pair from the azomethine group is illustrated by the differences in the values obtained for the rate constants of the various amino acids studied, with the most electronegative substituents having the highest rates: chloride followed by phosphate and sulfide. This result supports the idea that the β-elimination step is rate limiting. It is interesting to note that the differences in catalytic effects of di- and trivalent metal ions are quite small, whereas one would expect on the basis of first principles that their relative effects on α-hydrogen dissociation would be considerable, mainly because of differences in ionic charge. It is suggested that part of the large catalytic increase expected from an increase in the charge of the metal ion is considerably mitigated by the fact that increase of the charge would tend to slow down the removal of the electronegative β-substituent. Thus the experimental data indicate that both α-proton labilization and β-elimination steps are rate limiting.

The differences between the values of β-chloroalanine and β-chloro-β-aminobutyric acid rate constants point out the fact that the molecular groups attached to the α-carbon, other than the functional group, have considerable effect on the rate of the reaction. Electron-donating groups tend to slow down the reaction while electron-withdrawing groups aid the reaction. The differences in rate between α-proton labilization and β-elimination reaction would determine the relative importance of these individual steps in the overall elimination process.

The rate constants obtained for the metal-free β-chloroalanine and *O*-phosphoserine systems were of approximately equivalent magnitude for the β-elimination step and α-deprotonation. The rate constants of α-proton labilization of the β-chloro-α-aminobutyric acid system were 1.1 times larger than those obtained for β-elimination, and the differences are thus close to the limit of experimental error. The rates of α-proton labilization in the *S*-ethylcysteine system were less and were 1.1 times larger than those obtained for β-elimination. Because of the lack of a sufficient amount of Schiff base formation at low pDs for the metal Schiff base complexes, the relative rates of α-proton labilization and β-elimination could not be measured. The closeness of the β-elimination and α-hydrogen labilization rate constants that have been measured indicate the delicate balance between these two rate-limiting steps and the possibility that extensive constitutional changes could eliminate the second step without greatly influencing the first. The reverse influence is of course not possible.

Further experimental work is needed to elucidate the interplay between these two reaction steps in metal-free as well as in metal chelate systems and for the influence of the constitution of the amino acid on the absolute magnitudes and the relative values of the rates of these sequential steps in elimination reactions.

Acknowledgment. This research was supported by a grant, No. AM-11694, from the National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health.

Pyridoxal- and Metal-Catalyzed β-Elimination, Decarboxylation, and Dealdolation Reactions of β-Hydroxyglutamic Acid

Kuniyasu Tatsumoto[1] and Arthur E. Martell*

Contribution from the Department of Chemistry, Texas A&M University, College Station, Texas 77843. *Received November 21, 1980*

Abstract: Pyridoxal-catalyzed β-elimination and dealdolation reactions of β-hydroxylglutamic acid were found to occur simultaneously. The rate constants for the parallel elimination and dealdolation reactions in D_2O in the absence of metal ions were determined by proton NMR over the pD range of 4–10. The variations of the rates with pD and solution conditions were found to be similar to those previously reported for the β-phenylserine–pyridoxal system although the magnitudes of the rate constants were found to be much smaller. Sequential pyridoxal-catalyzed reactions consisting of β-elimination followed by δ-decarboxylation were observed in the pD range near the pK_a of the δ-carboxyl group. Metal ion catalysis was found to be less effective than proton catalysis in the promotion of γ-decarboxylation, and the catalytic effects of metal ions were found to decrease with increase in charge on the metal ion.

Metzler et al.[2] have described a general mechanism for the vitamin B_6-catalyzed reactions of Schiff bases of α-amino acids. The metal ion- and pyridoxal-catalyzed dealdolation reactions of β-hydroxy-α-amino acids have been described,[3-6] and probable reaction mechanisms have been proposed. Martell et al.[7,8] have described the kinetics and a proposed general mechanism for vitamin B_6-catalyzed β-elimination reactions of Schiff bases of α-amino acids having β-hydroxyl substituents. In the case of β-phenylserine β-elimination and dealdolation reactions were found to occur simultaneously.[9] For pyridoxal Schiff base model systems, decarboxylation of aspartic acid to alanine and of β-hydroxy aspartate to serine has been described.[10-12] The general mechanism proposed for δ-decarboxylation involves the ketimine Schiff base as an intermediate, thus requiring transamination of the aldimine Schiff base. The mechanisms proposed for these decarboxylation reactions involved consideration of the relative electron-withdrawing effects of the azomethine and pyridyl groups. Similarly, the capacity of the Schiff base to accommodate the electron pair shifted in the formation of an intermediate in the decarboxylation reaction was indicated as important in the proposed mechanism.[12] The present study of pyridoxal-catalyzed

(1) Abstracted in part from a dissertation submitted by K. Tatsumoto to the faculty of Texas A&M University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.
(2) Metzler, D. E.; Ikawa, M; Snell, E. E. *J. Am. Chem. Soc.* **1954**, *76*, 648.
(3) Metzler, D. E.; Longenecker, J. B.; Snell, E. E. *J. Am. Chem. Soc.* **1953**, *75*, 2786; **1954**, *76*, 639.
(4) Longenecker, J. B.; Snell, E. E. *J. Am. Chem. Soc.* **1957**, *79*, 142.
(5) Metzler, D. E.; Snell, E. E. *J. Biol. Chem.* **1952**, *198*, 353.
(6) Marcello, J. A.; Martell, A. E., in press.
(7) Tatsumoto, K.; Martell, A. E. *J. Am. Chem. Soc.* **1977**, *99*, 6082.
(8) Tatsumoto, K.; Martell, A. E. *J. Am. Chem. Soc.* **1978**, *100*, 5549.
(9) Tatsumoto, K.; Martell, A. E.; Motekaitis, R. J. *J. Am. Chem. Soc.*, preceding paper in this issue.
(10) Miles, E. W.; Meister, A. *Biochemistry* **1967**, *6*, 1734.
(11) Doctor, V. M.; Oro, J. *J. Mol. Evol.* **1972**, *1*, 326.
(12) Sakkab, N. Y.; Martell, A. E. *Bioinorg. Chem.* **1975**, *5*, 67.

0002-7863/81/1503-6203$01.25/0

Table I. Proton Association Constants for the β-Hydroxyglutamic Acid–Pyridoxal System (t = 25 °C; μ = 0.10 M(KNO_3))

compds	pK_1	pK_2	pK_3	pK_4
pyridoxal[a]	4.20	8.66	13.0	
HOglu[b]	2.09	4.08	9.06	
HOglu Schiff base[c]			6.35	10.20

[a] Reference 20. [b] HOglu = β-hydroxyglutamic acid. [c] log K_f = [HOglu Schiff base^{3-}] /([HOglu^{2-}] [pyridoxal$^-$]) = 0.64.

α-decarboxylation of β-hydroxyglutamic acid was undertaken because the electron shifts considered necessary for γ-decarboxylation, illustrated in Scheme I, would be accommodated in the intermediate formed by the elimination of the β-hydroxyl group. This paper reports the kinetics and a suggested mechanism for the nonenzymatic pyridoxal- and metal ion-catalyzed reactions of β-hydroxyglutamic acid.

Experimental Section

Pyridoxal hydrochloride was obtained from Mann Laboratories as Mann Analyzed grade. DL-β-hydroxyglutamic acid hydrochloride was obtained from Biochemical Laboratories. Glycine obtained from Sigma Chemical Co. was used without further purification. NaOD (40%), D_2O, and DCl (20%) were obtained from Diaprep Corp.; the purity of D_2O was 99.7%. NaOD and DCl were diluted to the appropriate concentrations under dry nitrogen. Stock solutions of aluminum(III) and zinc(II) were prepared by dissolving $Al_2(SO_4)_3$ and $Zn(NO_3)_2$ in D_2O and evaporating to dryness. After this procedure was repeated several times to remove residual H_2O, the solutions were diluted to the appropriate concentration and standardized by conventional chelatometric titration.[13] The gallium(III) solution was prepared by dissolving a specific amount of gallium metal in DCl and diluting to the appropriate volume.

The pH values of the solutions used for kinetic studies were measured with a Corning Model 101 digital electrometer fitted with a Beckman miniature combination glass electrode, and pH values were adjusted with NaOD. The instrument was calibrated before and after each kinetic run by the use of standard buffers and adjusted by the use of activity coefficients to read −log [H^+] directly. For D_2O solutions, the deuterium ion concentration was computed by adding 0.40 to the observed reading.[14] The temperature of the reaction was maintained at 31.5 ± 1.0 °C, the ambient temperature of the NMR probe. The ionic strength was maintained at μ = 1.0 M with potassium nitrate.

All kinetic runs were carried out in homogeneous systems. In the metal-free pyridoxal–amino acid systems, the analytical concentrations of amino acid and pyridoxal were 0.10 M. In the metal–pyridoxal–amino acid systems, the analytical concentrations of amino acid and pyridoxal were 0.10 M, and the concentrations of metal ions were set at 0.10 M to achieve an amino acid:pyridoxal:metal ion molar concentration ratio of 1:1:1. ^1H NMR spectra were obtained with a Varian HA-100 nuclear magnetic resonance spectrometer. The chemical shifts are reported in hertz with respect to the resonance of tetramethylsilane (Me_4Si), which was inserted into the experimental sample in a coaxial tube.

The fraction of Schiff base in the experimental solutions was determined by taking the sum of the integrals of the resonances of the 6-H, 4-CH, and 2-CH_2 groups of the pyridoxal moiety. The concentrations of the β-eliminated Schiff base were determined directly from the integral values and concentrations of formylacetic acid were determined from the mass balance (i.e., the difference between the initial (analytical) concentrations of amino acid and the sum of the $-CH_2-$ resonances of amino acid, amino acid Schiff base, unreacted starting material, and β-elimination product). The ratios of formylacetic acid and β-eliminated base concentrations were determined for each experimental NMR spectrum over the first 2 half-lives of the reaction.

Results and Discussion

Equilibria. Equilibrium data for the β-hydroxylglutamic acid–pyridoxal system were determined for the reaction conditions employed in this research and are presented in Table I. The values listed are analogous to those reported for other amino acid systems.[15-18] The equilibrium constant, K_f employed for expressing

Table II. The Observed Rate Constants for β-Hydroxyglutamic Acid–Pyridoxal (1:1)[a]

pD	$10^6k'_{obsd}$, $M^{-1}s^{-1}$	$10^6k'_{obsd_2}$, s^{-1}	$10^5k'_{obsd_3}$, s^{-1}	[Form]/[ESB]
3.88	3.28 ± 0.03			
5.02	3.89 ± 0.05			
5.67	4.53 ± 0.06			
6.27		5.8 ± 0.4	3.2 ± 0.4	1.81
7.79		7.9 ± 0.3	4.4 ± 0.3	1.80
8.16		8.6 ± 0.3	4.6 ± 0.2	1.87
8.82		9.9 ± 0.3	5.0 ± 0.3	1.98
9.54		8.8 ± 0.4	4.5 ± 0.3	1.96
10.36		7.2 ± 0.5	3.7 ± 0.5	1.95

[a] Standard deviations calculated as in ref 21.

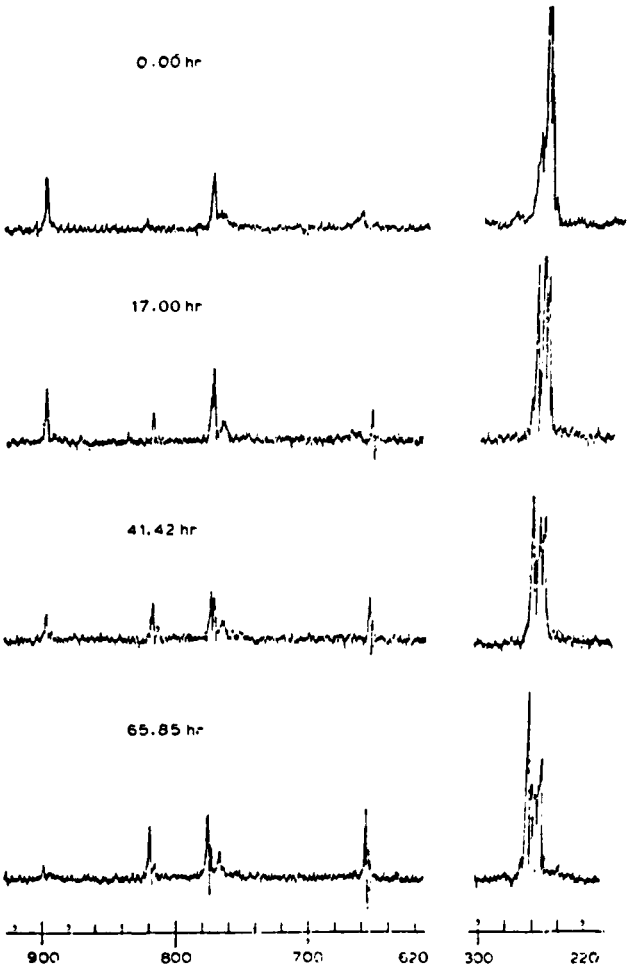


Figure 1. The 100-MHz NMR spectra of 0.10 pyridoxal and 0.10 M β-hydroxyglutamic acid at 31.5 °C in D_2O at pD 8.82 showing the changes observed during the course of Schiff base formation, β-elimination, and dealdolation reactions. The resonances shown are given in hertz with respect to Me_4Si.

Schiff base equilibria is given in the form used by Leussing[19] and is defined as

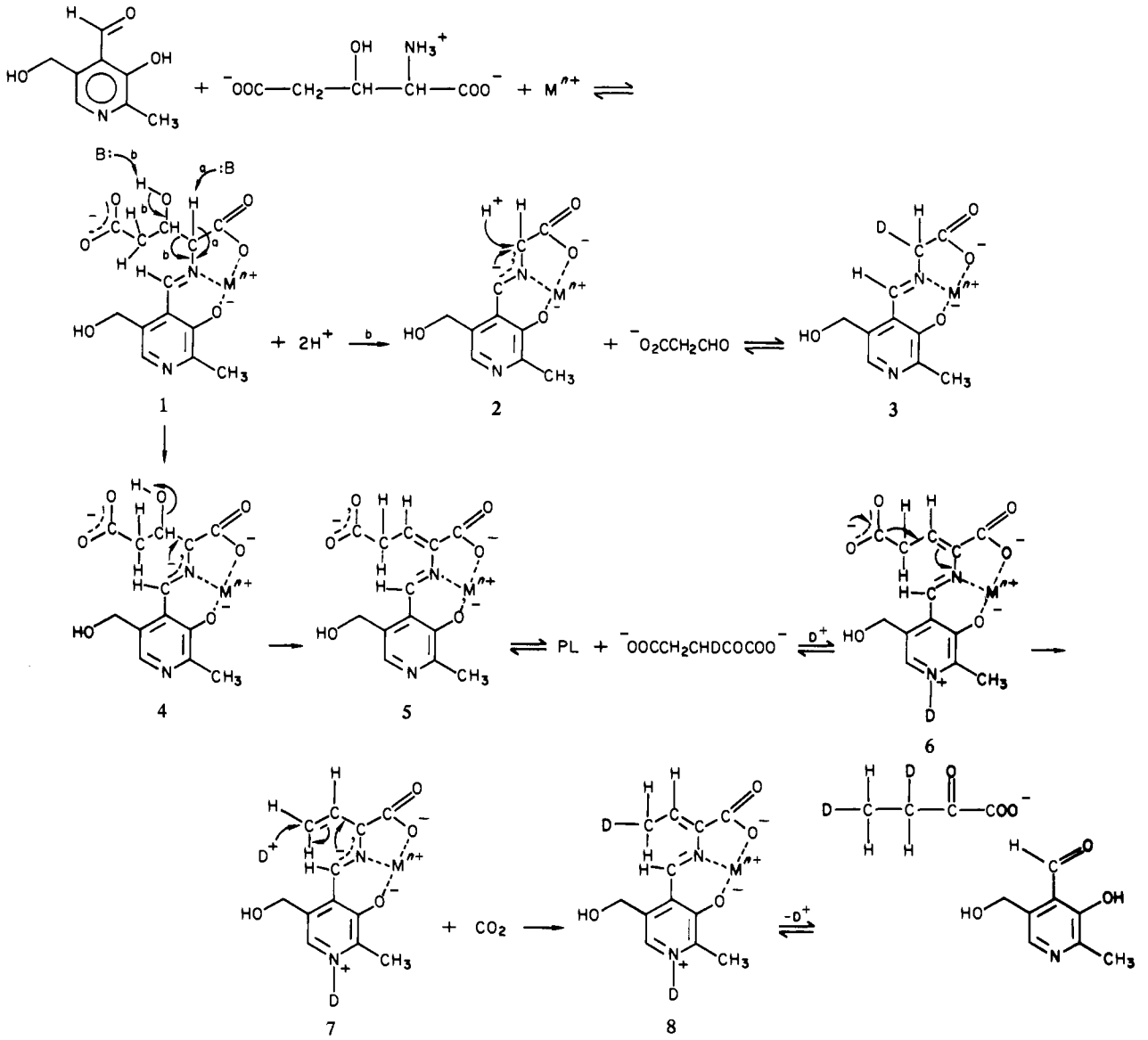
$$\text{pyridoxal}^- + \text{amino acid}^- + j\text{H}^+ \overset{K_{f_j}}{\rightleftharpoons} \text{Schiff base}\cdot\text{H}^{j-2}$$

$$K_{f_j} = \frac{[\text{Schiff base}\cdot\text{H}^{j-2}]}{[\text{pyridoxal}^-][\text{amino acid}^-][\text{H}^+]^j}$$

$$\log K_{f_0} = 0.64 \qquad \log K_{f_1} = 10.84$$

(13) Schwarzenbach, G. "Complexometric Titration"; Interscience: New York, 1957.
(14) Glasoe, P. K.; Long, F. A. *J. Phys. Chem.* **1960**, *64*, 188.
(15) Smith, R. M.; Martell, A. E. "Critical Stability Constants"; Plenum Press: New York, 1976; Vol. 1.
(16) Gansow, O. A.; Holm, R. H. *J. Am. Chem. Soc.* **1968**, *90*, 5629; **1969**, *91*, 573.
(17) Nagano, K.; Metzler, D. E. *J. Am. Chem. Soc.* **1967**, *89*, 2891.
(18) Felty, W. L.; Leussing, D. L. *J. Inorg. Nucl. Chem.* **1974**, *36*, 617.
(19) Felty, W. L.; Ekstrom, C. G.; Leussing, D. L. *J. Am. Chem. Soc.* **1970**, *92*, 3006.
(20) Metzler, D. E.; Snell, E. E. *J. Am. Chem. Soc.* **1955**, *77*, 2431.

Scheme I. Suggested Mechanism for Pyridoxal- and Metal Ion-Catalyzed Dealdolation, β-Elimination, and γ-Decarboxylation of β-Hydroxyglutamic Acid[a]



[a] M^{n+} may be a proton (deuteron), i.e., in the monoprotonated (deuterated) Schiff base, or a metal ion.

NMR Evidence for Intermediates and Reaction Products. The proton chemical shifts of pyridoxal and β-hydroxyglutamic acid and of the Schiff base have been previously described and may be readily assigned for the considitions employed in this study.[16] As indicated in Table II, NMR spectra were measured at several values of pD from 3.88 to 10.36. At pD values less than 5 in the presence of metal ions and at pD values less than 6 in the absence of metal ions, the NMR spectra of equimolar solutions of pyridoxal and β-hydroxyglutamic acid consist of resonances attributable entirely to the two components. All resonances shift to higher field with increasing basicity in accordance with the increasing negative charge of the organic compounds present. As the pD is increased above 6, the Shiff base resonances became apparent. Figure 1 illustrates the 100-MHz NMR spectra of 0.10 M pyridoxal and 0.10 M β-hydroxyglutamic acid at pD 8.82 showing the changes observed during the course of dissociation, Schiff base formation, and subsequent pyridoxal-catalyzed reactions. The disappearance of resonances at 663, 775, and 877 Hz corresponds to the disappearance of free pyridoxal and Schiff base, and the appearance of resonances at 657, 778 and 820 Hz corresponds to the formation of intermediates **2** and **4** illustrated in Scheme I. Figure 2 shows resonances near 150 Hz at pD 5.67 which are assigned to the γ-CH_2D group. The appearance of resonances at 150 Hz indicated that δ-decarboxylation has taken place. The reaction sequence indicated by **4**, **5** and **6** of Scheme I is suggested as the most reasonable mechanism for this reaction.

The δ-decarboxylation reaction is restricted to a narrow pD range since the γ-CH_2D resonances were observed only at pD 5.02 and 5.67. It is suggested that several factors determine whether decarboxylation occurs in these systems. For decarboxylation to be able to proceed, the carboxyl group must be deprotonated, a necessary condition, and the requirement is met when the solution pD is about or above the pK_a of the δ-carboxyl group (~4.1). The second requirement is that the pD must be high enough (pD ~5 or above) for considerable Schiff base to form. Also, there must be a pathway for the electron shift away from the carboxylate group, a requirement that is fulfilled only after the β-elimination reaction has taken place to produce an α,β-unsaturated intermediate (**5**, Scheme I). The β-elimination reaction would be expected to be quite slow, since the hydroxyl group is a poor leaving group. While the essential preliminary step in β-elimination, α-deprotonation, would be favored by protonation of both the azomethine and pyridine nitrogens, the increased positive charge in these protonated species would be expected to slow down the departure of the electronegative leaving group. At higher pD,

(21) Brooks, D. J.; Betteley, I. G.; Loxston, S. M. "Mathematics and Statistics"; Wiley: London, 1966; p 368.

however, where ring-protonation is not a factor, dealdolation becomes a competing reaction, so that the fraction of the Schiff base undergoing elimination would drop off as the pD is increased. Also, increased pD beyond the pK_a of the pyridine nitrogen would tend to slow down decarboxylation, which requires an electron shift in the direction opposite to that required for elimination. Thus the optimum pD range for sequential elimination and decarboxylation is relatively narrow and seems to be restricted to the range in which the pyridine ring of the Schiff base is present in both the protonated and deprotonated forms. In other words the electron shift required for elimination would be favored in the monoprotonated Schiff base, while the electron shift required for decarboxylation would occur in the diprotonated Schiff base. These requirements are met near the pK_a of the pyridine ring so that a facile protonation step can occur prior to decarboxylation. Thus, considering all the factors involved, the γ-decarboxylation reaction should occur at optimum rate in the pD range above the pK_a of the γ-carboxyl group and near the pK_a of the heterocyclic nitrogen, pD range 4.08–6.35.

The fact that NMR resonances were not observed for the formylacetic acid produced in the dealdolation reaction is not unexpected, since the protons on this molecule are activated by two adjacent carbonyl groups and should therefore undergo rapid proton–deuterium exchange with the solvent. Evidence for the dealdolation reaction was observed in the form of a decrease in the integrations of the γ-proton resonances in the pD range above that in which decarboxylation takes place. The concentration of dealdolation product was calculated from the difference between the sum of measured integration values and the values calculated for 100% protons present with the assumption of no dealdolation.

Reaction Kinetics. In this system the rate measurements were based on the differences in resonances, the disappearance of γ-CH_2 resonances of β-hydroxyglutamic acid and its Schiff base and the appearance of the γ-CH_2 resonances after β-elimination.

$$\text{PAL} + \text{HOglu} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{SB} \begin{cases} \xrightarrow{k_2} \text{Form} + \text{glySB} \\ \xrightarrow{k_3} \text{ESB} \end{cases} \quad (1)$$

For the metal–Schiff base (1:1) system, eq 2 was used. For certain

$$\text{PAL} + \text{HOglu} + \text{M}^{n+} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{SBM} \begin{cases} \xrightarrow{k_2} \text{Form} + \text{glySBM} \\ \xrightarrow{k_3} \text{ESBM} \end{cases} \quad (2)$$

cases where δ-decarboxylation reaction occurred, the additional reaction (3) was also considered. The terms employed in the

$$\text{ESB} \xrightarrow{k_4} \text{ABASB} \quad (3)$$

above equations, and their counterparts in Scheme I, are defined as follows: PAL = pyridoxal; HOglu = hydroxyglutamic acid; SB or SBM = β-hydroxyglutamic acid Schiff base with or without metal ion (**1**); Form = formylacetic acid; glySB = glycine Schiff base (**3**); glySBM = glycine Schiff base metal chelate (**3**); ESB or ESBM = β-eliminated hydroxyglutamic acid Schiff base with or without metal ion (**5**); ABASB = Schiff base of α-amino-2-butenoic acid (**7**), which produces α-ketobutyric acid (deuterated) on hydrolysis.

The rate equation 4 is obtained for the metal-free system in the absence of an appreciable amount of Schiff base.

$$-\frac{\text{d[HOglu]}}{\text{d}t} = \left(\frac{k_{-1}k_1}{k_2 + k_3 + k_{-1}} - k_1\right)[\text{PAL}][\text{HOglu}] = k'_{\text{obsd}}[\text{PAL}][\text{HOglu}] \quad (4)$$

Similarly, for the metal–Schiff base (1:1) systems

$$-\frac{\text{d[HOglu]}}{\text{d}t} = \left(\frac{k_{-1}k_1}{k_2 + k_3 + k_{-1}} - k_1\right)[\text{PAL}][\text{HOglu}][\text{M}^{n+}] = k^{\text{M}}{}_{\text{obsd}}[\text{PAL}][\text{HOglu}][\text{M}^{n+}] \quad (5)$$

For certain cases, where eq 3 is applicable

$$-\frac{\text{d[HOglu]}}{\text{d}t} = \left(\frac{k_{-1}k_1}{k_2 + k_3 + k_4 + k_{-1}} - k_1\right)[\text{PAL}] \times [\text{HOglu}] = k'_{\text{obsd}}[\text{PAL}][\text{HOglu}] \quad (6)$$

When the steady-state assumption is not applicable to Schiff base formation (i.e., when the accumulation of Schiff base in the solution is appreciable), the concentration changes in the Schiff base and some of its β-elimination reaction products can be followed by NMR. Thus eq 7 and 8 may be used. The reaction

$$\text{d[Form]}/\text{d}t = k'_{\text{obsd}_2}[\text{SB}] \text{ or } k^{\text{M}}{}_{\text{obsd}_2}[\text{SBM}] \quad (7)$$

$$\text{d[ESB]}/\text{d}t = k'_{\text{obsd}_3}[\text{SB}] \text{ or } k^{\text{M}}{}_{\text{obsd}_3}[\text{SBM}] \quad (8)$$

stoichiometry gives the relationship shown in eq 9, where $[\text{HOglu}_0]$

$$[\text{HOglu}_0] = [\text{HOglu}] + [\text{SB}] + [\text{ESB}] + ([\text{Form}] \text{ or } [\text{glySB}]) \quad (9)$$

is the initial concentration of the amino acid. In metal-catalyzed systems, the concentrations of Schiff bases in (9) are replaced by the concentrations of the Schiff base metal complexes. In simple cases, when Schiff base formed rapidly to nearly 100% of the theoretical value, first-order rate constants were determined from the slope of a plot of −ln [SB] vs. time. In more complex cases, when Schiff base was not fully formed and its concentration varied during the course of the reaction, a graphical solution was utilized wherein the instantaneous rate of reaction of Schiff base (d[product]/dt) was determined from the tangent of the curve of the plot of [product] vs. time. These values of d[product]/dt were then plotted against the [SB] values, for the corresponding points in time, to give the first-order rate constants directly from the slope of the straight line. Higher order rate constants were obtained under pseudo-first-order reaction conditions by dividing the pseudo-first-order rate constants by the values of the concentration of each reactant (pyridoxal and/or metal ion) whose concentration was maintained relatively constant during the reaction under consideration.

The observed rate constants for the β-hydroxyglutamic acid–pyridoxal system are reported in Table II for metal ion-free systems. The data are given in terms of first-order rate constants above pH 6, where measurable concentrations of the Schiff base exist. At low pH the second-order dependence is based on pyridoxal and amino acid concentrations. In this range, reaction rates cannot be apportioned among the three reactions that occur simultaneously, because of the limitations of the NMR method as applied to this system. In this pH range ($\lesssim$6.0) it is possible that dealdolation occurs as a competitive reaction, at least for the higher pH values of this range. Above pH 6 δ-decarboxylation does not contribute to a significant extent, and it is possible to resolve the observed rates into rates of β-eliminaton and dealdolation.

From the above discussion of the mechanism of δ-decarboxylation, it is obvious that the contribution of that reaction as a function of pH to the overall rate of disappearance of β-hydroxyglutamic acid must be in the form of a typical bell-shaped curve. Although there is a sigmoidal relationship between magnitudes of the rates of β-elimination and decarboxylation as a function of pH resulting from the equilibrium **5** ⇌ **6**, it is aparent that the occurrence of decarboxylation would not influence the observed rate when measured by the disappearance of the substrate and/or its Schiff base, since such measurements would reflect the cumulative results of reaction sequences **1** → **2** → **3** and **1** → **4** → **5**. The increase in the observed rate below pH 6 is probably due to increased reactivity of the monoprotonated Schiff base toward both β-elimination and dealdolation. It has been shown[9] for other amino acid substrates that at pH values as low as 6 the contribution of the monoprotonated form to the observed rate is comparable to that of the diprotonated species and increases rapidly with pH.

The observed first-order rate constants for both β-elimination and dealdolation increase with pH as the pH was varied from 6

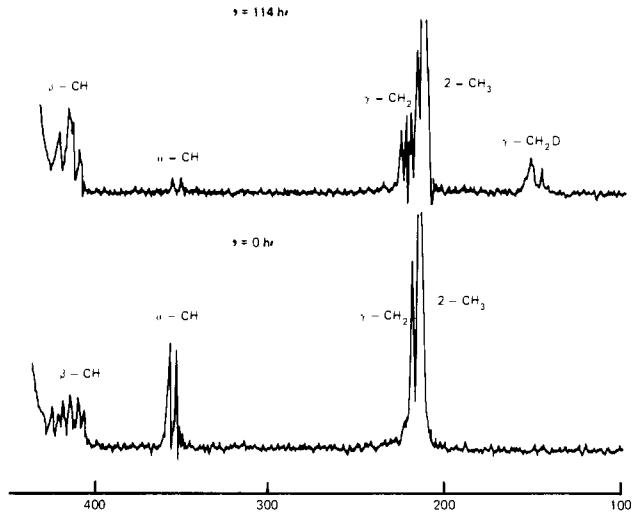


Figure 2. The 100-MHz NMR spectra of 0.10 pyridoxal and 0.10 M β-hydroxyglutamic acid at 31.5 °C in D_2O at pD 5.67 showing the appearance of the γ-CH_2D resonance near 150 Hz as an indicator of the γ-decarboxylation reaction. The resonances shown are given in hertz with respect to Me_4Si.

to 10. Since the concentration of the monoprotonated species is at its maximum value around pH 8, another type of mechanism must be involved for both dealdolation and β-elimination reactions. It has been shown[9] for β-chloro-α-aminobutyric acid that the reactivity (as well as the stability) of the nonprotonated Schiff base toward both β-elimination and dealdolation is probably much lower than that of the monoprotonated form and that the kinetic data may be interpreted in terms of base (hydroxide ion) catalysis of both reactions occurring through the monoprotonated form. Although the latter reaction route is kinetically indistinguishable from a simple reaction through the nonprotonated Schiff base species, it provides a much more satisfying interpretation of the results through a logical use of first principles. On this basis, one would expect that increasing pH would result in a levelling off of the observed rate if k_{HSB} and k'_{HSB}[OH] are comparable in magnitude.

$$\text{rate}_{obsd} = k_{HSB}[HSB] + k'_{HSB}[OH^-][HSB] \qquad (10)$$

Thus any decrease in the contribution of the first term with increasing pH would be compensated by an increase of similar magnitude of the second term. A small increase might be expected if unprotonated Schiff base were to have some activity of its own. The fact that a slight decrease is observed for both dealdolation and β-elimination reactions indicates that either k'_{HSB}[OH] is less than k_{HSB} or that increased dissociation of the Schiff base occurs at high pH because of the lower intrinsic stability of the nonprotonated Schiff base relative to the protonated species.

The increase in ratio of the rate of dealdolation to β-elimination with increasing pH is an indication that catalysis by hydroxide ion is more effective for the former reaction. The importance of base catalysis for dealdolation has been pointed out by Martell and co-workers.[6,8,22]

Metal Ion Catalysis. The influence of metal ions on the catalysis of decarboxylation of β-hydroxyglutamic acid was probed by determination of reaction products and studies of the rates of their formation on systems consisting of equimolar ratios of Ga(III), Al(III), or Zn(II) to substrate and pyridoxal, and observed rate constants are listed in Table III. Al(III) and Zn(II) were studied at pD 5.2 and 5.1, respectively, well above the pK of the terminal carboxyl group of β-hydroxyglutamic acid. Since the Ga(III) chelate of the Schiff base tended to precipitate (disproportionate to gallium hydroxide, the Schiff base, and its components) in this pH range, Ga(III) catalysis was studied under more acid conditions (pD 4.26). The extent of decarboxylation that occurred

(22) Marcello, J. A.; Martell, A. E., private communication.

Table III. Metal-Catalyzed Rate Constants for β-Hydroxyglutamic-Pyridoxal (1:1:1)

metal	pD	k^M_{obsd}, M^{-2} s^{-1}
Zn(II)	5.1	4.6×10^{-5}
Al(III)	5.2	1.4×10^{-6}
Ga(III)	4.26	3.8×10^{-6}

with the Ga(III) and Al(III) Schiff base chelates was not detectable by the NMR technique used in this investigation, so that the rates of this reaction for these systems may be considered to be at least 1 order of magnitude slower than the rates that occur in the presence of the protonated Schiff base in this range of pD.

For the Zn(II)–Schiff base system decarboxylation was found to occur at a rate comparable to the metal-free system; however the ratio of the product of dealdolation to that of β-elimination was found to be much lower than in the protonated Schiff base. These results show (1) the importance of favoring the β-elimination reaction as a condition for γ-decarboxylation and (2) that increasing the charge of the metal ion greatly lowers the tendency of Schiff base to undergo β-elimination. These effects are treated in detail elsewhere.[9] The greatly reduced tendency toward decarboxylation in the 1:1:1 metal–Schiff base chelate systems was contrary to expectations, and the possibility that the reaction was hindered by direct metal ion interaction (i.e., coordination of the δ-carboxylate group by the metal ion) will be the subject of further investigation.

Mechanistic Considerations. The mechanisms proposed for the interplay of dealdolation, β-elimination, and γ-decarboxylation are summarized in a general way by the reaction sequences in Scheme I. The factors influencing decarboxylation (**5** → **6** → **7** → **8**) in the metal-free systems are described above. Whether or not pyridine ring protonation is a contributing factor in the Schiff base metal chelates is still in question, but the pH employed is low enough to allow such interactions to take place. It is considered reasonable to assume that **6** is an intermediate for the metal ions investigated since the metal chelates would be much less protonated than the metal-free systems, in accordance with the observed reduction of the rates of decarboxylation in the metal chelates. This interpretation is also in accord with the fact that the reduction in the rate of decarboxylation by the Zn(II) ion is less than that observed for the Al(III) and Ga(III) chelates.

The interplay of the factors that determine the relative rates of dealdolation and β-elimination is also of interest. For a more complete understanding of the pH profile in the absence of metal ions to be obtained, these reaction systems should be resolved into three components: reaction rates of the diprotonated, monoprotonated, and completely deprotonated Schiff bases. Since details of these systems involving other amino acids as substrates are described elsewhere,[9,22] the factors discussed here are restricted to the observations that are unique to β-hydroglutamic acid Schiff bases and their metal chelates. The δ-decarboxylation reaction can be observed only over a limited range of conditions because it is dependent on two mutually exclusive requirements: increased protonation of the Schiff base and decreased protonation (monoprotonation in metal-free systems and nonprotonation in the metal chelates). Increased protonation resulting in positive charges on both the pyridine and azomethine nitrogens seems to be required to achieve facile decarboxylation in conjugated systems such as **6**. On the other hand the production of **6** and **5** through β-elimination is inhibited by pyridine ring protonation in systems where the azomethine nitrogen is either protonated or coordinated to a metal ion. This requirement is discussed in detail elsewhere.[9] The basis of this requirement is that increased positive charge in the Schiff base retards removal of the negative hydroxide-leaving group. Thus the formation of **5** and **6** in Scheme I as a precursor for decarboxylation requires the release of negative charge toward the leaving group, whereas the initial step **1** → **4** requires strong electron-attractive tendencies at the azomethine (and pyridyl) nitrogens. These conditions also favor the competing dealdolation reaction (**1** → **3**). The interplay of factors favoring dealdolation vs. elimination has been explored for amino acids with aromatic

substituents but are not yet predictable for aliphatic amino acids, except that high pH seems to favor dealdolation through stronger base catalysis. The situation is complicated by the electronic requirements of the leaving group. In any case high alkalinity precludes γ-decarboxylation, which is restricted to a relatively low pH range, in both the presence and absence of metal ions.

Acknowledgment. This work was supported by a grant, No. AM-11694, from the National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health.

Communications to the Editor

A Stereocontrolled Synthesis of Antineoplastic Podophyllum Lignans

D. Rajapaksa

Department of Chemistry, University of Waterloo Waterloo, Ontario N2L 3G1, Canada

R. Rodrigo*

Department of Chemistry, Wilfrid Laurier University Waterloo, Ontario N2L 3C5, Canada

Received April 27, 1981

Podophyllotoxin (**1**) with its four contiguous chiral centers, its rigid and strained trans B/C ring fusion, and its axially locked C-1 aryl substituent, has long been a challenging target for stereocontrolled synthesis. Kinetic reprotonation of the C-2 enolate of 4-*O*-(tetrahydropyranyl)picropodophyllin (**3**), accomplished with 38% C-2 epimerization and 51% recovery of picropodophyllin (**2**), was reported 15 years ago by Gensler and Gatsonis[1] as the culminating step of their synthesis of **1**. Subsequent refinements[2] of structural aspects of the synthetic problem have led to picropodophyllin again, but no method has yet been devised for avoiding the formidable thermodynamic hurdle of the Gensler epimerization. Consequently, no practical synthesis of **1**, **4**, or **8** yet exists. The renowned antineoplastic activity[3] of **1** and **4** and the recent clinical application[4] of two glycosides VM-26 (**5**) and VP-16-213 (**6**) in the treatment of lung and bladder cancer are other urgent reasons for solving the stereochemical problem. In a partial solution, illustrated with a recent synthesis[5] of (±)-deoxypodophyllotoxin (**7**), we disclosed strategies for stereocontrol at three chiral centers (C_1–C_3). We now present a comprehensive solution with syntheses of (±)-epipodophyllotoxin (**4**), (±)-neopodophyllotoxin (**8**), and (±)-podophyllotoxin (**1**). Moreover, since clinical agents **5** and **6** have been previously prepared[6] from natural podophyllotoxin, our current endeavors also constitute a formal synthesis of these materials.

The bicyclo precursor **9**[5] hydrogenolyzed with freshly prepared W-2 Raney nickel[7] gave a 77% yield of the tetralin **10** which was converted to the acetonide **11** by standard methods.[8,9] This reaction is not a mere protection of the diol system; it also transforms a tetralin (**10**) into what is essentially a benzo-*cis*-decalin (**11**) with stereochemically fruitful consequences. Basic hydrolysis of the methyl ester moiety of **11** now takes place *without* inversion[10] at C-2 to provide the acid **12**. We advance a thermodynamic argument for this remarkable resistance of the C-2 ester to epimerization.[11] Of the two chair conformers possible for the flexible acetonide, **11a** is severely destabilized by interactions involving both the C-1 aryl substitutent and the axial methyl group of the acetonide; epimerization of its axial ester moiety does little to alleviate such sources of strain. The overwhelming preponderance of conformer **11**, with its equatorially disposed C-2 ester group, is evident in the large diaxial coupling ($J_{2,3}$) and the "normal" chemical shifts of the acetonide methyl groups. Thus the simple expedient of ketalization is employed here to alter the thermodynamic properties of the system and thereby maintain[12] stereochemical integrity at a remote site (C-2).

Removal of the acetonide with very dilute acid in aqueous dioxane at room temperature was interesting. After 24 h epipodophyllic acid (**13**) can be crystallized.[13] Exposure of **12** to the same conditions for 48 h. produces (±)-neopodophyllotoxin (**8**) in 95% yield.[14] Since the latter has been previously converted[14] to podophyllotoxin (**1**) (two steps, 63% overall[16]), this concludes a synthesis of **1** with the final element of stereocontrol (at C-4). Lactonization of **13** with dicyclohexylcarbodiimide (DCC) proceeded uneventfully to yield[15] epipodophyllotoxin (**4**).

(1) Gensler, W. J.; Gatsonis, C. D. *J. Org. Chem.* **1966**, *31*, 4004.

(2) Kende, A. S.; Liebeskind, L. S.; Mills, J. E.; Rutledge, P. S.; Curran, D. P. *J. Am. Chem. Soc.* **1977**, *99* 6082. Murphy, W. S.; Wattanasin, S. *J. Chem. Soc., Chem. Commun.* **1980**, 262.

(3) For a recent review see: Jardine, I. *Med. Chem.* **1980**, *16*, 319.

(4) Radice, P. A.; Bunn, P. A.; Ihde, D. C.; *Cancer Treat. Rep.* **1979**, *63* 1231.

(5) Rodrigo, R. *J. Org. Chem.* **1980**, *45*, 4538.

(6) Keller-Juslen, C.; Kuhn, M.; von Wartburg; A., Stähelin, H. *J. Med. Chem.* **1971**, *14*, 936. Kuhn, M.; von Wartburg, A. *Helv. Chim. Acta* **1969**, *52*, 948. Kuhn, M.; Keller-Juslen, C.; von Wartburg, A. *Ibid.* **1969**, *52*, 944. These conversions were carried out with natural podophyllotoxin. Our product is racemic, of course, but we are attempting resolution at some suitable stage in the synthesis.

(7) Mozingo, R. "Organic Syntheses, Collect. Vol. 111"; Wiley: New York, 1955; 181. We have observed that yields decrease, and some inversion at C-1 results if aged samples of the catalyst are used. Diol **10** has been fully characterized previously. See ref 5 for data.

(8) ^{1}H NMR spectra were run at 80 or 400 MHz in the FT mode and are reported for the C-3a dideuterio derivatives. Coupling constants for H-1, H-2 and/or H-3, and H-4 are obtained directly from the spectra and used to monitor the stereochemistry of reactants and products. Assignments were confirmed by decoupling. The entire synthesis was repeated with the protonated analogues.

(9) With 2,2-dimethoxypropane and *p*-toluenesulfonic acid [yield 81%; m.p. 175 °C; δ ($CDCl_3$) 4.95 (d, H-4, $J_{3,4}$ = 3.71 Hz), 4.46 (d, H-1, $J_{1,2}$ = 6.05 Hz) 2.31 (q, H-3, $J_{2,3}$ = 12.2 Hz) 1.6 and 1.3 (s, 3 H each, CMe_2). H-2 was obscured by OMe at 3.5–3.8].

(10) Dilute sodium hydroxide in aqeueous dioxane at reflux for 6 h. [**12**, 82% yield; m.p. 190 °C; δ ($CDCl_3$) 4.95 (d, H-4, $J_{3,4}$ = 3.90 Hz), 4.49 (d, H-1, $J_{1,2}$ = 5.86 Hz), 2.27 (q, H-3, $J_{2,3}$ = 12.2 Hz). Deuterium incorporation at C-2 is observed under the same conditions (NaOD, D_2O).

(11) Under identical conditions the unprotected tetralin **10** was hydrolzed with complete inversion of C-2 to yield epipicropodophyllic acid (C-2 epimer of **13** [$\nu C{=}O^{KBr}$ 1700 cm^{-1}; δ (methanol-d_4) 4.90 (d, H-4, $J_{3,4}$ = 4.4. Hz), 4.43 (d, H-1, $J_{1,2}$ = 6.25 Hz), 3.12 (q, H-2, $J_{2,3}$ = 3.51 Hz), 2.49 br t, H-3)].

(12) The possibility that this method can be used not merely to maintain but to invert an unfavorable configuration at C-2 (e. g., in the C-2 epimer of **11**) has not escaped us. We are endeavoring to prepare such a compound.

(13) [**13**, 45% yield; m.p. 186 °C; δ (methanol-d_4) 4.93 (d, H-4, $J_{3,4}$ = 3,52 Hz), 4.46 (d, H-1, $J_{1,2}$ = 6.2 Hz), 2.33 (q, H-3, $J_{2,3}$ = 12.5 Hz) H-2 is obscured by residual methanol; $\nu_{C=O}^{KBr}$ 1690 cm^{-1}).

(14) Renz, J.; Kuhn, M.; von Wartburg, A *Liebigs Ann. Chem.* **1965**, *681*, 207. The infrared and ^{1}H NMR spectra of 8 were identical with spectra reproduced in this paper. Monitoring (TLC) of the reaction indicates that **13** is the initial product of hydrolysis. It presumably equilibrates to the C-4 epimer podophyllic acid which is irreversibly lactonized to **8**. A slow buildup of **8** is evident on TLC. No podophyllic acid was detected in admixture with **13** or in the recovered starting material.

0002-7863/81/1503-6208$01.25/0