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Claisen-Type Addition of Glycine to a Pyridoxal Iminium Ion in Water

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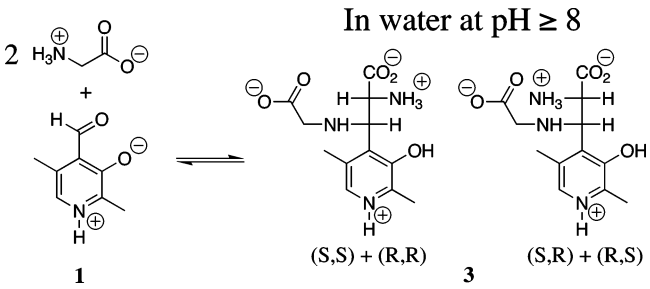

The 5′-deoxypyridoxal stabilized glycine carbanion has been generated in water at neutral and mildly basic pH. At pH < 7, this carbanion reacts mainly with the carbonyl carbon of **1** to form a stable Claisen-type adduct. At pH ≥ 8, this carbanion reacts with the iminium carbon of the pyridoxal−glycine iminium ion to form the second Claisen-type adduct **3** as the major reaction product.

Pyridoxal 5′-phosphate (PLP) is a cofactor for enzymes that catalyze a wide variety of reactions of amino acids.[1−3] The α-amino carbon of amino acids is only weakly acidic,[4−6] and the most important function of PLP is to stabilize the α-amino acid carbanion intermediates of enzyme-catalyzed reactions. The reactions of such PLP-stabilized carbanions in aqueous solution were thought to be well understood and to closely parallel enzyme-catalyzed processes. However, the reaction between the PLP analogue, 5′-deoxypyridoxal (**1**), and glycine in D_2O does not give the expected exchange of deuterium for the α-amino hydrogen of glycine from PLP-catalyzed deprotonation of glycine, but rather gives a quantitative yield of diastereomers **2a** and **2b** from the Claisen-type addition of glycine to **1** (Scheme 1).[7] We now report the formation of another pair of diastereomers **3a** and **3b** from the reaction of **1** (10 mM) and glycine (100 mM) at pH(D) > 8 (Scheme 1). These data reveal the richness of the reactions of the pyridoxal-stabilized amino acid carbanion **5** that has not been observed in studies to mimic the enzymatic reactions of PLP.

The reaction of glycine (100 mM) with **1** (10 mM) in D_2O buffered at pD 9.7 with 50 mM hexafluoroisopropanol (HFIP) at 25 °C (I = 1.0, KCl) was monitored by ^{1}H NMR as described in earlier work.[7] ^{1}H NMR analysis showed the disappearance of **1** and **4** over a period of several hours to give **2a** and **2b** as minor products and a pair of major products with spectra similar to those of **2a** and **2b**, but with signals for an additional methylene group. The following observations are consistent with our assignment of structures **3a** and **3b** to these diastereomers (Scheme 1), and they show that **1** is an effective catalyst of their breakdown to form **1** and glycine, as required by the principle of microscopic reversibility.

(1) An attempt to purify **3** by ion-exchange chromatography of the products that form in H_2O yielded only the starting material **1**. This shows that **3** is converted to **1** during the chromatography step.

(2) It was possible to purify **3** by ion-exchange chromatography when the carbonyl group at **1** that remains after a 5 h reaction time was reduced using sodium borohydride. A single major diastereomer of **3** was isolated and characterized by ^{1}H and ^{13}C NMR and by high-resolution mass spectrometry.

(3) ^{1}H NMR analysis of **1** (10 mM) in the presence of 100 mM glycine at early reaction times shows that the ratio of **1** and the glycine iminium ion **4** changes from [**4**]/[**1**] = 2.2 at pD 7.0 (50 mM phosphate buffer) to [**4**]/[**1**] ≈ 50 at pD 9.7 (50 mM HFIP buffer). Therefore, the change in product yields with increasing pD is due, at least in part, to the change in the relative concentrations of the electrophiles **4** and **1** that react with the pyridoxal stabilized glycine carbanion **5**.

(4) When the reaction of 10 mM **1** with 100 mM glycine was monitored at pD 9.7 in a solution buffered with 50 mM HFIP, **3** and **2** were formed in a ratio of [**3**]/[**2**] = 4.0 under conditions of kinetic control (Table 1). The two diastereomers of **3** were formed in a ratio of 2:1. The slow conversion of **3** to **2** was monitored over a period of 3 days. At the end of this time, the ratio [**3**]/[**2**] had decreased to 1.6 under conditions where the product yields are controlled by their thermodynamic stability.

(5) The breakdown of **3** to **1** was monitored directly in an experiment where **3** was prepared by the reaction of 20 mM **1** with 200 mM glycine for 5 h in HOH at pH 9.9 buffered with 50 mM HFIP. The small aliquot of this solution was then diluted by 100−400-fold into the same solvent, and the conversion of **3** back to **1** was monitored by following the increase in absorbance at 392 nm. The reactions of **3** are first-order for > 3 halftimes. The first-order rate constant for the breakdown of **3** increases 4-fold from 5.3×10^{-5} to 2.0×10^{-4} s^{-1} as the dilution is *decreased* 4-fold from 1/400 to 1/100. This provides direct evidence that the breakdown of **3** is catalyzed by the small amount of **1** that is in solution after generation of **3** and its dilution. *No reaction of* **3** was observed after dilution of **3**, when a 2-fold molar excess (relative to starting **1**) of sodium borohydride was first added to reduce the carbonyl group of remaining **1**.

Rate constant ratios k_{ald}/k_{im} (Scheme 1) for partitioning of **5** between addition to **1** and to **4**, calculated from the concentrations of reactants and products using eq 1, are also reported in

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SCHEME 1

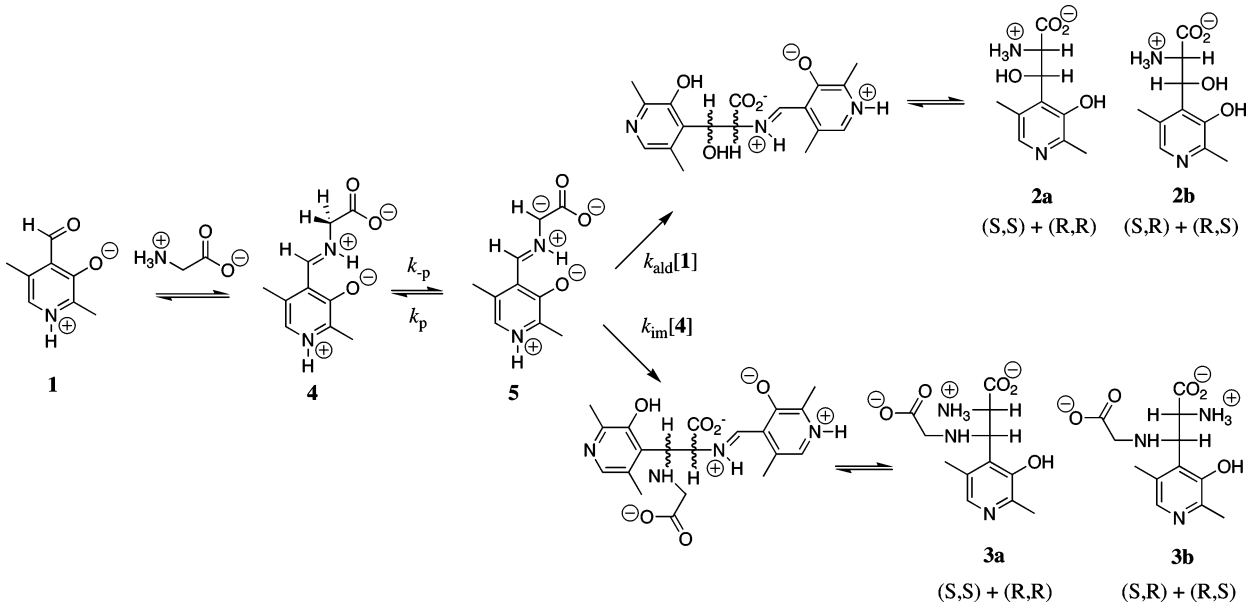

TABLE 1. pH Dependence of Product Yields for the Reactions of 1 with Glycine in D_2O at 25 °C at I = 1.0 (KCl)

pD[a]	[**4**]/[**1**][b,c]	[**3**]/[**2**][c,d]	k_{ald}/k_{im}[e]
5.1	0.91 (38%)	n. d.[g]	
7.0	2.2 (27%)	n. d.[g]	
8.0	9.0 (19%)	0.10	90
8.7	29 (18%)	0.33	88
9.2	38 n.d.[f]	1.3	29
9.7	48 n.d.[f]	4.1 (1.6)[h]	12

[a] The following buffers were used to maintain constant pD in D_2O: pD 5.1, 50 mM acetate buffer; pD 7.0 and 8.0, 50 mM phosphate buffer; pD 8.7, 50 mM triethanolamine buffer; pD 9.2 and 9.7, 50 mM HFIP buffer. [b] The ratio of the concentrations of **4** and **1** (carbonyl form). The numbers in parentheses give the percent of total **1** that is present as the carbonyl hydrate. [c] The concentration ratios were determined by ^{1}H NMR analysis as the ratio of the sums of the peak areas of the signals for every proton in each compound and correcting this ratio for a different number of protons in the two compounds. [d] The initial ratio of the concentrations of **3** and **2** determined after a reaction time of 90 min. [e] The rate constant ratios for partitioning of the glycine-stabilized carbanion **5** between addition to **1** and **4** calculated from eq 1. [f] No hydrate was detected by ^{1}H NMR. [g] No **3** was detected by ^{1}H NMR. [h] The ratio of **3** and **2** determined after a reaction time of 3 days.

Table 1. There is a ca. 8-fold decrease in k_{ald}/k_{im} from 90 to 12 as the pD is increased from 8.0 to 9.7. This shows that the increase in the yield of **3** is also caused by a decrease in the selectivity (k_{ald}/k_{im}) of **5** for addition to **1** as the pD increases. This decrease in selectivity is due mainly (or entirely) to the decrease in k_{ald} from deactivation of the carbonyl group of **1** by deprotonation at the pyridinium nitrogen with a pK_a of ca. 8.0 in HOH.[8−10]

$$\frac{k_{ald}}{k_{im}} = \frac{[\mathbf{2}][\mathbf{4}]}{[\mathbf{3}][\mathbf{1}]} \qquad (1)$$

These experiments to monitor the products of the reaction of **1** in D_2O have the following broader implications. (1) Protonation of the resonance stabilized carbanion **5** in D_2O to form pyridoxamine and glyoxylic acid or deuterium-labeled glycine is much slower than the dominant Claisen-type addition of this carbanion to low concentrations of a carbonyl or imine electrophile. By contrast, the protonation of unstable enolates in water, such as enolates of simple ketones, is known to be faster than the addition of these nucleophiles to 1 M solutions of simple carbonyl compounds.[11,12] (2) These data reported in Table 1 suggest the feasiblity of a broader study to determine the relative rate constants for partitioning of resonance stabilized carbanions between addition to aldehydes and imine/iminium ions.

We are not able to provide from these limited data a thorough kinetic and thermodynamic analysis of the reactions of **1** with Brønsted acids and the carbonyl group, which is required for a comparison of their Marcus intrinsic reaction barriers.[13,14] In fact, there are very few such analyses for carbanion−carbonyl group combination reactions. Our data support the qualitative notion that strong resonance delocalization of negative charge at a carbanion favors the reaction of the carbanion with carbon electrophiles compared to reaction with Brønsted acids. This suggests that there is a greater requirement for loss of resonance stabilization of the carbanion at the transition state for reaction with the Brønsted acid compared with carbonyl electrophiles, due to the greater nonsynchronization of changes in resonance and polar interactions in the former transition state.[15−17] An even more qualitative interpretation is that the reaction of *soft* carbon nucleophiles and electrophiles is preferred in comparison to the *soft* carbon nucleophile and *hard* Brønsted acids.[18] In conclusion,

(8) A pK_a of 8.0 has been reported for deprotonation of the pyridine nitrogen of **1** in H_2O at I = 0.10 (KCl) [ref 9]. This pK_a is expected to increase to ca. 8.5 in D_2O [ref 10].

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a modern theoretical treatment of carbanion−carboxyl group combination reactions would be useful.

Materials

5′-Deoxypyridoxal was prepared by a published procedure. The simple Claisen-type adducts **2a** and **2b** were characterized as described in previous work.[7] All other organic and inorganic chemicals were reagent grade and were used without further purification.

^{1}H NMR spectra (64 transients) at 500 MHz in D_2O at 25 °C were obtained using a 6000 Hz sweep width, a 6 s acquisition time, a 90° pulse angle, and a 70 s relaxation delay which was 7-fold greater than the longest T_1 for the protons of interest.

A sample of the major diastereomer of **3** was prepared by the addition of 30 mg of **1** to 10 mL of 200 mM glycine buffered with 50 mM hexafluoroisopropanol (80% free base at pH 9.9). After 5 h, either (a) the pH was adjusted directly to 1.8 using 6 M HCl and the solution applied to a Dowex 50 cation exchange column or (b) 70 μL of sodium borohydride in water [4.4 M in 14 M NaOH] was first added before adjusting the pH to 1.8. In both cases the products were eluted from the column using HCl. Only **1** was obtained in the first case, but in the second case, the major diastereomer of **3** was isolated by elution with 2 M HCl in a yield of 25%: ^{1}H NMR (500 MHz, D_2O, pD 9.8) δ 2.19, 2.32 (3H, s), 3.178, 3.190 (2H, AB, J = 17 Hz), 4.01 (1H, d, J = 6 Hz), 4.39 (1H, d, J = 6 Hz), 7.50 (1H, s); ^{13}C NMR (75.5 MHz, D_2O) δ 14.5, 15.8, 46.9, 53.8, 57.1, 127.5, 128.4, 130.6, 136.5, 142.8, 169.2, 170.5. Exact mass calculated for $C_{12}H_{17}N_3O_5+H^+$, 284.1247; found, 284.1241. Minor diastereomer, from ^{1}H NMR analysis of the mixture (500 MHz, D_2O, pD 9.8), δ 2.17, 2.29 (3H, s), 3.17, 3.20 (2H, AB, J = 16 Hz), 3.65 (1H, d, J = 7 Hz), 4.24 (1H, d, J = 7 Hz), 7.52 (1H, s).

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