, the product of the α- and β-deuterium isotope effect is 2.87/2.0 = 1.435. Thus, eq 7 correspnding to the data of Table III is shown in eq 13.

$$k_n = k_0(1 - n + n\phi_j)^{-2}\,[1 + (1.435 - 1)n]^{-1} \quad (13)$$

Equation 13 contains a correction term which takes into account the reduction in the concentration of glycine-d_5 and, thus, the α-deuterium isotope effect with the decrease in the atom fraction of deuterium in the solution. The use of ϕ_j = 1.418 obtained from the data of Table II and the experimental values of k_0 and n in eq 13 allows us to calculate a theoretical proton inventory for this model. These data, which are in excellent agreement with the experimental values, are collected in Table III.

The results obtained in the oxidative decarboxylation of alanine and valine differ significantly from those for glycine in four respects: (1) In addition to the carbonyl product a nitrile is formed as a major product with alanine and valine as has been shown earlier.[25] (2) The rate of the reaction is independent of substrate concentration at pH 3.7 and exhibits very little dependence at pH 5.0. (3) There is no inhibition of the rate with increasing substrate concentration at pH 5.0. (4) The pH–rate exhibits an inverted bell shape for both valine and alanine.

Alanine and valine differ from glycine only in containing a methyl or isopropyl group, respectively, at the α-carbon. The presence of these groups has very little effect on the pK_a of the carboxyl or amino groups. The above differences for alanine and valine are likely due to the involvement of the α-hydrogen in the rate-limiting step or in a step prior to the rate-limiting step. We propose Scheme III as the mechanism for these amino acids. This scheme incorporates Scheme II as path C but it also includes the abstraction of the α-hydrogen by the electrophilic bromine species as a possible route.

Scheme III accounts for the observed formation of a nitrile as a major product. In the case of glycine, path D and the crossovers from path C to path D are all absent so the rate is expressed by eq 10. But in the case of alanine and valine, the rate of formation of the imine is increased to the sum of the rate of hydride ion abstraction from the substrate, the rate of hydride ion abstraction from the bromo ester of the substrate, and the rate of the breakdown of the bromo ester of the substrate. Thus, k_2 in eq 10 and Scheme II equals the sum of k_2', k_2'', and k_3'' in Scheme III. The net result is an increase in the rate of substrate consumption. Although the product ratio can be determined, the partitioning ratio of the intermediates is not possible mainly because of the crossovers postulated in Scheme III.

The inductive push provided by the alkyl group at the α-carbon atom of the alanine and valine, which significantly accelerates the breakdown of the bromo ester C to the imines D and E, retards the reversion of the bromo

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Scheme III[a]

Path C Path D

[a] Electron movement in path D as well as electron movement associated with the crossover from path C to path D is shown by solid curved arrows while that associated with path C is shown by dashed curved arrows.

ester to the reactants (i.e., it decreases k_{-1}). The net result makes the k_{-1}/k_1 term in the denominator of eq 10 insignificant in comparison with substrate concentration even at low substrate concentration. Thus, the reaction reaches the maximum rate and exhibits zero-order dependence on substrate concentration.

The breakdown rate of the bromo ester is increased to the sum of the rate of conversion to the imine E and the rate of reaction with the oxidizing species. As a result the amount of bromo ester available for the dimerization to the inactive 2,5-diketopiperazine becomes significantly low and no inhibition of rate with an increase in substrate concentration at pH 5.0 is observed in contrast with what was seen with glycine.

The rate of hydride ion abstraction from the α-carbon of the substrate, the bromo ester, or the imine depends essentially on the oxidizing ability (i.e., the electrophilicity) of the species involved. At low pH the oxidizing species, HOBr, exists in its protonated form, H_2O^+Br, and the slow, rate-limiting abstraction of hydride ion occurs with ease. This, coupled with the inductive effect of the alkyl group at the α-carbon accounts for the larger value of the observed rate constant at lower pH. As the pH is increased, the concentration of the protonated electrophilic species continues to decrease and the hydride ion abstraction steps (i.e., the k_2'' and k_2''' steps) decrease. Although there is an increase in the breakdown of the bromo ester intermediate into the imine, it is not sufficient to offset the

larger decrease in the rate of hydride ion abstraction. Thus, there is a decrease in rate with increasing pH. Around neutral pH, the reaction proceeds mainly through path C in Scheme III. For the reasons stated earlier, there occurs a change in rate-limiting steps from the breakdown of the bromo ester (i.e., the k_2' step) to the disappearance of the imine (i.e., the k_3' and k_3'' steps). In the case of alanine and valine, the imine intermediate is converted into two sets of products by reacting with either water or by interacting with the oxidizing species. Of these, the reaction with water (i.e., the k_3' step) is an acid-catalyzed reaction and the other (i.e., the k_3'' step) is a base-catalyzed reaction. Hence, an increase in pH above the pK_a of the imine inhibits the reaction with water but enhances the interaction with the oxidizing species and causes a net increase in rate.

Conclusion

The mechanisms of oxidative decarboxylation of amino acids promoted by NBS have been shown to be significantly influenced by the presence of alkyl groups at the α-carbon. It will be interesting to see if this effect extends to other amino acids and to oxidizing agents other than NBS. It has been shown that the proton inventory technique can provide considerable information about the mechanisms of such oxidative decarboxylation reactions and its use in such studies should prove worthwhile.

Experimental Section

Materials. Glycine, glycine-d_5, alanine, DL-valine, citric acid, acetic acid, acetic acid-*d* (98 atom % D), glycine ethyl ester hydrochloride, and betaine hydrochloride (99%) were all commercial samples and were used as obtained. *N*-Bromosuccinimide (Aldrich) was recrystallized from water. Deuterium oxide (99.75 atom % D; Bio-Rad) was used as obtained. Water was twice glass distilled before use.

Citric acid–disodium hydrogen phosphate buffer solutions of desired pH were prepared by mixing the required volumes of stock solutions of 0.1 M citric acid and 0.2 M disodium hydrogen phosphate.

Acetic acid–sodium acetate buffer solutions of the desired pH(D) were prepared by mixing the required volumes of stock solutions of acetic acid (or acetic acid–*d*) and sodium acetate in protium oxide or deuterium oxide. The ionic strength of the stock solutions was maintained with sodium perchlorate at 0.5 M. Solutions of lower buffer concentration were prepared from the above buffer solution by dilution with 0.25 M sodium perchlorate solution. The pH(D) of the solutions was measured by using a Corning pH meter Model 130 equipped with a combination electrode.

Kinetics. The oxidative decarboxylations of glycine, alanine, and valine were monitored by following the decrease in absorbance at 240 nm on a Cary 118C UV–vis spectrophotometer equipped with a constant-temperature cell compartment and cell holder to maintain a constant temperature.

Reactions were initiated by injecting 25 μL of a stock solution of NBS in acetonitrile into 3.00 mL of the appropriate buffer solution containing the substrate. The runs were generally initiated exactly 75 s after the injection of the substrate solution. Reactions were followed to greater than 80% completion. Absorbance values at 5- or 10-s intervals were collected by using a Micromation computer interfaced to the Cary 118C spectrophotometer. The data were then analyzed by using a nonlinear least-squares computer program. Plots of log $(A_t - A_\infty)$ vs. time were used in a confirmatory fashion.

The reactions of alanine or valine with NBS (6×10^{-4} M) at pH 3.7 (acetic acid–sodium acetate buffer) at 25 °C exhibited no dependence on amino acid concentration over the range 0.6×10^{-2} to 18×10^{-2} M. The first-order rate constants were 5.54×10^{-2} s^{-1} and 1.54×10^{-2} s^{-1}, respectively, within experimental error. The concentration of NBS at pH 3.7 (ionic strength was 0.5 M) at 25 °C was varied from 1×10^{-4} to 8.4×10^{-4} M (glycine concentration was 1.22×10^{-2} M) and was shown to have no effect on the first-order rate constant for the oxidative decarboxylation of glycine ($k = 4.36 \times 10^{-3}$ s^{-1} within experimental error). Variation of the ionic strength from 0 to 0.28 M ($NaClO_4$) at pH 5 did not influence the rate constant ($k = 8.43 \times 10^{-3}$ s^{-1}) for the glycine decarboxylation.

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Registry No. Glycine, 56-40-6; DL-alanine, 302-72-7; DL-valine, 516-06-3; *N*-bromosuccinimide, 128-08-5.

Carbonyl Methylenation Using a Titanium–Aluminum (Tebbe) Complex[1]

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The titanium–aluminum (Tebbe) complex [$Cp_2TiCH_2ClAl(CH_3)_2$] is shown to be an effective methylenating agent for a variety of carbonyl groups. The reaction is unique in that the carbonyl groups of carboxylic acid derivatives are readily methylenated. Thus vinyl enol ethers are prepared from esters and enamines are formed from amides. The complex provides a method for methylenating hindered or base sensitive ketones that is advantageous to the Wittig reagent. Selective methylenation of dicarbonyl compounds is also accomplished.

Methylenation and general alkylidenation of ketones and aldehydes using the Wittig reaction is well established in the methodology of organic synthesis.[3] There are, however some limitations to the use of the technique. Wittig reactions are quite sensitive to the steric environment around the carbonyl group undergoing reaction.[4] Further, the

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