consider the quantity $[\Delta H(v \rightarrow S) - \Delta H(v \rightarrow c\text{-}C_6)]$, we find for the solvent MeOH that all five alkenes are more solvated by *polar* interactions than is *n*-octane by 0.32 ± 0.01 kcal/mol, and DMF by 0.56 ± 0.04 kcal/mol. Additional interactions in benzene amount to 0.42 to 0.03 kcal/mol. This implies that π-bond solvation in the octenes is unaffected by *cis* or *trans* geometry, or by location of the bond at a terminal or nonterminal position in the linear carbon chain.

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(24) NOTE ADDED IN PROOF. ΔH_v of 2,2,5,5-tetramethyl-3-hexene (10.22 kcal/mol) and of 2,2,5,5-tetramethylhexane (10.15 kcal/mol) have recently been measured in this laboratory by Dr. L. A. Peacock. $\Delta H(v \rightarrow S)$ in the four solvents are −8.93, −7.33, −8.79, −10.01, and −8.51, −7.20, −8.54, −9.93 kcal/mol, respectively. This confirms the discussion of steric hindrance to π-bond solvation of the alkene. In the discussion of steric effects on dispersion interactions with quaternary carbons, the solvation enthalpy of 2,2,5,5-tetramethylhexane is now found to be 1.80 ± 0.09 kcal/mol (0.90/C) less than that of *n*-decane, in close agreement with the results for the other three compounds having quaternary carbon atoms.

Rotational Isomerism in Leucine: Proton Magnetic Resonance Study of [γ-^{2}H]Leucine and Thermodynamic Analysis

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Abstract: The ^{1}H NMR spectrum of [γ-^{2}H]leucine has been measured and analyzed to yield values of $^3J(H^\alpha\text{-}H^\beta)$ over a range of pD and temperature. These coupling constants have been used to calculate populations of rotational isomers at a range of temperatures in the cationic, anionic, and zwitterionic states. The rotamer in which the side chain is gauche to the amino group and trans to the carboxylate group has the highest population under all conditions. The thermodynamic values for the interconversion of rotational isomers of leucine (about the $C^\alpha\text{-}C^\beta$ bond) are derived from experimental populations. For example, the interconversion between the most stable rotamer, in which the isopropyl group is trans to the carboxylate group and gauche to the amino group, and the least stable rotamer, in which the isopropyl group is gauche to both C^α substituents, is described for the leucine anion by $\Delta G°_{25}$ = 1000 cal/mol, $\Delta H°$ = 2200 cal/mol, and $\Delta S°$ = 4 eu. The enthalpies and entropies are discussed in terms of possible intramolecular forces and solvation effects. The free energies are compared to those observed in other amino acids. Differences in rotameric state entropies ranging from 1 to 5 eu are found. These results indicate that isotopic substitution of the γ position for the purposes of spectral simplification is effective and suggests that the technique may be generally useful for other amino acids with γ protons.

The side chains of most amino acid residues of peptides and those of the corresponding free amino acids are considered to rotate freely in solution. The determination of the distributions of rotational isomers of free amino acids is important in identifying some of the forces responsible for the distribution of rotational isomers of amino acids incorporated as residues in peptides. An understanding of these forces should aid our knowledge of the factors that control peptide conformations, for the role of side chains in determining peptide conformation is well recognized.[2]

For the common natural amino acids the predominant values of χ^1, the dihedral angle about the $C^\alpha\text{-}C^\beta$ bond, have been assumed to be the three staggered conformations designated rotamers, I, II, and III, as shown in figure 1. An analysis of relative rotamer populations p about the $C^\alpha\text{-}C^\beta$ bond can be based upon the values of the coupling constants between vicinal α and β protons, $^3J(H^\alpha\text{-}H^\beta)$, inasmuch as the measured values of $^3J(H^\alpha\text{-}H^\beta)$ are weighted averages of the coupling manifest in the three rotamers.[3-9] Of the factors contributing to the energies of rotational isomerism in amino acids, steric hinderance and Coulombic interactions have been regarded as the most significant,[4-13] although ion-induced dipole contributions, hydrogen bonding, and interactions with solvent may also be important in particular cases. The energetics of rotational

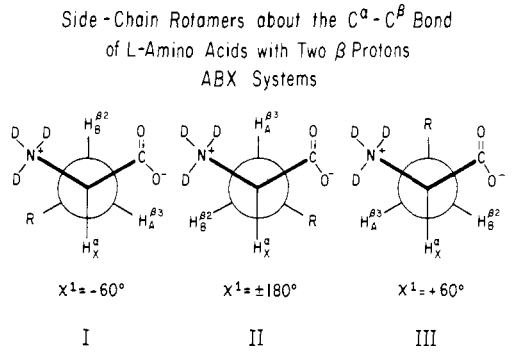

Figure 1. Molecular nomenclature for the Newman projections of the three staggered conformations about the C^{α}–C^{β} bond of an L-amino acid. The zwitterion state in D_2O is shown.

isomerism in aliphatic hydrophobic amino acids has not been studied by proton NMR in the past because of complexity of the spectra.

In those amino acids containing γ protons there is coupling between the β and γ protons as well as between the β and α protons, and it is then difficult, sometimes impossible, to analyze the ^1H NMR spectrum in order to obtain the desired values of $^3J(H^{\alpha}-H^{\beta})$. Coupling between vicinal β and γ protons may be eliminated by double-resonance methods or by replacing the γ protons by deuterons and neglecting the small couplings of the β protons to the γ deuterons.

This report is concerned with the analysis of rotamer populations about the C^{α}–C^{β} bond of the amino acid leucine (**1**). Direct electronic decoupling cannot be used to eliminate the coupling between each of the β protons and the single γ proton in this amino acid because of the small differences in chemical shift among these three protons, and for this reason the measurements were made on the deuterated isomer [γ-^2H]leucine (**2**).[14] The observed rotamer populations have been used to derive thermodynamic quantities.

$(CH_3)_2CHCH_2\underset{\displaystyle NH_3^+}{\underset{|}{C}}HCOO^-$ **1**

$(CH_3)_2CDCH_2\underset{\displaystyle NH_3^+}{\underset{|}{C}}HCOO^-$ **2**

Experimental Section

All 220-MHz ^1H NMR studies were performed on a Varian Associates HR-220 spectrometer equipped with a Nicolet Technology Corp. TT-220 FT/computer system. Spectra were accumulated by the pulse and Fourier transform technique.[15] The α and two β protons of [γ-^2H]leucine in D_2O comprise an isolated three-spin system. Analysis and simulation of the resonances for these three protons as an ABC spin system was performed by an implementation of LAOCN3,[16] and analysis as an ABX spin system was performed in accordance with previously published conventions.[17] DL-[γ-^2H]leucine (mol wt = 132.18) was prepared by the method of Sogn et al.[14] All samples were prepared in D_2O (99.97% D) and transferred to Wilmad No. 528-PP NMR tubes. Sodium [2,2,3,3-$^2H_4$]-3-(trimethylsilyl) propionate was added as an internal standard, from which all chemical shifts and resonance positions are reported in parts per million or hertz downfield.

Five samples were prepared in 1 M DCl for a concentration-dependence study covering the range 0.032–0.54 M. pD titration was performed by mixing various volumes of 0.076 M [γ-^2H]leucine in 1 M DCl with 0.076 M [γ-^2H]leucine in 2 M NaOD. The pD was determined directly in the NMR tube using an Ingold 6030-03 pH electrode and a Radiometer 28 pH meter calibrated in pD by standard deuterated buffers.[18]

Three samples for the temperature-dependence studies were prepared in 1 M DCl, 1 M NaOD, and NaD_2PO_4–Na_2DPO_4 buffer (pD 6.0 at 25 °C; phosphate concentration, 0.1 M). The concentration of [γ-^2H]leucine was 0.54 M in the cation and anion samples and 0.076 M in the zwitterion sample. At each temperature, ten spectral scans were taken for the cation and anion samples, and 256 for the zwitterion. Temperature was determined by measurement of the difference in chemical shift of the two signals from ethylene glycol in a capillary in the NMR tube and use of standard tables from the instrument manufacturer.

Rotamer Population Analysis. It is assumed in the usual way[2-9] that only the three staggered conformations (rotamers) about the C^{α}–C^{β} bond of leucine are present to any significant degree and that, on the NMR time scale, observed chemical shifts and coupling constants represent a weighted average of values characteristic of these three rotamers. Nomenclature has yet to be standardized, and our rotamer designations shown in Figure 1 agree with those used previously by some workers,[4] but not by others.[6,10]

The observed vicinal coupling constants were used to calculate populations of rotamers by methods well described previously[3-7] using values of the coupling constants for gauche and trans conformers ^3J_g and ^3J_t. It is assumed that ^3J_g and ^3J_t are constants that are independent of the β proton and rotamer involved. This assumption neglects the possibility that the effect of substituent electronegativity upon the strength of coupling may depend upon the orientation of the substituent group ($-ND_3^+$, $-COO^-$, and –R in Figure 1) with respect to the coupled nuclei, as well as the possibility that the true values of the dihedral angles are slightly different from the assumed staggered conformations. These are standard assumptions which are generally accepted because of the consistency of the results obtained.[3,19] Various workers have proposed values for ^3J_g and ^3J_t. The values proposed by Pachler[4] and those derived from the Karplus relation given by Kopple et al.[20] have both been used here and are given below.

	Values proposed by Pachler	Values proposed by Kopple et al.
^3J_g	2.60 Hz	3.25 Hz
^3J_t	13.56 Hz	12.40 Hz

Assignment of β Protons. It is not possible to assign chemical shifts unequivocally to specific β protons solely on the basis of ^1H NMR measurements. There is ambiguity in the assignment of the β protons to the A and B protons in the NMR analysis; either ($\beta 3 \equiv$ A and $\beta 2 \equiv$ B) or ($\beta 3 \equiv$ B and $\beta 2 \equiv$ A). As in previous work[10] it might be assumed that rotamer I is less hindered than rotamer II because in I the bulky $-COO^-$ group is trans to the bulky side chain –R group and is gauche to both of the β protons. The larger coupling constant between vicinal α and β protons would then be designated $^3J_{BX}$, and the smaller one, $^3J_{AX}$, so that $p_I > p_{II}$. In some other cases, this problem has been solved by stereospecific substitution of deuterium at the β position.[8,9] We have recently measured $^3J(^{15}N'-H^{\beta 2})$, $^3J(^{15}N'-H^{\beta 3})$, and ($^3J(C'-H^{\beta 2}) + {}^3J(C'-H^{\beta 3})$) in other isotopic isomers of leucine, and their values confirm that these assignments based on steric considerations are in fact correct.[21]

Thermodynamic Analysis. It is assumed that equilibrium populations of rotamers are obtained from coupling constants. Then $K_{I,II} = p_I/p_{II}$, $K_{I,III} = p_I/p_{III}$, and $K_{II,III} = p_{II}/p_{III}$, where p_I, p_{II}, and p_{III} denote the populations of rotamers I, II, and III as depicted in Figure 1. The free energies are given by $\Delta G°_{I,II} = -RT \ln K_{I,II}$, and similarly. Standard statistical methods of linear regression[22] were used to calculate the line of best fit between the $\Delta G°$'s and temperature. The slope of this line is $-\Delta S°$, and the intercept at absolute zero, $\Delta H°$.

Results

ABX analysis produced the appropriate coupling constants summarized in Table I. To confirm the spectral analysis, values obtained were used to simulate the spectrum. There is a good correspondence between the experimental spectra and spectra simulated using for each resonance a line width at half height of 2.5 Hz. Combination lines were not observed in the experimental spectra, were of negligible intensity in the simulated spectra, and therefore have been ignored.[23]

A sample of 0.076 M DL-[γ-^2H]leucine cation in 1 M DCl was titrated at a constant temperature of 30 °C in steps of 0.2–1.2 pD units until the amino acid was essentially in the anionic state. All 12 resonances in the α- and β-proton regions could be followed as the amino acid was titrated from the cation to the zwitterion, but the spectrum became broad in the transition region between the zwitterion and the anion and here individual resonances could not be followed.

Table I. Coefficients of the Quadratic Equations Describing the Dependence on Temperature of Chemical Shifts, Coupling Constants, and Derived Populations of Rotamers about the C^{α}-C^{β} Bond of [γ-^{2}H]Leucine

Constant[a]	State[b]	Coefficient[c] A	$B \times 10^2$	$C \times 10^4$
	Chemical Shifts, ppm			
δ^{α}	C	4.112	−0.04	0.02
	Z	3.745	*d*	*d*
	A	3.260	−0.04	0.09
$\delta^{\beta 2}$	C	1.752	0.01	*d*
	Z	1.694	−0.06	0.03
	A	1.379	−0.05	0.02
$\delta^{\beta 3}$	C	1.855	−0.01	0.03
	Z	1.729	0.04	0.01
	A	1.451	0.02	0.04
	Coupling Constants, Hz			
$^2J(H^{\beta 2}\text{-}H^{\beta 3})$	C	−14.48	*d*	*d*
	Z	−14.50	*d*	*d*
	A	−13.52	−0.09	*d*
$^3J(H^{\alpha}\text{-}H^{\beta 2})$	C	8.85	−1.7	0.43
	Z	9.92	−2.0	0.36
	A	8.92	−0.9	0.08
$^3J(H\text{-}H^{\beta 3})$	C	5.71	1.2	−0.29
	Z	4.97	0.7	−0.29
	A	5.87	−0.3	0.00
	Populations[e]			
p_{I}	C	0.570	−0.2	0.04
	Z	0.668	−0.2	0.03
	A	0.576	−0.1	0.00
p_{II}	C	0.283	1.1	−0.03
	Z	0.216	0.1	−0.03
	A	0.299	0.0	0.00
p_{III}	C	0.147	0.0	0.01
	Z	0.116	0.1	0.00
	A	0.125	0.1	0.00

[a] The value of constants for p are dependent on the choice of ^3J_g and ^3J_t; ^3J_g = 2.6 and ^3J_t = 13.6 Hz.[4] [b] C, cation; Z, zwitterion; A, anion. [c] The value of δ, J, or p at a temperature T (°C) is given by $A + BT + CT^2$. [d] These values could not be determined to an acceptable degree of accuracy. [e] I, II, and III are the rotamers illustrated in Figure 1.

For all three states of ionization the coupling constant between the α proton and the β proton with the more upfield chemical shift (~8.3–10.1 Hz) was larger than that between the α proton and the downfield β proton (~4.4–6.0 Hz). The larger and smaller coupling constants are associated with the $\beta 2$ (B) and $\beta 3$ (A) protons, respectively (see Figure 1). Hence, the upfield and downfield β-proton chemical shifts are assigned to $H^{\beta 2}$ (B) and $H^{\beta 3}$ (A), respectively.

The spectra of the cation, zwitterion, and anion were observed at a number of temperatures over the range 4–81 °C. There was no observable dependence of the values of $^3J_{AX}$ and $^3J_{BX}$ on concentration. The coefficients of the quadratic equations for the variation of coupling constants and populations with temperature are presented in Table I. Values for the geminal coupling constant ($^2J_{AB}$) and chemical shifts of the α and β protons for the three states of ionizations are also in Table I.

Relative values of free energy, enthalpy, and entropy for the interconversion among the three rotameric isomers of leucine in the anionic, zwitterionic, and cationic forms are tabulated in Table II. Figures 2 and 3 illustrate typical data found and represent the smallest and largest experimental scatter encountered in the measurements. These thermodynamic values are obviously dependent on the values chosen for ^3J_g and ^3J_t. Those used to obtain the results presented in Table II were ^3J_g = 2.6 and ^3J_t = 13.56 Hz,[4] which were preferred because they were derived from suitable model compounds in a convincing

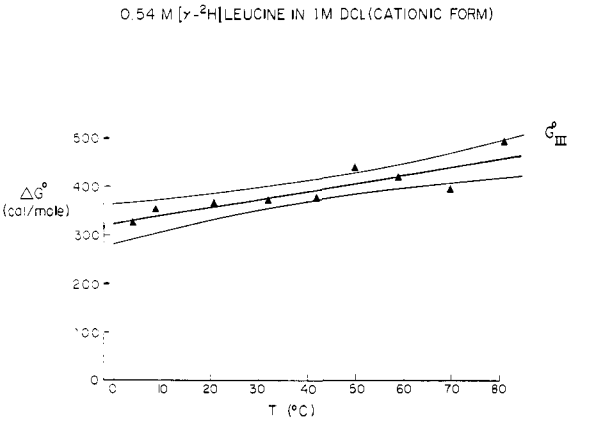

Figure 2. The free energy of rotamers III in leucine cation relative to rotamer I as zero. The heavier straight line is the calculated linear regression to the points derived from experimental data. The lighter curved lines are the quadratic approximation to the 95% confidence limit of the central straight lines. The lines are calculated using the values of ^3J_g and ^3J_t of 2.6 and 13.56 Hz.[4]

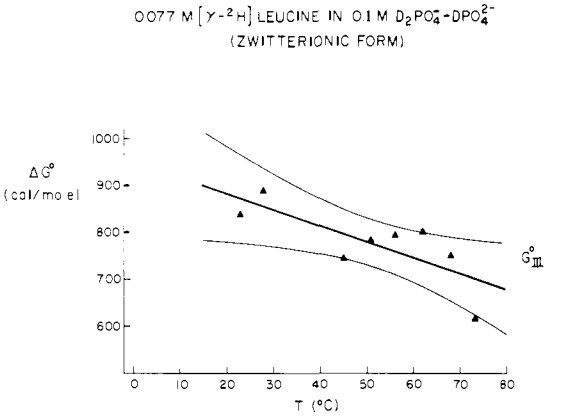

Figure 3. The free energy of rotamer III in the leucine zwitterion relative to rotamer I as zero. See legend to Figure 2.

Table II. Thermodynamic Values for Rotamers of Leucine in D_2O

Value	State of ionization[a]	I[b]	II	III
$G°_{25}$[e] cal/mol	C	0[c]	320 (10)[d]	720 (20)
	Z	0	610 (30)	950 (40)
	A	0	430 (10)	1050 (20)
$H°$, cal/mol	C	0	990 (60)	910 (160)
	Z	0	910 (300)	1890 (390)
	A	0	110 (130)	2190 (290)
$S°$, eu	C	0	2 (0.2)	1 (0.5)
	Z	0	1 (0.9)	3 (1)
	A	0	−1 (0.4)	4 (1)

[a] C, cation; Z, zwitterion; A, anion. [b] I, II, and III are the rotamers illustrated in Figure 1. [c] The free energy of the rotamer of lowest energy in each state of ionization is defined as zero in this table. The values of H and S for the rotamers of a given state of ionization are related in the usual way to the values of G for the rotamers of that state. The values of G, H, and S for the rotamers of different states of ionization, however, are not comparable because absolute values are unknown and are presumed to be different. [d] The figure in parenthesis is the standard error, representing the 67% confidence limit of the estimated value. [e] We have used the ° superscript to denote values derived from $\Delta G° = -RT \ln K$ in this table. Elsewhere we have omitted the superscripts, and the standard state should be inferred from the context.

Table III. Free Energies of Rotamers in Amino Acids

Amino acid	State of ionization[a]	Temp, °C	$G°$,[b] cal/mol I[c]	II	III	Ref
Serine	C	25	990	1400	0	8
	Z	25	460	1110	0	8
	A	25	350	760	0	8
Aspartic acid	C	25	240	760	0	8, 9
	ZA	25	0	950	320	8, 9
	diA	25	0	970	670	8, 9
Asparagine	C	25	90	680	0	8
	Z	25	0	810	160	8
	A	25	0	680	570	8
Aspartic acid γ-methyl ester	C	25	170	580	0	8
	Z	25	30	550	0	8
	A	25	0	250	340	8
Histidine	A	33	0	420	300	13
Phenylalanine	Z	25	0	560	380	12, 28
	A	25	0	420	300	12, 28
Tyrosine	C	25	0	340	300	8
	Z	25	0	510	300	8
	diA	25	0	400	180	8
	diA	33	0	440	200	12
Tryptophan	A	33	0	410	200	12
α-Aminobutyric acid	Z		70	70	0	11

[a] C, cation; Z, zwitterion; A, anion; ZA, cation/dianion; diA, dianion. [b] The free energy of the lowest energy state in each state of ionization is defined as zero in this table. Values were calculated from coupling constants in the original references by the procedures used in this paper. [c] I, II, and III are the rotamers illustrated in Figure 1. The stereospecific assignments of I and II are not available for histidine, tyrosine, and tryptophan.

fashion and have been used by others in previous analyses of this type.[4-13] In addition we have assumed that ^3J_g and ^3J_t are negligibly dependent on temperature and pH,[24] an assumption supported by the observed small temperature and pH dependencies of the H^α-H^β coupling constant in alanine, in which the three rotamers are identical.[6]

Discussion

The coupling of the γ deuteron to the β protons should be between 0.4 and 2 Hz, depending on the value of the dihedral angle involved. These splittings of the β resonances were not observed. The $^1H^\beta$-$^2H^\gamma$ and $^1H^\alpha$-$^2H'$ couplings can contribute substantially to the observed $^1H^\beta$ and $^1H^\alpha$ line widths.[25] Therefore, deuterium substitution for the purposes discussed here will be useful only when there is sufficient chemical shift separation between the two β protons. For example, if the difference in β chemical shifts of leucine were 0.05 ppm (11 Hz) or less, the analyses based on 220-MHz data described here would not be possible.

Because all 12 resonances in the α- and β-proton regions could be resolved and followed in the transition from cation to zwitterion, it was possible also to follow the chemical shifts of the α and β protons through the transition region. It was found that the β proton with the more upfield chemical shift in the cation was also the one with the more upfield chemical shift in the zwitterion. Consequently, there is no doubt that the population assigned to rotamer I or rotamer II in the cation corresponds to the one assigned to this same rotamer in the zwitterion. These assignments cannot be carried through to the anion, based solely on the titration, but they are confirmed by measurements of vicinal heteronuclear coupling constants.[21] The loss of spectral resolution in the transition region between zwitterion and anion probably reflects a relatively slow rate of exchange between these forms on the NMR time scale.

In considering the thermodynamic values of Table II, we examine first the relative enthalpies. It will be noted that H_{III} is either approximately equal to or greater than H_{II} and that H_I is always lowest. The transition from the anion to the zwitterion involving the protonation of the anionic group appears to increase by 800 cal/mol the relative enthalpy of rotamer II [the isopropyl group (R) trans to the amino/ammonium group (NH_2/NH_3^+)]. The transition from the zwitterion to the cation in which the carboxylate anion is protonated appears to decrease by 1000 cal/mol the relative enthalpy of rotamer III [R gauche to the carboxylate anion/carboxylic acid group ($COO^-/COOH$)].

We are unable to give a complete explanation of the values and relationships in Table II. The introduction of a charge into the C^α substituents may be expected to change the relative energetics of the rotamers by the interactions between the charge and the permanent and induced dipoles in the isopropyl group. Such changes are expected to be similar for the rotamers in which the isopropyl group (R) is gauche to the introduced charge and quite different for the rotamer in which it is trans. The effects of protonation of the ammonium group (A → Z) fulfill these expectations; the effects of protonation of the carboxylate group (Z → C) do not, in that the protonation affects the relative enthalpy of only one gauche rotamer (III, but not II). These electrostatic considerations are quite elementary, because a simple dipole model for these interactions neglects the differences of sizes of the ions, the orientations of R both as a whole and internally in the different conformers, the dependence of the size of induced dipoles on the inducing charge, and the nonadditivity of effects from the two charges in Z.

Steric repulsion will be an important interaction in the rotamers, particularly in III where there is close packing of R and both of the bulky substituents of C^α. If changes in charge indirectly cause changes in steric repulsion, for example by an increase in effective radius of the groups from changes in solvation, then a further complexity is introduced. There is, additionally, a direct contribution to the rotamer enthalpies from the exchange interactions between the atoms that are attached to the central pair of atoms[26] (in this case C^α and C^β); the same interaction is totally responsible for the barrier height in molecules like ethane. Finally, the enthalpy of each rotamer

of each state of ionization will differ because of the differences in polarization of the solvent by the different electric fields.[5] This phenomenon has been investigated for phenylalanine,[7] but the lack of clear correlation of rotamer population with dielectric constant suggests that the situation is highly complex.

The entropies in Table II are of a comparable magnitude to those observed in aromatic amino acids.[6,7,12,13] The variations of the entropies in Table II with the state of ionization are roughly parallel to the variations of enthalpies discussed above. It is noteworthy that in Z and A, the highest relative entropy occurs in the rotamer that appears to be most sterically hindered, III. We suggest that solvation contributes to this behavior, because we consider that no intramolecular effect could do so.

The free energies in Table II are comparable to those reported for other amino acids (Table III) and for other, analogous compounds.[27] The values of G are, with one exception, lower than their corresponding enthalpies. The parallelism observed in H and S with changes in the state of ionization is not present in G. The free energies are relatively less affected by changes in the state of ionization than their constituent enthalpies and entropies. In examining the values of Table III it will be noted that the energy of rotamer II is always highest, and that rotamer III is generally next in size, in contrast to our results in leucine. On the other hand, the free energies of rotamer III for the compounds listed in Table III are generally lower in C than in Z or A. This is in general agreement with our findings in leucine.

In conclusion, spectral simplification by isotopic substitution is effective for the purpose of examining rotational isomerism in leucine and should be similarly effective in comparable cases such as methionine, lysine, glutamic acid, glutamine, arginine, ornithine, and proline, as well as in peptides containing these amino acid residues.[29] This study of leucine shows that the energetics of rotational isomerism in molecules of this size are quite complex, but that simplifications emerge from examination of enthalpies and entropies rather than from considering free energies alone. This suggests that a substantial increase in understanding of the energetics of rotational isomerism in amino acids in general may result from temperature studies of their rotational isomerism.

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