24-9; o-$C_6H_4(CH{=}CHCH_2OPh)_2$, 104292-61-7; Bu_3SnOPh, 3587-18-6; PPh_3, 603-35-0; $PhCH_2CH_2CONHMe$, 940-43-2; $PhCH_2CH_2CN$, 645-59-0; $PhCH_2CH_2CO_2Me$, 103-25-3; $H_3CCHDCH_2CO_2Et$, 104292-55-9; diphenylsilane, 775-12-2; zinc chloride, 7646-85-7; triethylsilane, 617-86-7; 1-phenyl-3-[(triethylsilyl)oxy]butene, 82798-48-9; phenylsilane, 694-53-1.

Supplementary Material Available: Figure of observed and simulated NMR spectra of **22** and **22a** (1 page). Ordering information is given on any current masthead page.

Systematic pH Study on the Acid- and Base-Catalyzed Racemization of Free Amino Acids To Determine the Six Constants, One for Each of the Three Ionic Species

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Abstract: Computer analysis of pH profiles for racemization of four amino acids at 142 °C led to the determination of the six absolute rate constants, one for each ionic species of amino acid in aqueous solution catalyzed by hydronium and hydroxide ions. A comparison is made to show the effect of using all six constants to express the observed rate constants, as opposed to using only four in previous studies.[2] The analyses also allowed the calculation of amino acid pK_a values at elevated temperatures.

Amino acids are ubiquitous, and the L enantiomers of the amino acids have become associated with the presence of life. L-Amino acids racemize or epimerize in natue to their D isomer. When D/L measurements are carefully determined on fossils or samples of geological interest accompanied with suitable calibration, racemization and epimerization can be used as a method of dating. Samples of only a few years old and others as old as many hundreds of thousands of years have been studied. Racemization and epimerization of amino acids, peptides, and proteins occur at measurable rates in the laboratory at elevated temperatures (>100 °C) (see ref 2 and 3).

To accurately determine racemization or epimerization rates on amino acids found in shells, bones, or other geological material is difficult because many factors influence the rates of these reactions, e.g., temperature, hydrolysis, ionic strength, position of the amino acid in the peptide chain, moisture, pH, metal ions, and other environmental and structural factors. To more accurately apply racemization (or epimerization) studies to geological samples, a better understanding is needed of the fundamental chemistry of these reactions under laboratory-controlled conditions.

Absolute rate constants are pH independent. However, the observed rate of racemization (or epimerization) of an amino acid is pH dependent. The amino acid exists in three species (+, 0; +, −; and 0, −). Racemization (or epimerization) of these three species can be both acid and base catalyzed. As a consequence, there are six absolute racemization (or epimerization) rate constants involved in these reactions (k_1–k_6). As mentioned, the relative concentration of the three species is pH dependent. The observed rate constant can be expressed as a function of these rate constants, the concentration of each species, and the concentration of the hydronium and hydroxide ions, eq 1.

$$k_{obsd} = k_1\alpha_{+0}[H^+] + k_2\alpha_{+0}[OH^-] + k_3\alpha_{+-}[H^+] + k_4\alpha_{+-}[OH^-] + k_5\alpha_{0-}[H^+] + k_6\alpha_{0-}[OH^-] \quad (1)$$

Bada and Shou[2] reported calculated absolute rate constants for some amino acids at 142 °C. Their calculations involved only four absolute rate constants (eq 2).

$$k_{obsd} = k_1\alpha_{+0}[H^+] + k_2\alpha_{+0}[OH^-] + k_4\alpha_{+-}[OH^-] + k_6\alpha_{0-}[OH^-] \quad (2)$$

This prompted us to study the importance of considering all six constants when calculating values for the absolute racemization rate constants and pK_a's and predicting overall observed racemization rate constants.

Results and Discussion

The study of Bada and Shou[2] only involved analysis of isolated areas of the pH curve for racemization. They assumed that the ionic species of major concentration were the only species necessary to consider. As a result they could only calculate values for pK_2, not pK_1.

Our study allows the analysis of the entire system with all species of amino acids and catalysts being considered at every point on the pH profile. It also allows calculation of both pK's of the amino acids (see Experimental Section).

We have studied the applicability of this method by calculating the log k vs. pH curves to match the experimental curves for the racemization of Ala and Phe.[3] These figures are not reproduced here. However, the six absolute rate constants are included in Table I in order that their values can be readily compared with Bada and Shou's values and the reevaluated values we obtained by applying this method to their data. A comparison of the pK_a values is given in Table II. The two methods (Bada and this study) give similar but significantly different results for rate constants and pK values. Another method of calculating pK's of amino acids was applied at 142 °C by assuming that the classical empirical equation of Robinson and Stokes[4] would give accurate results for aqueous systems above the boiling point of water. The Robinson and Stokes equations gave reasonably similar results

(1) To be presented at the 192nd National Meeting of the American Chemical Society, Anaheim, CA, Sept. 7–12 1986. Baum, R. M.S. Thesis, Utah State University, 1985.

(2) (a) Bada, J. L.; Shou, M. Y. In *Biogeochemistry of Amino Acids*; Hare, H. E., Hoering, T. C., King, K., Jr., Eds.; Wiley: New York, 1980; p 235. (b) Shou, P. M.-Y.; Bada, J. L. *Naturwissenschaften* **1980**, *67*, S37. (c) Shou, P. M.-Y. Ph.D. Thesis, University of California, CA, Scripps Institution of Oceanography, 1979.

(3) Smith, G. G.; Sivakua, T. *J. Org. Chem.* **1983**, *48*, 627.

(4) Robinson, R. A.; Stokes, R. H. *Electrolyte Solutions*, 2nd ed.; Butterworths: London, 1965.

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Table I. Absolute Rate Constants for Racemization of Amino Acids at 142 °C (s^{-1})

	k_1	k_2	k_3	k_4	k_5	k_6
alanine						
Bada[a]	9.84×10^{-7}	1.05×10^{3}		3.55×10^{-2}		9.75×10^{-6}
this study[b]	9.9×10^{-7}	7×10^{3}	6×10^{-7}	3.6×10^{0}	1.4×10^{2}	8.0×10^{-6}
ref 3[c]	1.6×10^{-8}	1×10^{4}	1×10^{-4}	1.2×10^{-3}	5.2×10^{-3}	2.0×10^{-7}
valine						
Bada[a]	2.59×10^{-7}	3.72×10^{2}		1.32×10^{-2}		3.88×10^{-6}
this study[b]	2.5×10^{-7}	3×10^{4}	2.1×10^{-6}	1.6×10^{0}	3×10^{1}	3.3×10^{-6}
leucine						
Bada[a]	9.62×10^{-7}	7.08×10^{2}		3.09×10^{-2}		5.00×10^{-6}
this study[b]	9.5×10^{-7}	1×10^{5}	5.1×10^{-6}	2.3×10^{0}	3×10^{1}	3.9×10^{-6}
phenylalanine						
Bada[a]	8.97×10^{-7}	2.51×10^{3}		1.41×10^{-1}		1.41×10^{-5}
this study[b]	8.4×10^{-7}	3×10^{4}	6.9×10^{-5}	6.2×10^{0}	2×10^{2}	1.2×10^{-5}
ref 3[c]	8×10^{-7}	1×10^{7}	6.4×10^{-7}	4.5×10^{1}	3×10^{2}	2.6×10^{-4}

[a] Calculations by Bada and Shou[2a] using data from Bada and Shou.[2] [b] Calculations by this study using data from Bada and Shou.[2] The rate constants from this study have significant figures which represent the degree of variation during computer minimizations. [c] These values were determined by using data obtained by Smith and Sivakua, ref 3. The earlier studies were carried out at 120 °C.

Table II. pK_a Values of Amino Acids

		Bada and Shou[a]	Robinson and Stokes[b]	this study[c]	25 °C
alanine	pK_1		2.66	2.8	2.35
	pK_2	8.28	7.77	8.0	9.69
valine	pK_1		2.75	2.7	2.32
	pK_2		7.65	7.8	9.62
leucine	pK_1		2.72	2.8	2.36
	pK_2	8.40	7.65	8.0	9.60
phenylalanine	pK_1		2.59	2.5	1.83
	pK_2		7.22	7.8	9.13

[a] pK_2 values calculated by Shou[2a] on racemization data at 142 °C from Bada and Shou.[2] [b] pK_a values extrapolated from Robinson and Stokes[4] empirical formulas. [c] pK_a values calculated in this study on racemization data at 142 °C from Bada and Shou.[2]

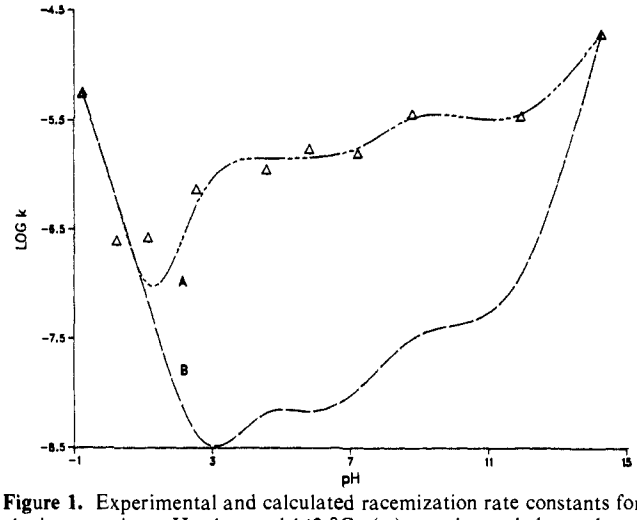

Figure 1. Experimental and calculated racemization rate constants for alanine at various pH values and 142 °C: (Δ) experimental observed rate constants by Bada and Shou;[2] (A) calculated observed rate constants using the six absolute rate constants from this study; and (B) calculated observed rate constants using the four absolute rate constants from Bada and Shou.[2]

of pK values. The greatest differences in absolute rate constants occurred in k_2 and k_4 (1–2 orders of magnitude). The differences appear to be due to Bada and Shou's neglect of k_3 and k_5. It is interesting to note that pK_1 values increased and pK_2 values decreased with increasing temperature and pressure in sealed tubes at 142 °C. Changes in pK_a with change in temperature are likely responsible, at least in part, for denaturation of peptides.

A computer analysis was performed to observe the racemization pH profiles obtained by using Bada and Shou's four rate constants and our six constants (Figures 1–4).

Figures 1–4 clearly illustrate the necessity of using all six absolute racemization rate constants for an amino acid to obtain a calculated curve close to the experimentally observed values in a pH profile study.

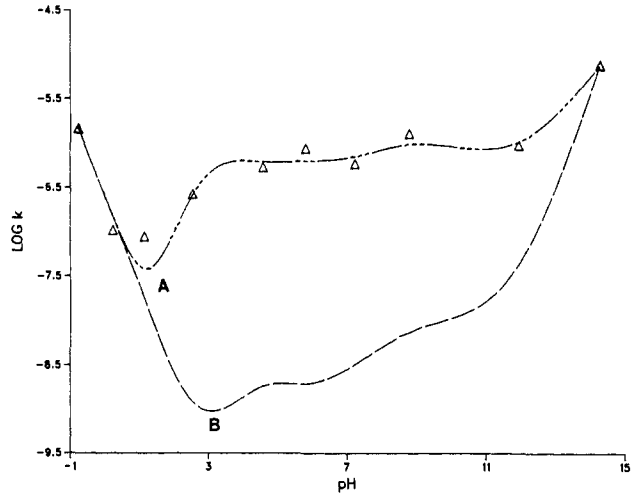

Figure 2. Experimental and calculated racemization rate constants for valine at various pH values and 142 °C: (Δ) experimental observed rate constants by Bada and Shou;[2] (A) calculated observed rate constants using the six absolute rate constants from this study; and (B) calculated observed rate constants using the four absolute rate constants from Bada and Shou.[2]

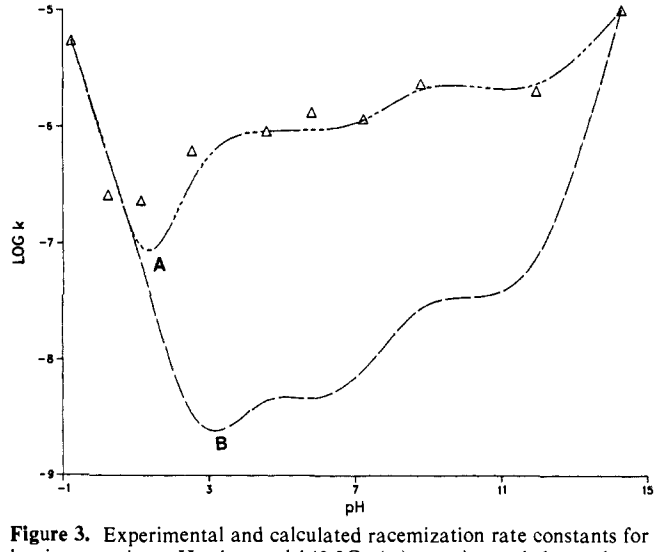

Figure 3. Experimental and calculated racemization rate constants for leucine at various pH values and 142 °C: (Δ) experimental observed rate constants by Bada and Shou;[2] (A) calculated observed rate constants using the six absolute rate constants from this study; and (B) calculated observed rate constants using the four absolute rate constants from Bada and Shou.[2]

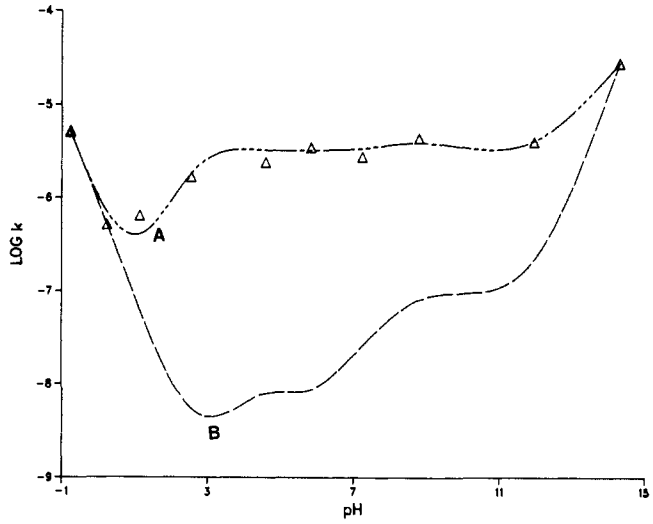

Figure 4. Experimental and calculated racemization rate constants for phenylalanine at various pH values and 142 °C: (Δ) experimental observed rate constants by Bada and Shou;[2] (A) calculated observed rate constants using the six absolute rate constants from this study; and (B) calculated observed rate constants using the four absolute rate constants from Bada and Shou.[2]

Bada and Shou's[2] data points are shown by the Δ's in all three plots. Curve B is the computer-calculated curve using only the four rate constants k_1, k_2, k_4, and k_6 calculated by Bada and Shou.[2] Curve A is the one obtained by using all six rate constants, which follows the experimental points precisely.

The pK_a values used in the calculations of both curves A and B were those obtained in this study using computer analysis. However, there was very little effect on the plots when using pK_a values reported by Bada and Shou[2] or obtained by applying Robinson and Stokes'[4] equations.

Experimental Section

Absolute Rate Constants and pK_a's. The values of different pH's and observed rate constants were entered into the computer. The concentrations of H^+, OH^-, and each ionic species of the amino acids (using the Henderson-Hasselbach equation)[5] were then calculated for each pH. After this, a minimization routine (ZXSSQ from the IMSL Library) using least-squares analysis was called to calculate the best six absolute rate constants. The pK's of the amino acids were determined by manually varying the pK values until the differences between the calculated and experimental values of observed rate constants were minimal.

Observed Rate Constants. The calculated observed rate constants were obtained by entering previously determined pK_a values and absolute rate constants in the computer program and then calculating the resultant observed constants at various values of pH. The pH profile data (Δ points in all plots) are those reported by Bada and Shou.[2] Curve B was obtained by using Bada and Shou's equation (2) with only their four rate constants. Curve A was obtained by using eq 1 with all six rate constants from this study.

Acknowledgment. We thank NASA for research Grant NSG-7038 and the Research Office at Utah State University for computer time.

Registry No. Alanine, 56-41-7; valine, 72-18-4; leucine, 61-90-5; phenylalanine, 63-91-2.

(5) Eisenberg, D.; Crothers, D. *Physical Chemistry with Applications to the Life Sciences*; Benjamin/Cumming: Menlo Park, CA, 1979; p 580.
(6) This is a companion paper to the following paper in this series.

Neighboring Residue Effects: Evidence for Intramolecular Assistance to Racemization or Epimerization of Dipeptide Residues[1]

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Abstract: Dipeptides, their methyl esters, diketopiperazines (DKP), and N-substituted derivatives were racemized at high temperatures (approximately 120 °C) in aqueous phosphate buffered solutions at pH values close to the pH of maximum racemization (approximately 8). The racemization of the dipeptides Ala-Gly and Gly-Ala followed reversible first-order kinetics. The initial rate of racemization of DKP was very fast but soon slowed down, supposedly due to hydrolysis. The resulting rate was similar to that of the dipeptides. Esters of dipeptides followed racemization patterns similar to DKP. The racemization rate constants of the dipeptides studied were shown to be independent of the concentration of the dipeptide and the concentration of buffer. A carboxy-terminal proline residue greatly increased the rate of racemization (epimerization) of the amino-terminal residue. Increasing the basicity of the N-terminal amino acid residue increased the rate of racemization (or epimerization) of the C-terminal residue unless the C-terminal residue was sterically hindered as the Ile and Val. Decreasing the basicity of the N-terminal amino acid residue decreased racemization or epimerization for nonhindered C-terminal amino acids. These results support the influence of neighboring groups in the racemization or epimerization of dipeptides. DKP formation is a competing reaction allowing racemization or epimerization in dipeptides. Dipeptide racemization or epimerization is proposed to be the result of a combination of intramolecular base assistance and DKP formation.

Dipeptides racemize or epimerize faster than free or protein-bound amino acids. Some possible explanations are inductive effects, intramolecular assistance, and diketopiperazine (DKP) formation. Each of these factors may have some effect on the racemization (epimerization) of dipeptides. The objective of this research was to study the extent of influence of each factor. With dipeptides, some amino-terminal (N-terminal) amino acids racemize or epimerize faster than carboxy-terminal (C-terminal) amino acids.[2] In others, the reverse is true. Some C-terminal amino acids racemize or epimerize faster than N-terminal ones.[2] This study helps explain why this is observed.

(1) (a) Presented in part at the Fifth IUPAC Conference on Physical Organic Chemistry, Santa Cruz, CA, Aug. 1980; No. B., p 18. (b) Additional data presented at the Utah Academy of Science Arts and Letters, Salt Lake City, UT, April 1984.

(2) (a) Smith, G. G.; de Sol, B. S. *Science (Washington, D.C.)* **1980**, *207*, 765. (b) Kriausakul, N.; Mitterer, R. M. *Science (Washington, D.C.)* **1978**, *201*, 1011. Kriausakul, N.; Mitterer, R. M. *Geochim. Cosmochim. Acta* **1980**, *44*, 753. (c) Steinberg, S.; Bada, J. L. *Science (Washington, D.C.)* **1981**, *213*, 544. (d) Mitterer, R. M.; Kriausakul, N. *Org. Geochem.* **1984**, *7*, 91.

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