repeated twice). Extraction of the two most intense bands (the fastest moving band contained 1) afforded 1 (0.060 g) and pure **3-azido-1-phenylcyclohexene (14)** (0.16 g) as a liquid: IR 2100 cm^{-1} (N_3); ^1H NMR δ 7.50–7.30 (m, 5 H, aromatic protons), 6.10 [dt, 1 H, $J_{2,3}$ = 3.85, and $J_{2,6}$ = 1.77 Hz, =CH (H_2)], 4.12 [m, 1 H, CHN_3 (H_3)], 2.60–2.40 (m, 2 H, methylenic H_6), 2.05–1.90 (m, 2 H, methylenic H_4), 1.87–1.78 (m, 2 H, methylenic H_5); double-resonance experiments showed coupling between H_2 and H_3, H_2 and H_6, H_3 and H_4, H_6 and H_5, H_4 and H_5; ^{13}C NMR 142.3, 140.7, 128.2, 127.6, 125.3 (aromatic carbons), 121.2 (C_2), 56.8 (C_3), 28.3, 27.4, and 19.8 ppm (methylenic carbons). Anal. Calcd for $C_{12}H_{13}N_3$: C, 72.33; H, 6.57. Found: C, 72.42; H, 6.70.

The above described reaction was also repeated in some different experimental conditions in order to evaluate the importance of the temperature and of the temperature-increasing rate on the dehydrohalogenation process leading to the azido olefin 14 vs the elimination process leading to olefin 1. In any case the proceeding reactions were followed by GLC. The slower the temperature of the reaction mixture increases in order to reach the refluxing temperature, the higher the percentages of olefin 1 that are obtained. In a run, for example, after 3 h at 50 °C, the reaction mixture had the following composition: 1 60%, 14 10%, starting product 30%. The same mixture after 15 min at 80 °C gave the following result: 1 80%, 14 15%, starting product 5%. In a different run the iodo azide 3 was directly added to the boiling ethanolic KOH: the reaction is complete in 15 min, yielding a mixture of 1 and 14 in a ratio about 70:30.

1-Azidocyclohexane (18). A pure sample of 18 was prepared by heating at 120 °C for 15 h a solution of bromocyclohexane (0.30 g, 1.84 mmol) in DMSO (10 mL) in the presence of NaN_3 (0.61 g, 9.4 mmol). Crude 18 was distilled to give pure 18 as a liquid: bp 38 °C (15 mmHg) [lit.[20] 68.5–69 °C (21 mmHg)]; IR 2095 cm^{-1} (N_3); ^1H NMR δ 3.32 [m, 1 H, CHN_3 (H_1)]; ^{13}C NMR 59.9 (C_1), 31.7, 25.4, and 24.3 ppm (methylenic carbons). Anal. Calcd for $C_6H_{11}N_3$: C, 57.57; H, 8.85. Found: C, 57.60; H, 8.95.

Attempts To Reduce the N_3 Group of 3 to an Amino Group. The following reactions were carried out:

(a) Iodo azide 3 (0.10 g, 0.3 mmol) in anhydrous THF (3 mL) was treated with BH_3-Me_2S complex (1 mL, 10 mmol), and the resulting mixture was left stirring at room temperature. After 30 days, the unreacted iodo azide 3 was completely recovered.

(b) Iodo azide 3 (0.165 g, 0.5 mmol) in EtOH (10 mL) was hydrogenated at room pressure for 12 h at 20 °C in the presence of 10% Pd/C (0.20 g). The analysis (GLC) of the crude reaction mixture showed the presence of 1 (40%), 1-phenylcyclohexane (20%), and starting material (40%).

(c) Treatment of a solution of 3 (0.10 g, 0.3 mmol) in anhydrous ether (15 mL) with $LiAlH_4$ (0.050 g) yielded almost completely olefin 1 (^1H NMR).

Dehalogenation Attempt. In order to remove selectively the iodine atom, iodo azide 3 (0.10 g, 0.3 mmol) in anhydrous THF (5 mL) was treated with Bu_3SnH (0.2 mL). After 4 h at room temperature, the reaction mixture consisted of a 30:70 mixture of olefin 1 and starting material 3 (^1H NMR and GLC).

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Effect of the Side Chain on the Racemization of Amino Acids in Aqueous Solution[1]

Grant Gill Smith* and G. Vanita Reddy

Department of Chemistry and Biochemistry, Utah State University, Logan, Utah 84322-0300

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The rate of racemization of 13 amino acids possessing hydroxy, carboxy, alkoxy, carboalkoxy, alkyl, aryl, and thioether side chains were compared. Reaction conditions were identical for all amino acids studied. Gas chromatography was used to determine the percent of D isomer present. Hydroxy amino acids racemized most rapidly, but conversion to an ether function reduced the rate considerably. The increased racemization rate of methionine (R = $CH_2CH_2SCH_3$) over Ala (R = CH_3) has been attributed to orbital overlap from the sulfur. Asp racemized faster than Glu, α-aminoadipic acid, and pyroglutamic acid. β- and γ-monomethyl esters of aspartic and glutamic acids, respectively, racemized only slightly faster than the corresponding free acids. The slight increase in rate appears attributable to a solvent change brought on by ester hydrolysis. Under the reaction conditions, pH 8 and 140 °C, hydrolysis of the esters competed favorably with racemization at the methine carbon. The relatively lower racemization rate observed in the case of Glu compared with Asp resulted from the slow formation of pyroglutamic acid. Pyroglutamic acid racemized at a considerably slower rate than acidic amino acids. The differences in the racemization rates with changes in the R group are discussed in terms of several factors, including intramolecular reactions, direct field effects, orbital overlap, and solvation effects, as well as inductive, resonance, and steric factors.

Introduction

Amino acid racemization studies are of interest in peptide synthesis,[2] geochronology,[3] geothermometry,[3] and nutrition.[4] They provide a system in which to study structure vs reactivity relationships and afford a method

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of determining the pK_1 and pK_2 values of amino acids at elevated temperatures[5] as well as the pK_a values of the α-methine hydrogen.[6]

Many factors are known to affect the rate of racemization of amino acids and the rate of isotope exchange; among them are pH,[5,7] ionic strength,[5,7b] buffer concentration,[7a] presence and absence of water,[8] and complexation with metal ions.[9] Amino acids are known to racemize in basic[10] and acidic[11] media and at neutral pH. Temperatures well above 100 °C are required for racemization at measurable rates.

A mechanism for racemization of free amino acids in aqueous solution was proposed by Neuberger[12] in 1948 as a modification of Dakin's[13] mechanism given for hydantion formation in 1910. Neuberger represented racemization as occurring from the anion $RCH(NH_2)COO^-$ rather than the zwitterion, $RCH(NH_3)^+COO^-$.

The initial step in racemization is loss of a proton from the α-methine carbon, resulting in the formation of a resonance-stabilized planar anion (eq 1). The negative charge on the carboxylate group inhibits dissociation of the methine proton. Conversion of the amino groups to amides and of the carboxylic acid to esters or amides are recognized as having a significant effect on the rate of racemization or exchange. Matsuo et al.[14] showed that esterification of the carboxylic group or amide formation increased the rate of deuterium exchange and racemization of Ala and Phe in basic solution as shown for Ala. Amino

$$CH_3CH(NH_3^+)COO^- > CH_3CH(NHAc)CONH_2 > CH_3CH(NHAc)COOR$$

acids bound in dipeptides racemize much faster than free amino acids.[15] The reason is not fully understood, but intramolecular effects and diketopiperazine formation seem to be important.[15,16] Amino acids bound in proteins undergo inversion 10 times faster than free amino acids.[4a]

The rate of amino acid racemization is related to the electronegativity and the size of the R group.[4a,5,14c,17,18] Alkyl groups have a retarding affect on the rate, inductively and sterically. Sato et al.[14c] attempted to show that racemization rates could be correlated by Taft σ^*-constants. Val did not follow the pattern, which reflects the steric influence. Bada and Shou[20] have pointed out that using k_{obs} is not justified but rather absolute rate constants are preferred, calculated for the individual ionic species at k^{+0}, k^{0-} and k^{+-}. The absolute rate constants for racemization of amino acids should be considered for both acid and base catalysis. However, absolute rate constants are difficult to obtain, especially for acidic and basic amino acids. Smith and Sivakua[5] used a modified Taft equation that included both inductive and steric effects, resulting in a high correlation coefficient. The side groups were limited to alkyl and aryl groups, however. Friedman and Masters,[4g] in a study of the racemization of amino acids in casein with 0.1 N NaOH at 65 °C, showed a σ^*-plot for Val, Leu, Ala, Glu, Phe, Asp, Gln, and Asn with a high correlation coefficient. Asp and Glu fell considerably off the line. Bound amino acids and free amino acids do not necessarily behave similarly. When heteroatoms are present in the side chain (R group), direct field and solvation effects also appear to play a significant role. Intramolecular effects (direct field effects, solvation, orbital symmetry) and intramolecular reaction (e.g. lactam formation) where hydroxyl, thioether, and carboxylic acid groups are present are not fully appreciated or understood. The purpose of this study was to evaluate the effect of carboxyl, hydroxyl, and thioether groups on racemization of amino acids containing these functional groups and their ether and ester derivatives. The reaction conditions were identical.

The abstraction of an α-proton as the initial and rate-limiting step in racemization reaction is demonstrated by the fact that the rates of racemization and isotope exchange are identical (within experimental error) for the amino acids and their derivatives.[5,14,19] Isotope exchange experiments were carried out in acidic and basic solutions. From a deuterium isotope study the abstraction of the methine hydrogen was found to be rate-controlling.[6] Smith and Sivakua[5] studied the racemization and methine hydrogen exchange of arylglycines at pH 10 and found that the rates for racemization and tritium exchange were the same. Because the rate of reprotonation (k_{rac}) is equal to the rate of tritium exchange (k_{ex}),[5] they concluded that there was no kinetic isotope effect in the reprotonation step of the carbanion and that the rate-determining step in racemization is indeed the removal of the methine proton.

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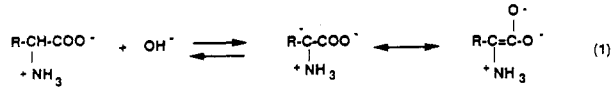
The positive ρ obtained in the Hammett plot of racemization of arylglycines supports an electron-rich transition state leading to a carbanion type intermediate. However, the magnitude of the ρ (+1.15) strongly suggests that the charge is not solely stabilized by the aryl ring. Smith and Sivakua proposed that the stabilization of the anion was primarily from the positive charge on the nitrogen in the zwitterion.[5] Neutral amino acids exist in three ionic forms (+0, +−, 0−). Smith and Sivakua[5] and Bada and Shou[20] were able to show that the absolute rate constant for base-catalyzed racemization of the +0 species was the largest. However, at near-neutral pH values, the zwitterion likely contributes most to the observed rate, as in eq 1.



(1)

Environmental factors, as well as structural changes, are critical to racemization; therefore, it was essential that environmental factors be carefully controlled during a study of the effect of structural changes. These factors have been reviewed recently and will be discussed only briefly.[4j,7b,17a,21,22]

Individual rate constants of racemization are independent of pH, but the observed rate varies with pH. This may occasionally lead to ambiguity concerning the effect of pH on racemization. In general, amino acids racemize faster in strong base than in strong acid. Little or no racemization occurs during the acid hydrolysis of peptides and proteins in 6 N HCl for 12 h at 110 °C.[23]

Bada[7a] and Bada and Shou[20] have reported pH profile studies in the range of −1 to 12 on the racemization of several neutral amino acids and Asp. They were reasonably successful in calculating the pH profile for the neutral amino acid but less successful for Asp. The mechanism for the racemization of Asp is considerably more complicated than for neutral amino acids.

The rate of racemization increases with increasing concentration of buffers.[7a,b] The effect of changes in ionic strength on the rates of racemization of both free and gelatin-bound Ala showed that changes in ionic strength had little effect at pH 7.6, but above pH 10 had an effect on both the buffered and unbuffered reactions.[7b,22] At higher pH values, the amino acid is essentially in the form of its anion [$RCH(NH_2)COO^-$]. When both reacting species have a negative charge, repulsions are more effectively shielded from one another by an increase in ionic strength.

There is some debate about the effect of surfaces, such as clays on reactions.[24] Metal complexes of both transition and nontransition metal ions catalyze racemization.[9] Nickel is the only metal that causes a retardation of racemization.[9c]

The effect of the R group has received attention and was reviewed recently by Smith and Evans[17a] and Bada.[4j] Two factors, steric and electronic, appear significant. However, we found that other factors are important when heteroatoms are present in the R group. Aryl groups stabilize the incipient carbanion by resonance, while aralkyl groups stabilize it through induction. Side groups which contain heteroatoms may influence the rate of the reaction by a direct field effect. For example, a carboxylate group may serve as an intramolecular base in removing the methine hydrogen, a hydroxyl group could stabilize the carboxylate anion of the amino acid while a sulfur-containing group could bring about carboxylate ion stabilization through orbital overlap.

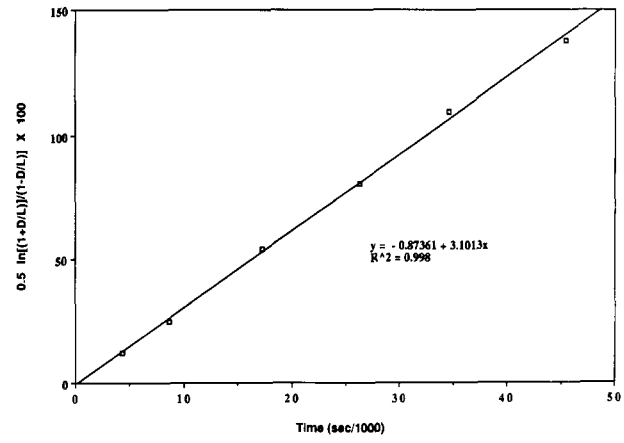


Figure 1. First-order rate plot for the racemization of methionine at 140 °C.

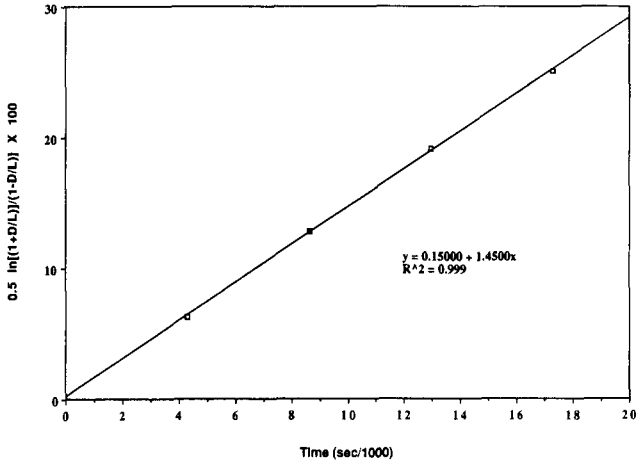


Figure 2. First-order rate plot for the racemization of γ-monomethyl glutamate at 140 °C.

The integrated rate equation for this reversible pseudo-first-order racemization is shown in eq 2. K is equal to 1 for racemization but may differ from 1 for epimerization. C is zero if the starting amino acids are free of the D isomer.

$$\ln \left[\frac{1 + [D]/[L]}{1 - [D]/[L]} \right] = (1 + K)\,kt + C \qquad (2)$$

Results and Discussion

For the amino acids and their derivatives studied here, the racemization reaction followed pseudo-first-order kinetics. A plot of 0.5 ln [(1 + D/L)(1 − D/L)] vs time was linear. Two representative plots are given in Figures 1 and 2, for methionine and γ-monomethyl glutamate, respectively. The rate constants for the racemization of the amino acids and their derivatives are shown in Table I. For ready comparison amino acids with similar structure are grouped together.

Heteroatoms definitely affect racemization rates, but the effect may be either positive or negative. Hydroxy amino

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Table I. Racemization of Amino Acids and Their Derivatives at pH 8.0 and 140 °C

amino acid	abbreviation	k (s^{-1}) × 10^6	rel reactivity
	Hydroxy Amino Acids		
serine	Ser	15.5	8.0
Ser *tert*-butyl ether	Ser *t*-Bu ether	10.0	5.1
threonine	Thr	3.98	2.0
Thr *tert*-butyl ether	Thr *t*-Bu ether	2.08	1.1
	Sulfur Amino Acid		
methionine	Met	3.08	1.6
	Neutral Amino Acids		
phenylalanine	Phe	2.35[a]	1.2
alanine	Ala	1.95[b]	1.0
	Acidic Amino Acids		
β-monomethyl aspartate	β-Me Asp	4.31	2.2
aspartic acid	Asp	4.00	2.1
γ-monomethyl glutamate	γ-Me Glu	1.46	0.74
glutamic acid	Glu	1.04	0.53
α-amino adipic acid	α-Aaa	0.63	0.32
pyroglutamic acid	Pga	0.19	0.10

[a] 3.48×10^{-6} (ref 5). [b] 1.77×10^{-6} at 139 °C (ref 5).

acids and their ether derivatives increase the rate, as does a thioether group. A second carboxylic acid functional group either increases or decreases the rate, depending on its location from the reactive site. When the carboxy group is on the β-carbon (Asp) the rate is doubled. However, when the carboxyl group is located further away the rate is retarded. For example, when the carboxyl functional group is one methylene group further away, as in Glu, the rate is decreased by half. When it is two methylene groups removed, as in α-aminoadipic acid, the rate drops to one-third the value observed for Ala. These results may be explained by the conversion of acidic amino acids into acidic lactams through intramolecular reactions. The lactams, with the positive charge removed from the nitrogen, are less reactive than the standard amino acid, Ala.

Hydroxy Amino Acids. The hydroxyl groups located on the methylene group adjacent to the methine carbon are the most effective in increasing reactivity. Ser and Thr racemized the fastest of the amino acids studied, and Ser racemized 8 times faster than Ala. Conversion of the alcohol function in Ser to its *tert*-butyl ether reduced its reactivity to 65%; the *tert*-butyl ether of Thr reacted only 52% as fast as Thr. However, all four derivatives reacted faster than Ala (Table I). Several factors contribute to the increased reactivity: First, the adjacent oxygen lowers the free energy of activation by stabilizing the anion through an inductive effect along the σ system. Second, the hydroxyl group "solvates" a water molecule or hydroxide ion needed for the base in the reaction. Third, the hydroxyl group can also stabilize the carboxylate ion by reducing its negative charge, which in turn increases the acidity of the methine hydrogen. Fourth, the hydroxyl group may help to increase the concentration of the zwitterion, which is known to be one of the most reactive species present in the equilibrium mixture.[5]

Bada et al.[18a,20] have strongly supported the importance of the inductive effect on racemization. They have attempted to show that the relative order of reactivity can be correlated with Taft's σ values. Schroeder and Bada[25] suggested that Ser reacts faster than Thr because it shows a greater electron-withdrawing effect, $\sigma_I = 0.56$ for CH_2OH in Ser, $\sigma_I = 0.46$ for $CH(CH_3)OH$ in Thr. The hydroxyl and alkoxy groups have nearly the same inductive effect,

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Table II. Eyring Parameters for the Racemization of Free Amino Acids in Aqueous Solution[a-c]

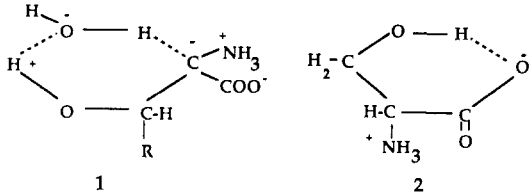
amino acid	ΔH^*, kcal/mol	ΔS^*, eu
Ser	25.8 ± 0.5	−17.4
Thr	25.0 ± 0.7 (K = 0.9)	−22.4
Ala	28.6 ± 0.4[d]	−16.3
Phe	23.0 ± 0.4[d]	−28.4
Asp	28.2 ± 0.1[d]	−15.4
Glu	30.9 ± 0.02[d]	−15.1

[a] From ref 22. [b] 0.05 M phosphate buffer, pH 7.6. [c] Ala = 27.6 ± 0.6, Phe = 27.3 ± 0.2 (ref 5). [d] Ala, Phe, Glu ranged from 28.2 to 32.4 and Asp was 20.8 kcal/mol in alkaline-treated casein (ref 4g).

and we expected that they would cause the same, or nearly the same, increase in rate, but this was not the case (Table I).

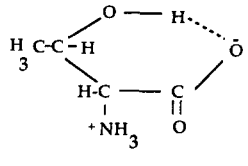
Although the inductive effect was recognized as important by Smith and de Sol,[16] they suggested that the solvating effect of the hydroxyl group was also important. They proposed that the hydroxyl group in Ser "solvates" the base required to remove the α-hydrogen. This action is reflected in a more favorable ΔS^* and a lower ΔG^*. Our data support this hypothesis. Regardless of its position in dipeptides (C or N terminal), Ser racemized rapidly,[16] suggesting that solvation of the base by the hydroxyl group contributes to increasing racemization.

Table II shows activation parameters for six of the amino acids studied. Inductive and resonance effects are reflected in ΔH^* values. The ΔH^* values for Ser and Thr are 25.8 and 25.0 kcal/mol, respectively. As the enthalpies of activation for the racemization of Ser and Thr are the same, the hydroxyl inductive effects in Ser and in Thr are similar (σ_I 0.56 vs 0.46, respectively). This suggests that the increased reactivity in racemization of Ser and Thr over Ala is attributable to factors other than just the inductive effect of the R group as proposed by Bada and Shou[20] and Schroeder and Bada.[25] The entropy of activation suggests an explanation for the difference in the reactivity of these two hydroxy amino acids. The difference in entropy values ΔS^* (−17.4 eu for Ser and −22.4 eu for Thr), suggests it may be due to steric and/or solvation effects. The steric effect of the methyl group in the side chain of Thr could hinder the attack of the base at the α-carbon. "Solvation effects" acting through a six-membered cyclic transition state are described in formula **1**. A greater solvation effect by the hydroxyl group in Ser over that found by the hydroxyl group in Thr would show a more favorable entropy of activation resulting in a faster reaction for Ser.



1

2



3

R = H, ser; R = CH_3, thr

Ser and Thr may also increase the rate of reactivity over that of Ala because their hydroxyl group could reduce the negative charge on the carboxylate ion as shown in formulas **2** and **3**. Hydrogen bonding decreases the electron

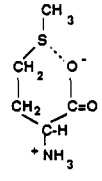
density around the carboxylate anion and spreads out the charge, allowing a greater negative charge to develop at the methine carbon. Because Thr racemizes slower than Ser, we assume that the methyl group in Thr reduces the extent of hydrogen bonding (inductively or sterically) over the hydrogen in Ser, preventing delocalization of charge to occur to the same extent. Other branched-chain amino acids (Val, Ile) also show reduced racemization rates.

Ser racemized 1.6 times faster than its *tert*-butyl ether. The σ_I effect for the alkoxy group is approximately the same as for the OH group. The major difference in reactivity could be attributed to the degree of solvation of the base and/or solvation of the carboxylate anion by the hydroxyl group. *tert*-Butyl groups are bulky, which may contribute to reducing reactivity. The methyl ether was not studied.

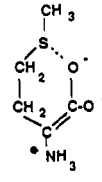
Solvation of the base and partial removal of the charge on carboxylate ion, as well as the inductive effects, are employed to explain why suitably located hydroxyl groups are effective in increasing the rate of racemization. Ethers are less effective because solvation does not take place as readily. The fact that ethers activate racemization demonstrates the importance of inductive effects.

Thioether Group. We did not obtain a direct comparison of the sulfhydryl group with the hydroxyl group at the same location [$XCH_2CH(NH_2)COOH$] because cysteine [$HSCH_2CH(NH)_2COOH$] decomposes at the reaction temperature (140 °C). Methionine [$CH_3SCH_2CH_2CH(NH)_2COOH$] (a thioether) racemizes almost as readily as Thr and more readily than Thr *tert*-butyl ether (Table I). It is unlikely that this effect can be attributed to the inductive effect of the sulfur because it is too far from the methine carbon, nor is it likely that this group contributes to the entropy of activation by solvating the base used in removing the methine hydrogen.

Kovacs et al.[26] in explaining the enhanced racemization rates in active esters of Cys and Met suggested that orbital overlap enhances the stability of the enolate, resulting in an increased rate of racemization. A similar explanation may be proposed for the increased reactivity of Met over Ala (**4**). A dispersed negative charge on the carboxylate anion again contributes to increasing reactivity.



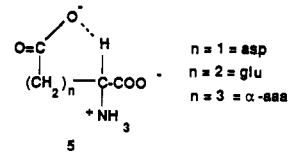
4a



4b

Neutral Amino Acids. The increased rate of racemization of Phe over Ala can be attributed to an inductive effect. However, the increase is not as great as expected. The ρ value in the racemization of arylglycine, where the aryl group is closer to the reactive site, was only 1.15.[5] As shown in Table II, Phe has the most negative $\Delta S^{\ddagger}$ value. Negative entropy factors are generally associated with ordering of the system. Because of the bulky nature of the phenyl group, the base does not attack the methine hydrogen as readily as it does in Ala. Thus with Phe there appears to be a trade-off between inductive and steric effects.

Acidic Amino Acids. Asp is known to be more reactive than the neutral amino acids, and the inductive effect has been considered sufficient to explain its reactivity.[20] Perhaps this is true when the β-carboxyl group is protonated, which occurs around pH 3, but the much faster rate for Asp in neutral solutions where the β-carboxyl group is in the anionic form is not explained by the inductive effect alone. Also the inductive effect alone does not explain why Asp in neutral solutions reacts faster than Ala while Glu and α-amino adipic acid react much slower. In 1984[1b] it was proposed that the Asp β-carboxylate can act as an intramolecular base as shown in **5**. Lairdon and



5

Lederman[4a] recently proposed the same intramolecular effect to explain why Asp racemizes much faster than expected. Intramolecular neighboring group participation of the carboxylate anion is common, particularly at a carbonyl carbon, as in the hydrolysis of esters.[27] Gaetjen and Morawetz[28] reported that hydrolysis of *p*-nitrophenyl glutarate proceeded through a six-membered ring intramolecular attack of the free carboxylate anion on the ester, resulting in the formation of phenol and glutaric anhydride.

To check the hypothesis that the carboxylate anion in Asp acted through an intramolecular base assistance (IMBA), the acid was derivatized to its β-monomethyl ester, and the two were studied under identical conditions. The ester did not react slower. The relative reactivity of these compounds was about the same (2.2 vs 2.1, Table I). A product study, however, showed that the ester hydrolyzed to the acid anion at the reaction temperature (140 °C). Hydrolysis of the ester to the acid was followed by ^1H NMR analysis. Studies at a lower temperature (120 °C) showed less hydrolysis, and the acid was found to react 1.7 times faster than its β-monomethyl ester measured at pH 7.6. These results strongly support a carboxylate anion acting as an intramolecular base through a six-membered ring, formula **5**. The measured activation studies also support an intramolecular mechanism (Table II). The entropy of activation for Asp racemization is one of the least negative of those studied.

It was expected that the γ-carboxylate anion in Glu would activate racemization through an intramolecular base. However, Glu reacted only half as fast as Ala (Table I). The explanation for this reduced reactivity is that another reaction is taking place simultaneously with racemization. ^1H NMR studies of the reaction mixture during heating showed that Glu disappeared, and pyroglutamic acid (Pga, formula **6**) was formed. Studies of the racemization of Pga showed that it racemized five times slower than Glu and 10 times slower than Ala. ^1H NMR and GC studies of Pga before and after heating demonstrated that no change occurred in structure other than racemization. Pga reacts slowly because there is no positive charge remaining on the amino nitrogen, confirming the importance of the positive charge on nitrogen for a rapid reaction. Formation of Pga from Glu was not immediate, however. Had the conversion to Pga been complete, Glu and Pga would have shown the same racemization rate. The results obtained for the γ-monomethyl ester appear to be complicated. ^1H NMR studies showed that the ester cyclized to Pga; however, its rate of racemization was not as slow

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(27) (a) Garret, E. R. *J. Am. Chem. Soc.* **1957**, *79*, 3401. (b) Zimmering, P. E.; Westhead, E. W.; Morawetz, H. *Biochem. Biophys. Acta* **1957**, *25*, 376.

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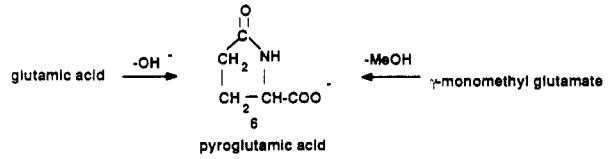
Table III. Effect of Methanol on the Racemization of L-Pyroglutamic Acid (Pga) at pH 8.0 and 140 °C

amount of methanol, mL	amount of 0.02 M Pga solution, mL	% D Pga[a]
0.2	0.8	2.56
0.4	0.6	4.48
0.6	0.4	7.83

[a] Determined by GC.

as that of Glu. Cyclization of the ester to Pga produced methyl alcohol, which increased the rate of racemization of Pga (Table III). This may explain why γ-monomethyl glutarate reacts faster than Glu. Methyl alcohol may also have assisted in the racemization of Asp, explaining why β-monomethyl aspartate was found to racemization slightly faster than the free Asp.



α-Aminoadipic acid (α-Aaa) racemized slower than Glu but faster than Pga (Table I). This is because cyclization of α-Aaa to its lactam was faster than Glu to Pga. To establish that cyclization occurred to the lactam, α-Aaa was heated for 20 h in a sealed tube under the same condition as used for the racemization of α-Aaa using a procedure described by Meister for the preparation of pyro-α-aminoadipic acids (piperidonecarboxylic acid).[29] Purification, however, was by sublimation rather than extraction. Elemental analysis, NMR, and melting point determinations established the structure of the lactam from α-Aaa, 128–129 °C; (lit.[29] mp 128 °C). Greenstein et al.[30] showed that heating α-Aaa forms an equilibrium mixture of the lactam and the acid (73% lactam, 27% acid). The fact that they observed no racemization when an aqueous solution of the acid was heated under reflux for 3 h also illustrates that the lactam is slow to racemize. The same observation was obtained by Meister[29] who prepared L-oxopiperidinecarboxylic acid by a similar method. The chemical shifts of the methine proton of both lactam were observed downfield from the methine proton of the corresponding acids. The chemical shift for Pga moved from 3.8 to 4.2 δ while the chemical shift for α-Aaa changed from 3.7 to 4.0 δ. Determination of racemization kinetics of δ-monomethyl-α-aminoadipate was not possible; all attempts to synthesize the monoester yielded the diester.

To summarize the findings with acidic amino acids, Asp racemizes at neutral pH values faster than Ala because of a differential inductive effect and also because the β-carboxyl group acts as an intramolecular base assisting in removing the methine hydrogen. Lactams racemize slower than the acids because the positive charge is no longer present on the nitrogen. α-Aminoadipic acid racemizes slower than Glu because lactam formation is faster for α-Aaa than Glu. Esterification of Asp at the β-position and Glu at the γ-position did not reduce the rate of racemization as much as expected because at 140 °C the esters readily hydrolyze to acids. At lower temperatures (120 °C) hydrolysis is retarded and esterification reduces racemization. Methyl alcohol causes a slight increases in the rate of racemization of Pga.

(29) Meister, A. *J. Biol. Chem.* **1954**, *210*, 17.
(30) Greenstein, J. P.; Birnbuam, S. M.; Otey, M. C. *J. Am. Chem. Soc.* **1953**, *75*, 1994.

Experimental Section

All L-amino acids, their derivatives, and trifluoroacetic anhydride (TFAA) were obtained from commercial sources; purity was checked by ^{1}H NMR spectroscopy. Buffer solutions of 0.05 M mono- and tribasic phosphates were prepared by dissolving these salts in deionized water. A 30% TFAA solution (v/v) in dichloromethane was prepared by mixing 30 mL of TFAA with 70 mL of dry, cold (10° C) dichloromethane. This mixture was stored at 10° C.

All samples were prepared as 25 mL of 0.02 M solution of the amino acid or derivative in deionized water. The pH was adjusted to 8.0 using 0.05 M buffer solution. One-milliliter aliquots of the solution were sealed in 8-mm Pyrex glass tubes.

Racemizations were performed by heating the sealed tubes first to 70 °C and then placing them in a constant-temperature oil bath at 140 °C. Tubes were cooled before opening. The constant temperature bath consisted of synthetic aircraft turbine oil (Texaco 7730) encased in 6 in. of treated cellulose insulation. The bath was regulated via a proportional temperature controller (RFL Industries, Inc., Model 70-115), and temperature was measured using an iron-constantan thermocouple connected to a potentiometer. The thermocouples were calibrated against an NBS-calibrated platinum-resistant Thermometer.

Treatment of the samples for GC and the GC analysis were performed as described by Smith and Wonnacott.[31] Isomers of hydroxy amino acids were found to separate better on a single phase (*N*-docosanoyl-L-valyl *tert*-butyl amide), while the other amino acid isomers were found to separate best on a 150 ft × 0.02 in. capillary column, loaded with a mixed phase of *N*-docosanoyl-L-valyl *tert*-butyl amide and *N*-octadecanoyl-L-valyl-L-valyl cyclohexyl ester.[31] Thr derivatives exhibited four peaks as they formed diastereomeric pairs.

Reversible first-order rate constants for the racemization were obtained by plotting 0.5 ln $([(1 + D/L)/(1 - D/L)]$ vs time, where amounts of D and L enantiomer were determined by GC.

^{1}H NMR and ^{13}C NMR spectra of the compounds were obtained before and after racemization on a JOEL 90 FX instrument at room temperature. The compounds (25 mg) were dissolved in 98% deuterium oxide. Sodium 3-(trimethylsilyl)propionate was used as an internal standard. ^{1}H NMR for the pyro-α-aminoadipic acid (oxopiperidinecarboxylic acid) was run on a Nicolet 300-MHz instrument.

Pyro-α-aminoadipic acid (oxopiperidinecarboxylic acid) was prepared according to the method of Meister[29] by heating α-Aaa in an aqueous solution in a sealed tube for 20 h. The water was remmoved by evaporation; the residue was sublimed. The sublimed and the unsublimed portions had the same melting point, 128–129 °C (lit.[29] mp 128 °C), mmp 127–128 °C. Anal. Calcd for $C_6H_9NO_3$: C, 50.32; H, 6.34; N, 9.78. Found: C, 50.42; H, 6.44; N, 9.54. ^{1}H NMR (D_2O 300 MHz): δ 1.54–1.66 (m, 2 H), 1.68–1.84 (m, 1 H), 1.88–2.6 (m, 1 H), 2.18 (t, J = 6.6 Hz, 2 H), 4.04 (t, J = 6.3 Hz, 1 H).

Conclusions

Heteroatoms in the R group of amino acids alter the racemization rates. When a hydroxyl group is present, solvation of the base and/or stabilization of the carboxylate anion have been proposed to explain the increased reactivity. Hydroxyl groups and alkoxy groups can activate through an inductive effect. The carboxyl group in Asp causes an increased reactivity which likely is not simply an inductive influence; the β-carboxyl group most likely acts as an intramolecular base (proposed in 1984[1b]). In a Taft $\sigma-\rho$ plot Asp falls off the line. (We are investigating intramolecular effects by determining the eight absolute rate constants in Asp racemization.) Glu reacted slower than Ala because it dehydrated to form the lactam, pyroglutamic acid (Pga), which reacted slower because it lacks a positive charge on nitrogen. α-Aminoadipic acid reacted more slowly than Ala because it also formed a lactam more rapidly than Glu formed Pga. Met racemized more rapidly

(31) Smith, G. G.; Wonnacott, D. W. *Anal. Biochem.* **1980**, *109*, 414.

than Ala because of the orbital overlap stability of the carboxylate anion.

Acknowledgment. Research support from the National Aeronautics and Space Administration (NGS 7038) is gratefully acknowledged as are funds from Utah State University Research Office for the purchase of the JOEL spectrometer. Our thanks to Dr. G. S. Reddy for carrying out some of the experiments. This research was supported by the Utah Agricultural Experiment Station, Utah State University, Logan, UT 84322-4845. Approved as journal paper no. 3807.

Registry No. Ser, 56-45-1; Ser(*t*-Bu), 18822-58-7; Thr, 72-19-5; Thr(*t*-Bu), 4378-13-6; Met, 63-68-3; Phe, 63-91-2; Ala, 56-41-7; Asp(OMe), 2177-62-0; Asp, 56-84-8; Glu(OMe), 1499-55-4; Glu, 56-86-0; α-Aaa, 1118-90-7; Pga, 98-79-3.

Reaction of Novel Imide Reducing Reagents with Pyrrolizidinediones

Edward W. Thomas,* Ronald H. Rynbrandt,† David C. Zimmermann, Larry T. Bell, Christine R. Muchmore, and Ernest W. Yankee

Research Laboratories, The Upjohn Company, Kalamazoo, Michigan 49001

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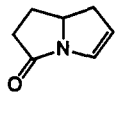
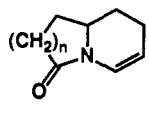
The reduction of pyrrolizidinediones **4a** and **4b** with $(i\text{-Bu})_2AlH$ and $LiBHEt_3$ affords the corresponding hexahydro-5-hydroxy-3*H*-pyrrolizin-3-ones **8a** and **8b** in good yield. $LiBHEt_3$ also reduces *N*-methylglutarimide (**18**) in 53% yield. The combination of $NaBH_4/MeOH/Ac_2O/CH_2Cl_2$ selectivity reduces an imide in the presence of an ester. Hexahydro-5-(methylthio)-3*H*-pyrrolizin-3-ones are products of the $NaBH_4$ reduction of pyrrolizidinediones in $MeSH/CH_2Cl_2/Ac_2O$. The reduction products, thioethers (**5a–c**) and lactamols (**8a–c**), are intermediates in the synthesis of pyrrolizinones **1a–c**.

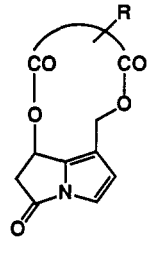
The pyrrolizidine alkaloids comprise a large class of natural products which have drawn synthetic interest due to their interesting structures and biological properties.[1,2] Although the pharmacologic properties of these compounds have ranged from antitumor activity[3] to hepatotoxicity,[4] no report has appeared describing their lipid altering or antiatherosclerotic activity. In conjunction with our search for lipid lowering and antiatherosclerotic medicinal agents,[5] we desired to synthesize **1a**. Similar 5–6 and 6–6 membered ring systems containing enamides of type **2** have been synthesized, and by virtue of their methods of synthesis the olefin is confined to the 6-membered ring.[6] Naturally occurring pyrrolizidine alkaloids are known which also contain the enamide group,[1] for example **3**.[7] However, the synthetic routes to these compounds were not directly applicable to **1**.[8] Recently, a [3 + 2] annulation approach to nitrogen heterocycles has afforded compounds similar to **1**, which contained tetrasubstituted olefins.[9]



1a



2, n = 1 or 2



3

Imide **4a** seemed a logical precursor to the desired target **1a**, via a reduction–elimination sequence. One attraction for this moiety is its ease of synthesis.[10,11] Another feature of the imide group is its ready conversion by reduction to hydroxy lactams, which are direct precursors to acyl imminium ions.[12] The reactive acyl imminium ions add diastereoselectively to olefins, affording complex molecular arrays.[13] The first reproducible and high-yielding method for the reduction of succinimides and glutarimides was introduced by Speckamp and consists of $NaBH_4$ in acidic EtOH.[14] Another reagent for the reduction of succinimides was introduced by Chamberlain and consists of $NaBH_4$ in MeOH at −5 °C.[15] A third method employs

† Deceased June 29, 1981.

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