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Mechanism of the Racemization of Amino Acids. Kinetics of Racemization of Arylglycines[1]

Grant Gill Smith* and Thipamon Sivakua

Department of Chemistry and Biochemistry 03, Utah State University, Logan, Utah 84322

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In a study of the rates of racemization of substituted arylglycines [$ArCH(NH_2)COOH$] at pH 10, the Hammett σ value was found to be surprisingly low, 1.15, suggesting a concerted reaction or charge stabilization in a manner other than by the substituent. The rate of methine hydrogen exchange was, however, the same as the rate of racemization, which argues against a concerted reaction mechanism. A pH profile study demonstrated that the most reactive species was the zwitterion [$^+NH_3CH(C_6H_5)CO_2^-$] in basic media. The racemization reaction showed general-base catalysis when the buffer concentration was changed at constant ionic strength. Within the aryl series, the entropy of activation was more significant than the enthalpy of activation. The ΔS^* ranged from −24.5 to +29.0 eu, while ΔH^* values ranged from 19.9 to 20.4 kcal. Racemization of arylglycines followed reversible first-order kinetics similar to that found for aliphatic amino acids in solution. The extent of racemization was studied as a function of pH. The details of the mechanism of this reaction are presented in light of these data.

Amino acid racemization studies are of interest to diverse specialists. Analytical chemists find it a challenge to measure the rate of racemization using small quantities while synthetic peptide chemists take precautions to avoid the process. On the other hand, geochemists relate racemization to the age of fossils, while evolutionists study racemization in their search for the origin of life. This report concerns the physical organic chemistry of the mechanism of racemization.

The research attempted to answer five questions: (1) Does the racemization of arylglycines follow reversible, psuedo-first-order kinetics? (2) What are the sign and magnitude of ρ in a Hammett plot of the racemization of *C*-arylglycines, and how do they relate to the reactive intermediate? (3) Do racemization and tritium exchange take place at the same rate? (4) Is racemization primarily entropy or enthalpy controlled? (5) What is the most reactive species in the racemization and exchange of aryl glycines? The results include new insight into the mechanism of amino acid racemization. Phenylglycine and its derivatives ($ArCHNH_2COOH$) afforded an ideal structure to use in studying substituent effects and the transition state in racemization.

Hare and Abelson[2] were among the first to study racemization in fossils, and since then several reviews on the application of racemization to geochronology and geothermometry have appeared.[3-8] It is reported that environmental factors, e.g., temperature,[9] pH,[10-12] metal ions,[13] buffer,[11] and moisture content,[4,14-17] as well as structural changes,[18] affect the rate of racemization.

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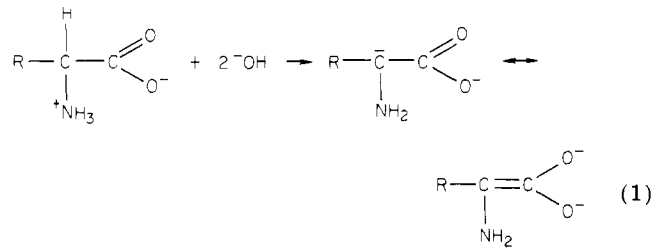
Neuberger[19] proposed the mechanism shown in eq 1 for

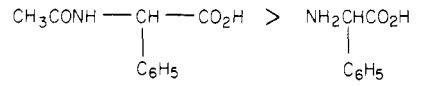


(1)

racemization in 1948. This represents a modification of the mechanism described by Dakin[20] in 1910 in a paper on hydantoins. Dakin suggested that, for base-catalyzed racemization, the initial rate-limiting step involves removal of the proton attached to the α-carbon, leaving behind a planar carbanion. Because the negative charge already on the carboxylate group would oppose dissociation of the methine proton, Neuberger hypothesized that a "very high concentration of base" would be required to bring about racemization of free amino acids. He also suggested that "any substitution on the carboxylate group, which abolishes charge, would facilitate ionization of the hydrogen and consequently racemization". Likewise, he stated that introduction of an electronegative substituent on the α-carbon atom or on the amino group would promote racemization. These predictions have been reasonably well verified although high concentrations of base are not required at high temperatures. Even though it was not clear what the reactive species is, his mechanism implies that two protons are lost from the zwitterion, one from the NH_3^+ and one from the methine carbon.

Several investigators have shown that the rate of racemization is a function of the electronegativity of the R group in the amino acid structure, $RCH(NH_3^+)COO^-$. Matsuo et al. (1967) showed, in a semiquantitative fashion, that phenylglycine (R = Ph) reacts faster than alanine (R = Me).[21] The effect of changes in the R group on racemization have been studied more recently by several research groups and reviewed by Smith and Evans.[18] Substitution on the carboxylate removes the negative charge from oxygen and enhances racemization. This may be accomplished either by protonation (lowering the pH) or by transformation into a carboxylic acid derivative. The work of Matsuo et al.[21] revealed that an ester group had a small accelerating effect while substitution of a carboxamide group for the carboxylate group greatly enhanced the rate. Carboxylic acid derivatives are all characterized by inductive and resonance effects which reduce electron density at the α carbanion without exerting an appreciable negative field effect. An α-NH_2 group reduced the rate of racemization.[21]

A protonated amine, NH_3^+, withdraws electrons inductively and stabilizes the incipient carbanion.[19] Where a NH_3^+ group is present, the electrostatic field effect is favorable, as unlike charges are in close proximity. Thus, a protonated amine will favor racemization. At pH values above the isoelectric point, however, the amount of protonated amino acid is reduced as the base concentration is increased. This complicates a study of the pH effect, but, nevertheless, it has been studied in detail recently by Bada and Shou[12] for aliphatic amino acids and is reported also in this paper for phenylglycine and alanine. Substitution by an electronegative group on nitrogen further accelerates the rate of racemization. Matsuo et al.[21] reported that *N*-acyl derivatives of both phenylglycine and alanine showed increased rates of racemization as measured by deuterium exchange. Overall, the following se-



quence of reactivity of the α-hydrogen was reported

$$R'C(O)NHCH(R)C(O)NH_2 \gg R'C(O)NHCH(R)CO_2H$$

$$H_2NCH(R)CO_2R'' > H_2NCH(R)CO_2H$$

Matsuo et al. did not evaluate reversible first-order kinetics on arylglycines or a pH profile study. Therefore, they could not determine a linear free energy relationship for racemization nor Arrhenius parameters or the most reactive species. Our studies were designed to accomplish these goals.

The kinetics of the racemization of six *C*-arylglycines [$ArCH(NH_2)COO^-$] were studied by a tritium-incorporation method and, where possible, checked by gas chromatography (GC) using an optically active stationary phase, *N*-docosanoyl-D-valyl-*tert*-butylamide. These studies were carried out under carefully controlled conditions (pH 10, ionic strength 0.50, phosphate buffer 0.05 M, amino acid concentration 0.01 M). By examination of the two methods, the rate of exchange and the rate of racemization were directly comparable. The rate data for the arylglycines were also compared to data from the racemization of four aliphatic amino acids (Ala, Val, Ile, Leu) and phenylalanine (Phe). Although racemization data for these amino acids have been reported previously from our laboratory[11] and elsewhere,[13e] these amino acids were studied again for two reasons: first, to establish accurate values for the activation parameters as there has been some discrepancy in the values reported for them; second, to directly compare the data on the arylglycines to the data on the aliphatic amino acids under precisely identical conditions.

Because arylglycines are considerably more reactive than aliphatic amino acids, a lower temperature range could be used to determine their activation parameters. As the substituted phenylglycines were not readily soluble at pH 7.6, these acids were studied at pH 10. Phenylglycine and alanine were studied over a pH range of −1 to 13.4 at constant ionic strength. This pH profile study made possible the evaluation of the six rate constants for the three species present in the amino acid solution each catalyzed by both acid and base. Activation parameters were determined at two pH values. Bada and Shou[12a] have suggested that discrepancies in activation parameters may result from differences in pH studies. These data question this interpretation, as will be discussed. The temperatures and pH at which the activation parameters were determined were as follows: 104.0–138.9 °C at pH 7.6 and 10.0 for phenylglycine (Pg) and phenylalanine (Phe); 124.6–175.4 °C at pH 7.6 for Ala, Val, Ile, and Leu; 80.2–110.3 °C at pH 10.0 for all arylglycines.

The racemization was studied in triplicate. For the aliphatic amino acids, Phe, and phenylglycine (Pg) triplicate GC analyses were also run on each sample, making a total of nine determinations for each amino acid. However, the substituted arylglycine derivatives ($CF_3CONHCH(Ar)COO$-*i*-C_3H_7) were not sufficiently volatile to use GC as a method of analysis. In these cases the triplicate analyses were studied by tritium exchange at the methine hydrogen. Multiple lyophilization was used to reexchange the tritium on the more labile position. The

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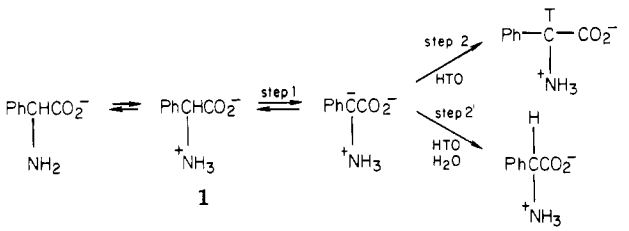
Table I. Racemization of Phenylglycine As Measured by ^3H Incorporation and Gas Chromatography[a]

temp, °C	time, h	^3H incorp method,[b] % incorp	GLC method, % racemization
80.2	15	14.5	15.2
	20	18.2	19.2
	25	23.2	23.4
90.1	3	8.4	9.2
	8	17.0	19.8
	13	28.2	30.2
	18	37.1	38.4
110.3	1	10.7	11.6
	2	19.7	20.8
	3	29.9	29.6
	4	36.4	37.8

	rate constant, 10^7k, s^{-1}	
temp, °C	^3H incorp	GLC method
80.2	16.0	16.4
90.1	35.4	36.4
110.3	160.3	162.1

[a] Average of triplicate determination. [b] The rate constant.

Scheme I



experimental error in the GC analyses was determined to be ±0.2% of the enantiomer formed. For the tritium exchange it was 0.4%. The pH profile for phenylglycine and alanine are shown in Figures 4 and 5. As expected from theory, the slope levels off at pH values near the pK_a of the COOH acid and the NH_3^+ acid (4 and 9, respectively) and continues to increase above these pH values. At 120 °C the estimated pK_a for NH_3^+ is 1–2 pK_a units lower than it is at 25 °C.

Results and Discussion

Exchange vs. Racemization. The rate constant of tritium exchange for phenylglycine (k_{ex}) was essentially identical with that of racemization (k_{rac}) at three different temperatures (Table I). For this reason any mechanism which involves a different rate of racemization than tritium incorporation (e.g., a push–pull mechanism, B···H···C···H···B) is questionable. This is discussed in more detail subsequently. From a considerably less rigorous study Matsuo et al.[21] also reported that $k_{ex} = k_{rac}$ for phenylglycine.

The removal of the methine proton from 1 (Scheme I) is most likely rate determining, with a rapid subsequent uptake of tritium (step 2) or hydrogen (step 2′) by the carbanion. The ratio of free base (anion) to zwitterion for arylglycine is approximately 100:1 at pH 10, but the rate constant for the zwitterion over the anion is roughly 100 000:1 (Table IV). The fact that racemization did not go faster than tritium incorporation indicates that there was no measurable isotope effect in step 2. An experiment to determine the kinetic isotope effect in step 1 is under investigation.

Reversible First-Order Kinetics and Hammett Study. Figure 1 demonstrates that the racemization of

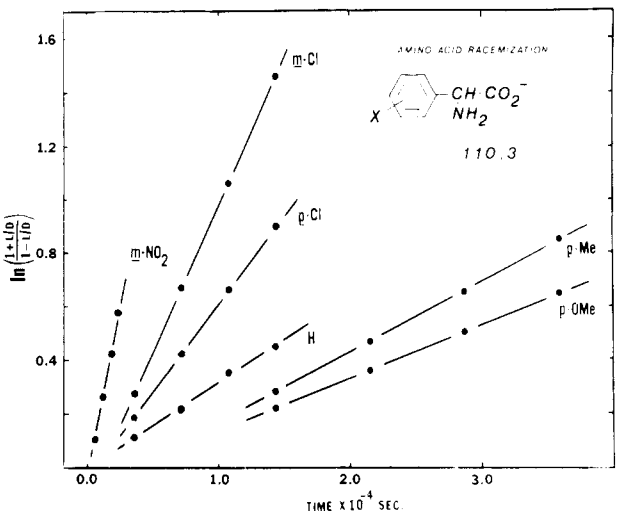


Figure 1. First-order rate plots for the racemization of arylglycines at 110.3 °C.

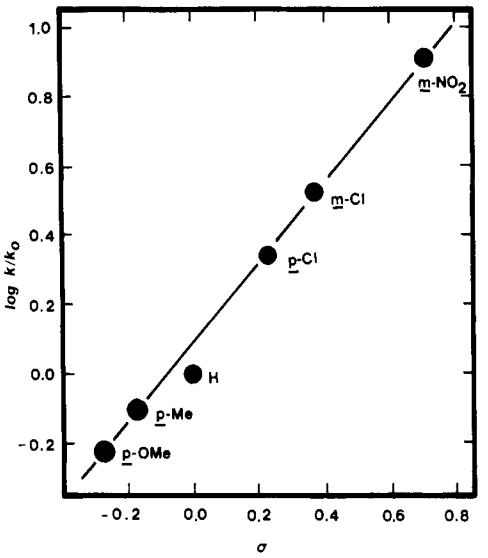


Figure 2. Hammett σ plot for the racemization of arylglycines.

arylglycines follows reversible first-order kinetics. This has been shown previously for aliphatic amino acids.[11]

Electron-withdrawing substituents (e.g., m-NO_2) cause a slight increase in the rate of racemization and electron-releasing groups cause a small decrease. The positive ρ from a Hammett plot (Figure 2) supports a charge-deficient transition state leading to a carbanion-type intermediate and directly substantiates a carbanion intermediate. Neuberger[19] and others have proposed it as an intermediate.[18] The magnitude of ρ (1.15) affords information about the nature of the carbanion. A value of 1.15 is small for a fully developed negative charge located on the atom adjacent to the ring if there is considerable change delocalization by ring substituents. It is generally observed that reactions that proceed through a charged intermediate (e.g., in the ionization of phenols, ρ = 2.11) show an appreciably higher ρ value. The ρ value for the ionization of benzoic acids at 25 °C in water is arbitrarily set at 1.00. In the racemization of arylglycines either very little charge is developed or stabilization comes from sources other than the ring substituents, e.g., the reaction medium, an ion pair, or perhaps other groups attached to the incipient carbanion (NH_3^+). We propose that the major stabilization comes from the reaction medium and the NH_3^+ as is discussed under the heading Arrhenius parameters. However, two others factors are considered.

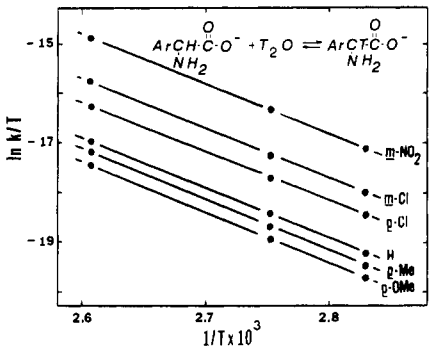


Figure 3. Arrhenius plot for the racemization of arylglycines at 80.2–110.3 °C.

In the ionization of $ArCH_2NO_2$, a case somewhat similar to $ArCHNH_2CO_2^-$, delocalization to the nitro (NO_2) group has been proposed to stabilize the incipient carbanion. In the ionization of $ArCH_2NO_2$ a ρ value of 0.83 in water at 25 °C was reported on the basis of the meta-substituted compounds.[22,23] This implies that the aliphatic nitro group may play an important role on charge stabilization. This may not be the complete explanation, however, because the dinitro compound, $ArCH(NO_2)_2$, gave a ρ value of 1.47 in water at 20 °C.[24] Two nitro groups will increase the magnitude of the charge on the carbanion but should stabilize the incipient carbanion more than one nitro group. Therefore, the dinitro compound would not require extensive stabilization by the ring substituents.

The σ_I constant for NH_2 is only 0.12[25] which is considerably less than 0.60 for the protonated (NH_3^+) state. Inductive effects of the NH_3^+ groups would stabilize the incipient carbanion. The electron-releasing resonance effect from the NH_2 group, however, would destabilize the charge as would a carboxylate (CO_2^-) group (σ_I = –0.35).[25] The zwitterion as the reactive intermediate would show a low ρ value because the NH_3^+ group would give considerable stabilization to the incipient carbanion. The temperature effect offers another explanation for the low ρ value. Jaffé points out that values generally follow an inverse linear relationship with the absolute temperature.[26] By use of Jaffé's method, a value of 1.6 was calculated for ρ at 25 °C, up from the value of 1.15 at 100 °C. Little temperature affect was observed, however, for the racemization of arylglycines between the temperatures of 80 and 110 °C. The ρ remained constant at 1.15; apparently, temperature is not a major factor for the ρ value.

Activation Parameters. Because substituents some distance from the reaction site (in the meta and para positions) are thought to alter electronic factors while leaving steric factors essentially constant, it was expected that $\Delta G^{\ddagger}$ would be influenced more by $\Delta H^{\ddagger}$ than $\Delta S^{\ddagger}$. Arrhenius plots for the racemization of arylglycines are shown in Figure 3 and computer (PDP8) calculated activation parameters are present in Table II for both the aliphatic and aromatic amino acids. Differences in $\Delta H^{\ddagger}$ (within the arylglycines) were very minor (19.0–20.4 kcal/mol^{-1}), while differences in the $\Delta S^{\ddagger}$ ranged from –24.5 to –29.0 eu. This negligible difference in $\Delta H^{\ddagger}$ as substituents are changed together with a significant change in $\Delta S^{\ddagger}$ suggest that entropy, not enthalpy, is responsible for the difference in rates with substituent changes in the ring

Table II. Activation Parameters for the Racemization of Four Aliphatic Amino Acids, Phenylalanine, and Six Arylglycines

amino acid	E_a, kcal mol^{-1} this work	Smith et al.[a] (pH 7.6)	Dungworth[b] (pH 7.0)	Bada[c] (pH 7.6)	$\Delta H^{\ddagger}$, kcal mol^{-1}	$\Delta H^{\ddagger}$,[a] kcal mol^{-1}	$\Delta S^{\ddagger}$, eu	$\Delta S^{\ddagger}$,[a] eu
alanine	28.4 ± 0.5	28.5	29.4	30.9	27.6 ± 0.6	28.6 ± 0.4	–18.7 ± 1.3	–16.3 ± 1.0
valine	28.6 ± 0.3	28.6	29.1		27.8 ± 0.3	28.0 ± 0.4	–20.4 ± 0.7	–19.8 ± 1.0
isoleucine	27.9 ± 0.6	27.9	28.9	31.4	27.1 ± 0.6	27.1 ± 0.6	–21.6 ± 1.4	–21.6 ± 1.4
leucine	28.1 ± 0.5	27.7	29.3		27.3 ± 0.5	27.5 ± 0.3	–20.2 ± 1.1	–19.8 ± 0.8
phenylalanine	24.0 ± 0.2	24.0		28.6	23.2 ± 0.2	23.0 ± 0.3	–28.0 ± 0.5	–28.4 ± 0.8
phenylglycine	20.7 ± 0.0				19.9 ± 0.0		–29.0 ± 0.1	
m-nitrophenylglycine	20.8 ± 0.1				20.1 ± 0.1		–24.5 ± 0.4	
m-chlorophenylglycine	20.7 ± 0.3				19.9 ± 0.3		–26.5 ± 0.9	
p-chlorophenylglycine	20.3 ± 0.3				19.6 ± 0.3		–28.4 ± 0.8	
p-methylphenylglycine	21.1 ± 0.1				20.4 ± 0.1		–28.3 ± 0.3	
p-methoxyphenylglycine	21.1 ± 0.1				20.3 ± 0.1		–28.9 ± 0.3	

[a] Reference 11. [b] Dungworth, G.; Vincken, N. J.; Schwartz, A. W. In "Advances in Organic Geochemistry"; Tissot, B., Bienner, F., Eds.; Editions Technip: Paris, 1973; p 689. [c] Reference 13e. [d] The analytical data for the arylglycines follow. *m*-Nitrophenylglycine: mp 171 °C. Anal. Calcd for $C_8H_8N_2O_4$: C, 48.99; H, 4.08; N, 14.29. Found: C, 48.48; H, 4.22; N, 14.05. *m*-Chlorophenylglycine: mp 223 °C. Anal. Calcd for $C_8H_8ClNO_2$: C, 51.75; H, 4.31; N, 7.55. Found: C, 52.12; H, 4.42; N, 7.35. *p*-Chlorophenylglycine: mp 228.5 °C. Anal. Calcd for $C_8H_8ClNO_2$: C, 51.75; H, 4.31; N, 7.55. Found: C, 51.25; H, 4.30; N, 7.49. *p*-Methylphenylglycine: mp 234 °C. Anal. Calcd for $C_9H_{11}NO_2$: C, 65.45; H, 6.67; N, 8.48. Found: C, 65.42; H, 6.65; N, 8.33. *p*-Methoxyphenylglycine: mp 234 °C. Anal. Calcd for $C_9H_{11}NO_3$: C, 59.67; H, 6.08; N, 7.73. Found: C, 59.77; H, 6.17; N, 7.55. Analyses were performed by M-H-W Laboratories (MI).

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Table III. Rate Constants and Relative Rates of Racemization of Four Aliphatic Amino Acids Phenylalanine and Six *C*-Arylglycines[a]

amino acid	10^7k, s^{-1} (139.0 °C)	rel rate, k_{aa}/k_{Ala}	arylglycine X	10^7k, s^{-1} (139.0 °C[b])	rel rate, k_{aa}/k_{Ala}
alanine	17.7	1.0	*p*-OMe	686.4	38.8
valine	5.9	0.3	*p*-Me	981.4	50.9
isoleucine	7.8	0.4	H	1063	60.1
leucine	11.7	0.7	*p*-Cl	2135	121
phenylalanine	34.8	2.0	*m*-Cl	3616	204
			m-NO_2	8761	495

[a] Results are the average of triplicate experiments and triplicate GC and tritium exchange analyses. [b] The values reported for the arylglycines are calculated values from data obtained at 110.3 °C by using activation parameters (see Table II).

($k_{ex(m\text{-}NO_2)}/k_{ex(p\text{-}OMe)}$ = 12). The importance of entropy differences in both rates and equilibrium are well-known. For example, the importance of entropy over enthalpy was observed in the ionization of carboxylic acids. Bolton and Hepler,[27] in their thermodynamic study of the ionization of aqueous substituted phenols at 25 °C, showed entropy to be more important than enthalpy. They stated that there is no simple, easily interpreted relationship existing between ΔH_0 values and substituent effects. The variation in entropies (ΔS_0) of ionization is the result of the considerable variations in distribution of charge density between substituents and reaction centers. In a series of substituted acetic acids, ΔH_0 differs little from one acid to another, while the observed change in ΔG_0 along the series is largely due to variations in ΔS_0. These are thought to result from solvation differences.[27]

The enthalpies of activation for the racemization of aliphatic amino acids are all considerably higher than $\Delta H^\ddagger$ for the aromatic amino acids (Table II). The opposite is true of $\Delta S^\ddagger$. These data point to the importance of both enthalpy and entropy in racemization. The enthalpy of activation ($\Delta H^\ddagger$) reflects differences in bond energies (including strain, resonance, and solvation energies) between the ground state and the transition state. The $\Delta S^\ddagger$ term relates to changes in randomness in the reacting molecules and the transition state and, of course, particularly with ionic reactions in polar solvents, in the ordering of the solvent. These results point to the importance of environmental factors to racemization.

Comparison of Racemization of Aliphatic and Aromatic Amino Acids. The rates and relative rates of six arylglycines, four aliphatic amino acids, and phenylalanine are shown in Table III. The values for the arylglycines were calculated from k values obtained at temperatures below 122 °C by using activation parameters. These data again show that both enthalpy and entropy are important to this difference (~60×) in rate. This is not surprising as the aryl group can serve as an electron sink, stabilizing the incipient carbanion in the transition state and reducing $\Delta H^\ddagger$. The $\Delta H^\ddagger$ factor is somewhat counterbalanced, however, by the less favorable entropy ($\Delta S^\ddagger$) in the aryl series, which likely was caused by more selective ordering of the medium (buffered water) with the bulkier arylglycines. If enthalpy changes were the only factor affecting the free energy of activation, the rate of racemization of the arylglycines would be about 40 000 times faster than racemization of aliphatic amino acids. As the enthalpy values are lowered by 9 kcal/mol, the entropy values become more negative by 8–10 eu. At 127 °C this contributes more than 3 kcal to $\Delta G^\ddagger$.

Effect of pH, Ionic Strength, and Buffer Concentration. As amino acids are polyfunctional and the natures of the functional groups change with a change in pH, it is not surprising that numerous factors influence the rate of their racemization, with some operating in a rather complex way. Reaction rates of charged species are expected to be influenced by changes in ionic strength. Theory predicts a much larger effect on reactions of anions than zwitterions with an increase in ionic strength. As expected, it has been shown that changes in ionic strength (μ) markedly influence the racemization of alanine at pH 10 and above but have little effect at pH 7.6[11] At pH 7.6 Ala is principally in the zwitterion form. At a pH value of 10, large quantities of sodium chloride increased the extent of racemization of phenylglycine. The percent L isomer formed from heating a phosphate-buffered solution of D-phenylglycine at 120 °C for 3 h increased from 22.5% to 25.5% where sufficient sodium chloride was added to make a 1.0 N solution. At pH 7.6 phenylglycine is essentially in the zwitterion ion form. At this pH the effect of increasing the ionic strength on racemization was modest. For example, when the ionic strength was increased from 0.25 to 0.50, the rate constants for the racemization increased only from 7.22×10^{-5} to 7.89×10^{-5}.

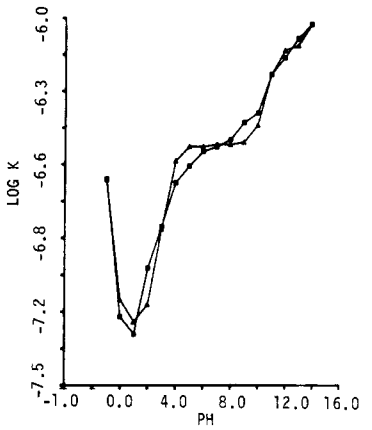


Figure 4. Rate of racemization L-alanine at 120 °C as a function of pH: ■, experimental; ▲, computer correlation.

The pH effect on the racemization rates of aliphatic amino acids has been studied by several researchers.[10-12] As theory predicts, the log k vs. pH flattens out near the pK_a of the amino acid and rises above this pH. Bada and Shou[12a] report another plateau at higher pH values. We have studied the pH–racemization profile for phenylglycine and alanine. At high pH values, the rise for phenylglycine parallels that for alanine (Figures 4 and 5) and does not plateau. Racemization, as noted above, is also susceptible to changes in ionic strength, and unless this factor is carefully considered, the pH profile is not properly observed. To minimize any observable effect by changes ionic strength, we calculated, using activity coefficients, the amount of potassium chloride necessary to bring the ionic strength to a constant value of 0.1193 at each pH value studied. pH values below 1 and above 12 were not buffered. For this reason the regions at the extremes in Figures 4 and 5 are less accurate than the regions between. Theory

(27) Bolton, P. D.; Hepler, L. G. *Q. Rev., Chem. Soc.* **1971**, *25*, 521.

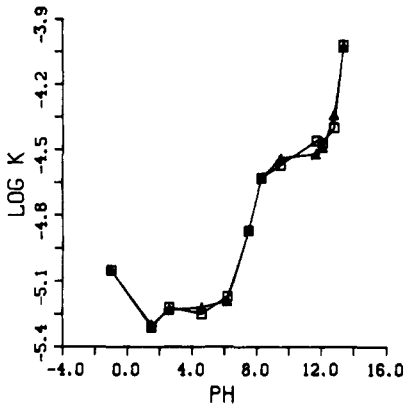


Figure 5. Rate of racemization of D-phenylglycine at 120 °C as a function of pH: □, experimental; ▲, computer correlation.

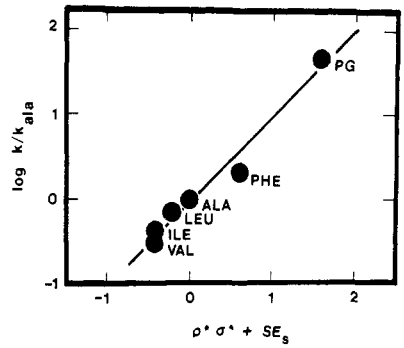


Figure 6. Taft $\rho^*\sigma^*$ + SE plot for racemization of aliphatic amino acids.

predicts that the zwitterion ($^+NH_3CHRCO_2^-$) with the $^+NH_3$ group attached to the methine carbon [$\sigma(NH_3^+)$ = 0.60][25] would racemize faster than the anion ($NH_2CHRCO_2^-$) [$\sigma(NH_2)$ = 0.12].[25] This was observed to be true from the computer-calculated values for individual rate constants. The ratio of k_4/k_6 was roughly 100 000 (Table IV). This clearly shows that the most reactive species is the zwitterion in basic media. The racemization was shown to follow general-base catalysis. By maintaining the pH constant at 8.7 and the ionic strength constant at 0.290, the D/L ratio varied from 0.151 to 0.171 to 0.245 as the phosphate buffer concentration was changed from 0.05 to 0.10 to 0.25 M, respectively (Table IV).

Steric Effects. Smith, Williams, and Wonnacott[11] suggested that steric as well as electronic effects cause a rather modest change in rate of racemization as the R substituent is changed from methyl to isopropyl, *sec*-butyl, and isobutyl. Their data were confirmed by this study, and a thorough investigation of the results, using the expanded Taft equation ($\log k/k_0 = \rho^*\sigma^* + SE_s$), assisted in evaluating the steric influence in semiquantitative terms. The parameter σ^* measures the polar substituent effect; ρ^* indicates the sensitivity of the reaction to changes in the polar effect. E_s indicates the steric effect introduced by the presence of a substituent, while S evaluates the sensitivity of the reaction to this effect. The Taft plot of $\log k/k_{Ala}$ for phenylglycine (Pg), Phe, Ala, Leu, Ile, and Val is shown in Figure 6. A linear regression analysis showed that ρ^* = 2.463 and S = 0.0960. The small value for S implies that the steric effect is small; however, a slightly better correlation was obtained by using both σ^* and E_s rather than just σ^* values (correlation coefficients of 0.970 and 0.958, respectively). Phenylalanine does not fall on the line. When this point is excluded, the correlation coefficient is 0.997. There is no obvious rationale, however, for excluding Phe. According to Bada and

Table IV. Computer-Calculated Rate Constants for the Individual Reactive Species in Amino Acid Racemization and pK_a Values for Amino Acids[a]

amino acid	25 °C		78 °C		120 °C		k(RCHN$^+$H$_3$COOH)		k(RCHN$^+$H$_3$COO$^-$)		k(RCHNH$_2$COO$^-$)	
	pK_1	pK_2	pK_1	pK_2	pK_1	pK_2	k_1	k_2	k_3	k_4	k_5	k_6 [b]
alanine	2.35	9.69			3.3 (2.52)[c]	10.7 (8.06)[c]	1.6×10^{-8}	1×10^4	1×10^{-4}	1.2×10^{-3}	5.2×10^3	2.0×10^{-7}
phenylglycine			2.85[d]	8.35[d]	0.8	7.8	8×10^7	1×10^7	6.4×10^{-7}	4.5×10^1	3×10^2	2.6×10^{-4}

[a] The rate constants and pK_a were calculated from $pK_a k_{obsd}$ values as a function of pH. [b] k_1, k_3, and k_5 are from acid-catalyzed reactions; k_2, k_4, and k_6 are from base-catalyzed catalyzed reactions. [c] These values were calculated from the pK_a values reported for alanine at 25 °C by using the Robinson and Stokes equation (Robinson, R. A.; Stokes, R. H. "Electrolytic Solutions", 2nd ed.; Butterworth: London, 1965). [d] Phenylglycine is not soluble in water at 25 °C. These pK_a values were determined by titrating Pg from pH 2 to 12 with 0.943 N sodium hydroxide solution and from pH 12 to 2 with 0.992 N hydrochloric acid.

Shou,[12a] a Taft plot should be applied to only one form of the amino acid, the singly or the doubly protonated form. Their plots, however, had the Phe point farther off the line than was found in the extended Taft plot (Figure 6). Steric factors, in addition to electronic factors, no doubt both contribute to the order Ala > Leu > Ile > Val. Alanine, with the smallest R group, has the least negative $\Delta S^{\ddagger}$ value (−18.7 eu) and racemizes the fastest. The $\Delta S^{\ddagger}$ for isoleucine (where R is *sec*-Bu) has the most negative $\Delta S^{\ddagger}$ value (−21.6 eu) of the strictly aliphatic amino acids. Perhaps its slightly faster rate of racemization, relative to valine (R = *i*-Pr), is due to the larger polarizability effect of the bulkier alkyl groups. Hine[28] has stated that a polarizable group will interact in a stabilizing manner with a nearby charge whether that charge be positive or negative. He also describes all alkyl groups as being more polarizable than hydrogen. The nature of the steric effect is not understood but is thought to include steric effects on solvation. Steric hindrance of solvation has been proposed as accounting for the decrease in the acidity of electrically neutral acids. Newman and Fukunaga[29] measured the ionization constants of highly hindered aliphatic carboxylic acids and found a clear tendency for the acid strength to decrease with increasing hindrance.

A detailed study of substituent effects in the racemization of arylglycines has shed further light on the relative importance of enthalpy and entropy effects. A change in substituents at the meta and para positions alters the electronic influence with little or no change of steric effects. As stated earlier, the data on the racemization of arylglycines are linearly correlated with a Hammett $\rho\sigma$ plot, with the value being only 1.15. This small ρ value demonstrates that the substituents have only a modest influence on stabilization.

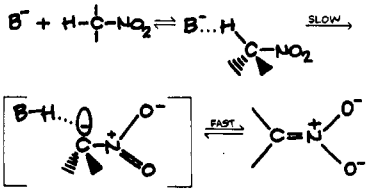
Mechanism. The details of the mechanisms of racemization are better understood in light of these data. A similarity seems to exist between the mechanism of ionization of nitroalkanes and the mechanism of racemization of amino acids. Racemization follows reversible first order kinetics.

$$\text{L-Amino Acid} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{D-Amino Acid}$$

$$\text{when} \quad k_1 = k_2$$

$$0.5 \ln \left[\frac{1 + D/L}{1 - D/L}\right] = kt + c$$

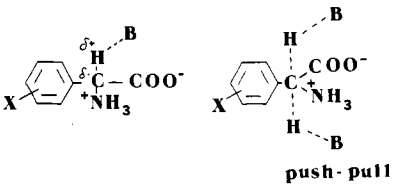
Bordwell and Boyle,[23] in a very careful study of the ionization of nitroalkanes [$ArCH(CH_3)NO_2$], using kinetic and solvent isotope studies, have proposed the following mechanism for the ionization:



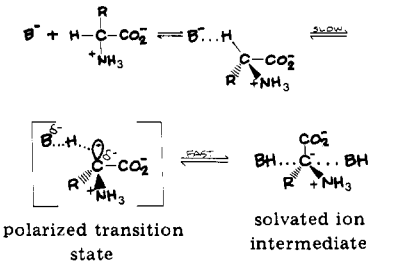
Bordwell and Boyle (1975)

They determined the ρ for this ionization with hydroxide ion in water at 25 °C to be 1.18, a value very similar to the 1.15 reported here for the racemization of arylglycines. This does not take into account the difference in temperature (25 °C vs. 80 °C). When corrected for temperature, the ρ for racemization was calculated to be 1.6 at 25 °C, but, as reported, the effect of temperature on ρ in racemization is small.

The mechanism for arylglycine racemization must concur with the following facts: (1) reversible pseudo-first-order kinetics, (2) a positive ρ in the Hammett plot, (3) a low ρ value (1.15), (4) a modest steric hindrance effect, (5) a modest pH dependence near neutral pH with the zwitterion showing the greatest reactivity, (6) ionic strength dependence at pH >10, (7) rate of exchange identical with the rate of racemization. On consideration of only the first six factors, a push–pull S_E2 mechanism involving transition



state **2** is attractive. The push–pull, S_E2 mechanism (**2**) would show reversible pseudo-first-order kinetics and a positive ρ with a modest magnitude and like S_N2 reactions would be subject to steric hindrance. It would be pH dependent and in the anion form ($PhCHNH_2CO_2^-$) would show an ionic strength effect at a pH above the isoelectric point of the amino acid. The rate of hydrogen exchange by this mechanism, however, would be only half as fast as the rate of racemization. In fact, the rate of exchange has been shown to equal the rate of racemization (Table I). This fact has also been observed by others.[21a,30] The S_E1 mechanism, patterned after the mechanism for the ionization of nitroalkanes, concurs with all the known facts, including the rate of exchange being identical with the rate of racemization (see below). A solvent molecule (BH)



polarized transition state — solvated ion intermediate

could be replaced by a cation (Na^+) if an ion pair is significant in stabilization. The mechanism for racemization of dipeptides is more complicated, involving intramolecular effects or perhaps dioxopiperazine formation, and is discussed elsewhere.[31,32]

Experimental Section

Synthesis. Arylglycines, except phenylglycine, were not commercially available. *m*-Nitrophenylglycine was prepared by direct nitration of phenylglycines as reported by Friis and Kjaer.[33] The other arylglycines were synthesized by careful alkaline hydrolysis of the appropriate 5-arylhydantoins[34] which were synthesized essentially as described by Bucherer and Leib.[35]

(28) Reference 25, p 151.
(29) Newman, M. S.; Fukunaga, T. *J. Am. Chem. Soc.* **1963**, *85*, 1176.
(30) (a) Matsuo, H.; Kawazoe, Y.; Sato, M.; Ohishi, M.; Tatsuno, T. *Chem. Pharm. Bull.* **1970**, *18*, 1788.
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(33) Friis, P.; Kjaer, A. *Acta Chem. Scand.* **1963**, *17*, 2394.
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Analytical data are given in Table II.

Temperature. Temperatures of the samples were controlled to ±0.5 °C by immersion in thermostatically controlled baths of synthetic aircraft turbine oil (Texaco 7730), encased in six inches of glass wool insulation. The baths were regulated via proportional temperature controllers (RFL Industries, Inc., Model 70-115), and the thermocouples were calibrated against an NBS calibrated platinum resistance thermometer.

Racemization Procedure. Sample Preparation. Phenylglycine solutions were carefully prepared for racemization as described by Smith, Williams, and Wonnacott.[11] The final concentration of amino acid was 0.01 M, and the phosphate buffer (NaH_2PO_4) concentration was 0.05 M. The pH was adjusted to 10.0 after application of a temperature correction of −0.0028 pH units/degree.[36] The ionic strength was 0.5. Aliquots (1.0 mL) of this solution were sealed into glass tubes and subjected to three temperatures ranging from 80.2 to 110.3 °C for the required period of time. All studies were performed in triplicate.

Sample Derivatization for GC. After heating, phenylglycine solutions were derivatized to *N*-(trifluoroacetyl)phenylglycine methyl esters as described by Smith and Wonnacott.[37] Triplicate GC analyses were performed on each sample.

Gas–Liquid Chromatography. The GC analyses were run under isothermal conditions by using a stainless steel capillary column (150 ft × 0.02 in.) coated with an optically active phase, *N*-docosanoyl-D-valine-*tert*-butylamide.[37,38] In all cases, base-line resolution was obtained for the D and L enantiomeric amino peaks. The gas–liquid chromatograph was an HP 5830 A equipped with an HP automatic injector and electronic integrater.

Tritium Incorporation Method. Tritiated water (1 Ci/g) was obtained in 0.5-mL lots from New England Nuclear Corp. (Catalog No. NET-001E). Arylglycine solutions were prepared similarly to the phenylglycine solution for GC study. The pH was adjusted to 10.0 and the ionic strength to 0.5. The tritiated water (1 Ci/g) was diluted and added to the prepared arylglycine solutions so that the final solutions had a specific activity of 5 mCi/g, a phosphate buffer concentration of 0.05 M, and an arylglycine concentration of 0.01 M. Aliquots (0.5 mL) of arylglycine solutions (5 mCi/g) were transferred to 5-mL ampules. The ampules were sealed and heated to bring about racemization.

After being heated, the arylglycine ampules were cooled and opened. The samples were lyophilized to dryness. The dry samples were redissolved in 0.5 mL of distilled water and lyophilized again. This procedure was repeated five times to remove the tritium at the labile positions (NH_2, CO_2H).

The percentages of tritium incorporation were determined from the specific activity of arylglycines (cmp/μmol). The counts per minute of each was determined by a Packard Tri-carb liquid scintillation counter (Model 3255) using Aquasol (NEN Catalog No. NEF 934) as a scintillant. The micromolar concentration was determined by measuring optical densities of each solution at 570 nm with a Bausch and Lomb spectrophotometer (Spectronic 20) after reacting an aliquot of arylglycines with ninhydrin in citrate buffer.[39,40]

pH Profile and Buffer Concentration Study. The pH profile studies on the racemization of L-alanine and D-phenylglycine were from pH −1.0 to 13.4. The ionic strength was carefully adjusted to 0.1173 between pH 2.6 and 12.1. In this range the solutions were buffered with 0.5 M phosphate buffer. The ionic strength was calculated in the usual way by using the equation $\mu = 0.5\sum Mz^2$. The activity coefficients of 0.836, 0.593, and 0.333 were used for $H_2PO_4^-$, HPO_4^{2-}, and PO_4^{3-}, respectively. A potassium chloride solution was added in the amount necessary to raise the μ to 0.1173. The amino acid concentration was 0.01 M. The pH was adjusted to the desired value by adding either 0.05 M hydrochloric acid or 0.05 M sodium hydroxide solution.

Racemizations were carried out as described above. The individual rate constants of the three ionic species present in aqueous solutions (catalyzed by acid and base) were calculated, and the pK's of the amino acids at the racemization temperature were obtained simultaneously. The observed rate constants and pH data were processed by a minimizing routine to obtain the "best fit" for eq 2. The results are shown in Table IV and in Figures 4 and 5.

$$k_{obsd} = k_1\alpha_{0,+}[H^+] + k_2\alpha_{0,+}[OH^-] + k_3\alpha_{-,+}[H^+] + k_4\alpha_{-,+}[^-OH] + k_5\alpha_{-,0}[H^+] + k_6\alpha_{-,0}[^-OH] \quad (2)$$

The buffer concentration–racemization study on L-alanine was carried out at constant pH (8.7), constant ionic strength (0.290), and constant temperature (118.8 °C) and with the phosphate buffer concentration varying from 0.05 to 0.10 to 0.25 M. The racemization was allowed to proceed for 163 h.

The pK_a values for phenylglycine (Pg) were not available from the literature. (The value which is given is for *N*-phenylglycine.) Because Pg is not soluble in water at 25 °C the study was made at 78 °C by titrating 200 mg of Pg dissolved in 60 mL of water with 0.943 N sodium hydroxide solution from pH 2 to 12. A similar plot was also carried out by using 0.992 N hydrochloric acid and going from pH 12 to 2. Both approaches gave essentially the same results (Table IV).

Acknowledgment. This research was supported by a research grant from NASA (NSG-7038) for which we express our sincere thanks and appreciation. Appreciation is also given to Eric Stroud and Rocky Baum for doing the pH profile and ionic strength studies.

Registry No. L-Alanine, 56-41-7; L-valine, 72-18-4; L-isoleucine, 73-32-5; L-leucine, 61-90-5; L-phenylalanine, 63-91-2; D-phenylglycine, 875-74-1; D-*m*-nitrophenylglycine, 4885-81-8; D-*m*-chlorophenylglycine, 25698-37-7; D-*p*-chlorophenylglycine, 43189-37-3; D-*p*-methylphenylglycine, 69501-56-0; D-*p*-methoxyphenylglycine, 24593-49-5.

(35) Bucherer, H. T.; Leib, V. A. *J. Prakt. Chem.* **1934**, *141*, 5.
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(40) Hirs, C. H. W., Ed. In "Methods in Enzymology"; Academic Press: New York, 1967; Vol. II, Sect. 1, p 329.