

Conformational Equilibria of β -Alanine and Related Compounds as Studied by NMR Spectroscopy

Françoise Gregoire, Sindy H. Wei, Erik W. Streed, Kenneth A. Brameld, Diana Fort, Linda J. Hanely, Jamie D. Walls, William A. Goddard,* and John D. Roberts*

Contribution from the Gates and Crellin Laboratories of Chemistry and the Materials and Process Simulation Center of the Beckman Institute, California Institute of Technology, Pasadena, California 91125

Received December 22, 1997. Revised Manuscript Received May 13, 1998

Abstract: The vicinal proton–proton couplings of the dipolar form of β -alanine in water, alcohol–water, and dimethyl sulfoxide–water solutions indicate little conformational preference and are consistent with an essentially statistical equilibrium of the *gauche* or *trans* conformations. The position of the equilibrium is only slightly affected, over a temperature range of about 130°, by changes in dielectric constants ranging from 30 to 80 or by massive changes in ionic strength. Quantum-mechanical calculations at the HF/6-31G** and LMP2/cc-pVTZ levels were found to give rather good parallels with experiment, although suggesting the *gauche* conformation to be 2–3 kcal/mol more stable in water or methanol than actually observed. A number of related compounds, such as *N,N,N*-trimethyl- β -alanine and *N,N*-diethyl- β -alanine, as well as the conjugate acid and conjugate base of β -alanine, also show no significant conformational preference in water solution. In conformity with these results, the zwitterionic form of piperidine-3-carboxylic acid (nipecotic acid) has about the same preference for equatorial carboxylate as cyclohexanecarboxylic acid itself. Taurine shows no significant conformational preference except in basic solution, where the couplings indicate about 53% of the *gauche* conformation. In contrast, *N,N,N*-trimethyltaurine is predominantly *trans* in acidic or neutral solution. The conformational equilibria of the *N,N,N*-trimethyltaurine species are most likely governed by steric hindrance, because there are rather large tetrahedral groups at each end of the ethano chains. Yet, even here the energy difference between *gauche* and *trans* is only about 1.2 kcal.

Introduction

An enormous amount of research has been done on conformational analysis and equilibria of substances with C–C single bonds since the pioneering work of Barton and Hassel, and for most relatively nonpolar compounds in relatively nonpolar solvents, much is understood about the factors that determine the position of the conformational equilibria and the barrier heights. In general, molecular-mechanics calculations can be expected to provide useful information about the interactions involved, because steric effects tend to dominate. Although the important amino acids have been extensively studied, much less seems to be known about the role of polar, hydrogen–bonding, and charge effects for ethane derivatives of the type X–CH₂–CH₂–Y in water solution.

Our interest in such problems was initially concerned and continues with conformational analysis of solutions of 1,3-butanedioic acid and its corresponding mono- and dianionic forms in water¹ and nonpolar media. Here, where hydrogen bonding might be expected to be especially important for the monoanion, the conformational equilibria in water was found to be essentially statistical, and if hydrogen bonding tends to strongly favor the *gauche* conformer, there must be a strong counterbalancing effect favoring the *trans* conformation. The simplest view is that, in water solution, the forces that would favor one conformation over the other are muted by solvation and the high dielectric constant.

In this context, we were much intrigued by several reports^{2–5} that proton NMR spectra of β -alanine in acid, neutral, or basic solution are consistent with little conformational preference, even though our *ab initio* calculations and ones previously reported^{6,7} indicate that the dipolar ion **1** with a *gauche*-like conformer should be more stable than the *trans* conformer by about 23–25 kcal in the gas phase. Figure 1 shows the overall calculated energy and its components for β -alanine as a function of rotational angle ϕ at the HF/6-31G** level. A very significant feature of Figure 1 is that it suggests, if not an actual hydrogen bond, about 3 kcal of stabilization at a rotational angle of 50° that arises from an energetically favorable mutual polarization of the NH₃⁺ hydrogens by the CO₂[–] group. Even if we were to assume that the calculated gas-phase energy difference between the *gauche* and *trans* rotamers is only a ballpark figure, there must be very sizable differences in solvation energy for the *gauche* and *trans* conformers to reduce ΔG for the conformational equilibrium in water to near zero. In crystalline β -alanine, the intermolecular forces must be large (the melting point is about 200 °C) and ϕ has the odd value of

(2) Kostromina, N. A.; Konunova, T. B.; Gulya, A. P.; Venichenko, A. S. *Teor. Eksp. Khim.* **1975**, *11*, 548–552.

(3) Mikhova, B.; Simeonov, M. F.; Spassov, S. L. *Magn. Reson. Chem.* **1985**, *23*, 475–577.

(4) Abraham, R. J.; Loftus, P.; Thomas, W. A. *Tetrahedron* **1977**, *33*, 1227–1234.

(5) Abraham, R. J.; Hudson, B. D.; Thomas, W. A. *J. Chem. Soc., Perkin Trans. 2* **1986**, 1635–1640.

(6) Gresh, N.; Pullman, A.; Claverie, P. *Theor. Chim. Acta* **1985**, *67*, 11–32.

(7) Ford, G. P.; Wang, B. *J. Am. Chem. Soc.* **1992**, *114*, 10563–10569.

(1) Lit, E. S.; Mallon, F. K.; Tsai, H. Y.; Roberts, J. D. *J. Am. Chem. Soc.* **1993**, *115*, 9563–9567.

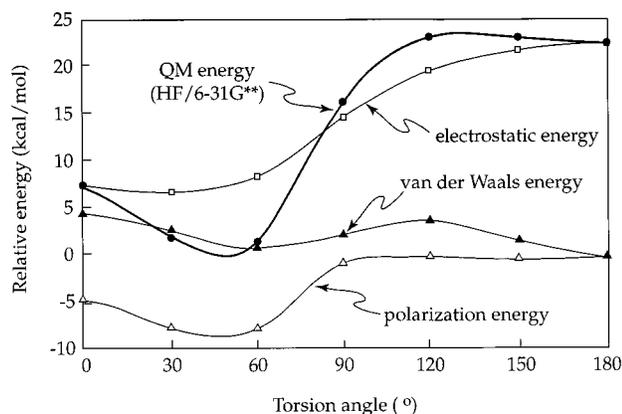


Figure 1. Component energy analysis for rotation about the central C–C bond of β -alanine in the gas phase. The absolute quantum-mechanical (HF/6-31G**) energy at a torsion angle of 0° is -321.81537 hartree. The absolute electrostatic energy at a torsion angle of 0° is -67.55 kcal/mol. Polarization energy is taken to be the difference between the quantum mechanical energy and the van der Waals plus electrostatic contributions.

84° .^{8,9} Although the bond angles and distances for β -alanine are such as to allow for intramolecular hydrogen bond formation between a carboxylate oxygen and an ammonium hydrogen, this is not a structural feature in β -alanine crystals. The ammonium hydrogens are indeed hydrogen bonded, but only intermolecularly to other carboxylate groups.^{8,9}

With this background, it was our intention to determine whether one could find conditions in which the equilibria could be substantially perturbed in hope of elucidating the factors that are responsible for what seemed to us to be an extraordinary lack of conformational preference. As will be seen, we have been rather unsuccessful in this endeavor. Consequently, it is of substantial interest to determine whether current theories are adequate to deal with all aspects of the lack of conformational preference with β -alanine and related compounds. Before elaborating on that, it will be advantageous to review the experimental results.

Experimental Section

Materials. Many of the compounds and solvents used in this work were commercial materials used without purification, except for being dried when appropriate in a vacuum desiccator over phosphorus pentoxide prior to use. *N,N,N*-Trimethyl- β -alanine was prepared by the procedure of Rahal and Badache,¹⁰ and *N,N,N*-trimethyltaurine in aqueous solution was made as described by Le Berre and Delacroix.¹¹

NMR Spectra. Variable-temperature NMR spectra were taken with a Bruker AM-500 NMR spectrometer. All other NMR spectra were taken with the same Bruker AM-500 or with GE QE-300 NMR spectrometers. Because the variable-temperature experiments were essentially qualitative, the NMR probe temperatures of the Bruker AM-500 NMR spectrometer were taken to be those indicated by the spectrometer readings. The *J* couplings were extracted from the experimental spectra either by our version of LAOCN3¹² or by gNMR 3.6.2 (Cherwell Scientific).

Calculations. All *ab initio* quantum-mechanical (QM) calculations were carried out with the Jaguar 3.0, Release 16^{13,14} software package

(8) Jose, P.; Pant, L. M. *Acta Crystallogr.* **1965**, *18*, 806–810.

(9) Papavinasam, E.; Natarajan, S.; Shivaprakash, N. C. *Int. J. Pept. Protein Res.* **1986**, *28*, 525–528.

(10) Rahal, S.; Badache, L. *Tetrahedron Lett.* **1991**, *32*, 3847–3848.

(11) Le Berre, A.; Delacroix, A. *Bull. Soc. Chim. Fr.* **1973**, 2404–2408.

(12) Bothner-By, A. A.; Castellano, S. M. *Computer Programs for Chemistry*; W. A. Benjamin, Inc.: New York, 1968; pp 10–53.

(13) Greeley, B. H.; Russo, T. V.; Mainz, D. Z.; Friesner, R. A.; Langlois, J.-M.; Goddard, W. A., III; Donnelly, R. E.; Ringnalda, M. N. *J. Chem. Phys.* **1994**, *101*, 4028–4041.

from Schrödinger, Inc. Full unconstrained geometry optimizations (HF/6-31G** and LMP2/cc-pVTZ(-f)), starting from both the *gauche* and *trans* conformations of β -alanine, were performed including the effects of solvent (in water and methanol). The effects of solvent polarization were included self-consistently in the QM wave function using the Poisson–Boltzman continuum description ($\epsilon = 80.37$) outside the solvent-accessible surface as determined with a probe radius of 1.2 Å.¹⁵ In addition, the torsion potential about the central C–C bond was determined by constraining the N–C–C dihedral angle, followed by optimization of all other degrees of freedom. In this way, solvated (H_2O) energies and optimum geometries (HF/6-31G**) were determined in 15° intervals from 0° to 180° . A full geometry optimization for the gas phase, without constraints, leads to a neutral species in which the proton has been transferred from the amine to the carboxylate. To avoid such complications, single-point gas-phase energies (HF/6-31G**) were calculated for the optimum geometries in solution. As a test of the HF and LMP2 methods, a density functional (DFT) geometry optimization was carried out for the solvated *gauche* and *trans* conformations. The Jaguar default Becke-3LYP method was used which includes the following functionals: exchange–exact HF, Slater local functional,¹⁶ Becke’s 1988 nonlocal gradient correction;¹⁷ correlation–Vosko, Wilk, and Nusair¹⁸ (VWN) local functional and Lee, Yang, and Parr¹⁹ local and nonlocal functional.

Component energies for the torsion potential in both the gas and solution phase (H_2O) were estimated empirically. The van der Waals (vdW) energy was calculated using molecular mechanics in the Biograf program (MSC-Biograf/Polygraph which is MSC version 3.3 of the software originally distributed by Molecular Simulations Inc.) and the Dreiding force field.²⁰ Gas-phase electrostatic energies were determined for each conformation (30° intervals) with the Delphi program.²¹ For these calculations, a charge of $\pm 0.78e$ on N and $C\beta$ was used, as determined from a fit of the electrostatic potential derived from the quantum mechanical calculations. The solvation/electrostatic component energies were also calculated using Delphi and a charge of $\pm 0.81e$ for N and $C\beta$. Because a static charge model was used for the Delphi calculations, changes in the charge distribution (polarization of the charge), arising from hydrogen bond formation and conformational differences, are not expected to be accounted for in the electrostatic components. Therefore, the difference between the total QM energy and the electrostatic, solvation, and vdW components comprises the polarization energy.

Results and Discussion

The 300 MHz proton spectrum of the $-CH_2-CH_2-$ grouping of β -alanine in neutral aqueous solution shows two simple triplets with the typical field-dependent inequality of the heights of the inner and outer peaks of the triplets. As a 0.1 M solution in D_2O , assuming an A_2B_2 spin system, the coupling constant at room temperature is 6.70 Hz with a line width of 0.7 Hz. The simplicity of the spectrum is such as to lead one to conclude that there is little, if any, preference for the *gauche* or the *trans* conformations. The accuracy of determining the conformational preference is at least somewhat compromised by the fact that we have no assurance that the rotational angles for the *gauche* and *trans* conformations are exactly 60° and 180° , respectively. As a result, estimating what the coupling constants should be

(14) Murphy, R. B.; Friesner, R. A.; Ringnalda, M. N.; Goddard, W. A., III. *J. Chem. Phys.* **1994**, *101*, 2986–2994.

(15) Tanner, D. J.; Marten, B.; Murphy, R.; Friesner, R. A.; Sitkoff, D.; Nicholls, A.; Ringnalda, M.; Goddard, W. A., III; Honig, B. *J. Am. Chem. Soc.* **1994**, *116*, 11875–11882.

(16) Slater, J. C. *Quantum Theory of Molecules and Solids, Vol. 4: Self-Consistent Fields for Molecules and Solids*; McGraw-Hill: New York, 1974.

(17) Becke, A. D. *Phys. Rev. A: At., Mol., Opt. Phys.* **1988**, *38*, 3098.

(18) Vosko, S. H.; Wilk, L.; Nusair, M. *Can. J. Phys.* **1980**, *58*, 1200.

(19) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B: Solid State* **1988**, *37*, 785.

(20) Mayo, S. L.; Olafson, B. D.; Goddard, W. A., III. *J. Phys. Chem.* **1990**, *94*, 8897–8909.

(21) Honig, B.; Nicholls, A. *Science* **1995**, *268*, 1144–1149.

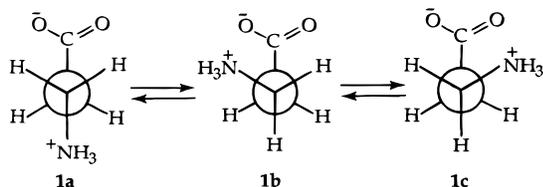
Table 1. Predicted Experimental Vicinal Proton-Proton Coupling Constants^a and Calculated *Gauche* Populations for Perfectly Staggered *Gauche* and *Trans* Conformations of β -Alanine **1** and Its Conjugate Acid and Base and for *N,N,N*-Trimethyl- β -alanine **3** and Its Conjugate Acid

J_t^t	J_g^t	J_t^g	J_g^g	$J_{g'}^g$	J_{13}	J_{14}	% <i>gauche</i> , calcd ^b
13.73	4.02	13.73	3.50	2.98	6.91 ^c	6.91 ^c	62, 69
13.77	4.02	13.77	3.51	3.00	6.93 ^c	6.70 ^d	61.5, 69
13.36	4.17	13.36	3.35	2.53	6.68 ^c	6.70 ^d	67, 66.5
13.78	4.01	13.78	3.51	3.01	6.94 ^c	6.70 ^d	62, 69
13.75	4.02	13.75	3.51	3.00	6.92 ^c	6.70 ^d	61, 69

^a Procedure of Altona.²³ ^b The first value given is calculated for J_{13} and the second from J_{14} . ^c Average calculated values for a 1:1:1 mixture of *gauche* and *trans* conformations. ^d Experimental values.

from published correlations^{5,22,23} of rotational angle and substituent electronegativities is correspondingly uncertain. However, it should be clear that little reliance can be put on estimates of the positions of conformational equilibria that do not agree with observed multiplicities and line widths of observed resonance signals. Thus, if the spectrum is actually representative of an AA'BB' spin system, then J_{AB} , $J_{AB'}$, $J_{AA'}$, $J_{BB'}$, and the chemical shift must have values such as to give line shapes that are consistent with the observed line widths. We have used our version of LAOCN3¹² to show that if the J_{13} and J_{14} coupling constants were to have the average value of 6.70 Hz at 300 MHz and a line width of 0.7 Hz, they could differ by no more than 0.8–0.9 Hz, without this being evident by visual inspection of the observed spectra.

For evaluation of the positions of conformational equilibria of the several β -alanine species, we have chosen to use the semiempirical procedures recently published by Altona and co-workers²³ because these procedures allow for much better assessment of the influences of specific substituent groups than do the procedures of Abraham⁵ and Haasnoot.²² Table 1 shows the predicted Altona coupling constants for perfectly staggered conformations for β -alanine. From these, we can estimate that a 1:1:1 mixture of **1a**, **1b**, and **1c** should have J_{13} , J_{14} , and their



averages all equal to 6.91 Hz, a value that agrees with our experimental value within the uncertainties of both the Altona procedure and the measurements. However, it is instructive to ignore the uncertainties and to calculate the two independent

values for the composition of the conformer mixture with the coupling constants of Table 1 and the values of J_{13} and J_{14} using eqs 1 and 2, respectively. Here, J_t^t and J_g^t are J_{14} and J_{13} , respectively, in **1a**, while J_t^g , J_g^g , and $J_{g'}^g$ are J_{13} , J_{14} , and J_{13} , respectively, in **1b**, **1b(1c)**, and **1c**.

$$f_g^{13} = \frac{2(\text{obsd } J_{13} - J_t^g)}{(J_{g'}^g + J_t^g - 2J_g^g)} \quad (1)$$

$$f_g^{14} = \frac{(J_t^t - \text{obsd } J_{14})}{J_t^t - J_g^g} \quad (2)$$

Thus, from J_{13} we get 62% *gauche* and from J_{14} , 69% *gauche*, as shown in Table 1. If we average these values, the average of 65.4% corresponds to the essentially negligible energy difference between the conformations of $\Delta G = -0.04$ kcal/mol.

One would hope that we could refine these estimates of composition that give different and independent results from J_{13} and J_{14} by using the angle dependencies of the predicted couplings to find the optimal angle for agreement of the percentages calculated from each couplings. We have attempted to use the Altona correlation²³ for this purpose on the basis that angle corrections should only be necessary for *gauche* conformations where the substituent interactions would be most evident. However, as was found previously for the butanedioate monoanion,¹ the differences in sensitivity of the J values to the rotational angle ϕ are usually so small that quite unreasonable deviations of 20° or more from the perfectly staggered values are required to bring the calculated percentages even significantly closer together. In any case, it should be noted that the 0.2 Hz experimental difference between J_{13} and J_{14} is well within the ± 0.36 Hz rms deviation found by Altona and co-workers²³ for their correlation of several hundred experimental and calculated J values. For these reasons, we accept the actual conformational equilibria for the dipolar ion, its conjugate acid, and its conjugate base as all being essentially statistical, which is not what we expected.

As mentioned earlier, ab initio calculations suggest that, in the gas phase, the *trans* conformation should be less stable than the *gauche* by 20 or more kcal/mol.^{6,7} Further, the predicted rotational angle between the NH_3^+ and CO_2^- groups for the *gauche* conformation is not 0°, which would bring these groups in closest proximity to take advantage of the electrostatic effect, but is much larger (about 50° from Figure 1 and from other calculations, 55°⁶ and 44°⁷). Figure 1 shows that this value is a compromise between attractive and steric repulsion influences.

It is not unreasonable to expect that, while water would diminish the electrostatic effect by virtue of its high dielectric constant, the strong gas-phase preference for *gauche* would be maintained in water solution. Such expectations do not take into account the fact that, when the NH_3^+ and CO_2^- groups are in close proximity, they are not likely to be as effectively solvated by water as when they are farther apart, in accord with the Born charging model for ions.²⁴ In fact, the change in dielectric constant from gas phase to almost any solvent is expected to substantially reduce the difference in electrostatic solvation energy between the *gauche* and *trans* conformers and make them have more nearly equal energies.²⁵ This line of

(22) Haasnoot, C. A. G.; de Leeuw, F. A. A. M.; Altona, C. *Tetrahedron* **1980**, *36*, 2783–2792.

(23) Altona, C.; Francke, R.; de Haan, R.; Ippel, J. H.; Daalmans, G. J.; Westra Hoekzema, A. J. A.; van Wijk, J. *Magn. Reson. Chem.* **1994**, *32*, 670–678.

(24) Born, M. Z. *Phys.* **1920**, *1*, 45.

(25) For a perceptive discussion see: Leffler, J. E.; Grunwald, E. *Rates and Equilibria of Organic Reactions*; John Wiley & Sons, Inc.: New York, 1963.

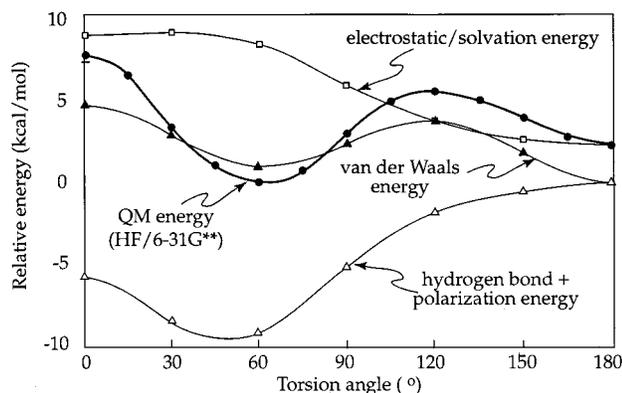


Figure 2. Component energy analysis for rotation about the central C–C bond β -alanine in the aqueous phase. The absolute quantum-mechanical (HF/6-31G**) energy at a torsion angle of 0° is -321.90137 hartree. The absolute electrostatic energy at a torsion angle of 0° is -82.08 kcal/mol. Polarization energy is taken to be the difference between the solvated quantum-mechanical energy, which is calculated rigorously using a Poisson–Boltzman continuum solvent description, and the van der Waals plus electrostatic/solvation contributions as determined using molecular modeling and Delphi.²¹

Table 2. β -Alanine Full Geometry Optimization (kcal/mol)^a

level of theory	<i>gauche</i>	<i>trans</i>	optimum angle, deg
HF/6-31G** (H ₂ O)	0.000	2.387	62.5
LMP2/cc-pVTZ(H ₂ O)	0.000	2.747	60.7
B3LYP/cc-pVTZ(H ₂ O)	0.000	2.715	58.0
HF/6-31G** (CH ₃ OH)	0.000	2.914	59.7
LMP2/cc-pVTZ (CH ₃ OH)	0.000	3.234	58.6

^a Relative energies for the unconstrained geometry optimization of the zwitterionic form of β -alanine including solvation. Reading down in the table, the respective calculated absolute energies in hartrees for the *gauche* conformers are -322.024 389 411 23 , -323.077 294 651 66 , -323.899 708 0 , -322.017 023 634 39 , and -323.073 647 720 70 .

reasoning is well supported by AM1 calculations made by Ford and Wang⁷ and by Figure 2, which shows how the energy of β -alanine in water is predicted to change with rotational angle as computed at the HF/6-31G** level. The LMP2/cc-pVTZ(-f) procedure gave very similar results, as shown in Table 2. To be sure, the *gauche* conformer is predicted to be more stable in aqueous solution than the *trans* conformer by 2–3 kcal/mol, but this is a relatively small discrepancy compared with the more than 20 kcal/mol difference between the conformers predicted for the gas phase. Clearly the solvation energy of the *trans* form is enormously greater than that for the *gauche*. The calculations gave no definitive evidence for or against internal hydrogen bond formation in water solution. For small molecules in the gas phase, it is claimed²⁶ that conformational differences can be calculated to $+0.37$ kcal/mol compared to experiment. For solutions as calculated here, the most likely possible shortcoming for the QM methods is the use of a continuum solvation model which does not include any explicit solvent molecules. For aqueous solutions containing β -alanine, there may be a structured solvent shell which favors the *gauche* conformation. More detailed QM calculations on cluster models, which include explicit water molecules, could well lead to closer agreement with experiment.

In an effort to learn more about the influence of the aqueous solvent, we determined the coupling constants of the dipolar ion of β -alanine in solutions with 5 M concentrations of “salting-

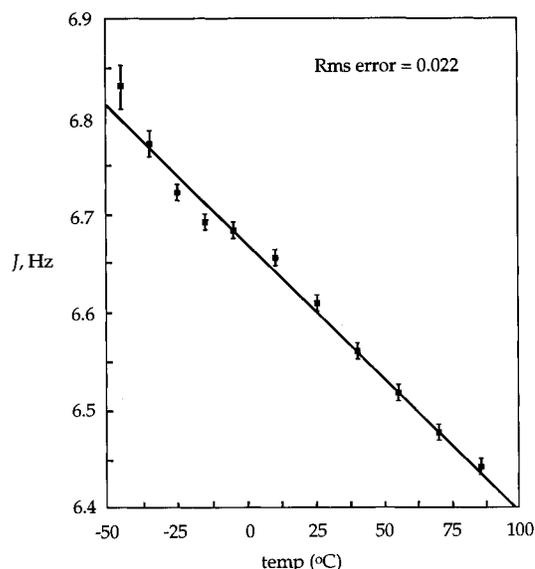


Figure 3. Variation of the $^3J_{\text{HH}}$ coupling constant of β -alanine as a function of temperature in 64/36 dimethyl sulfoxide/water solution.

in” or “salting-out” substances, such as guanidinium chloride, lithium chloride, and guanidinium perchlorate. None of these salts changed the observed coupling constants by more than 10%. The significance of these experiments is not wholly clear, but it must follow that the ions so introduced either do not complex strongly with β -alanine or that whatever changes they make in water structure are not ones that create significant differential solvation of the *gauche* and *trans* conformers of β -alanine.

While it is clear from what has been said so far that ΔG for the equilibrium between the conformers is nearly zero, the question remains as to the relative magnitudes of ΔH and $T\Delta S$. To this end, we have investigated the temperature dependence of the β -alanine conformational equilibrium. To be able to study this over a substantial temperature range, a 64/36 dimethyl sulfoxide (DMSO)/D₂O mixture was chosen as solvent, because it allowed a much larger operating range than water by not freezing at temperatures down to -50°C . We have measured the proton–proton coupling constant of β -alanine from -45°C to 85°C . The solvent becomes quite viscous at low temperatures,²⁷ and the viscosity changes result in substantial broadening and overlap of the individual triplet CH₂ resonances. The consequence is to make the line separations smaller than they would be if there were no overlap. It was possible to correct for the overlap by matching the experimental line shapes to ones generated by our version of LAOCN3¹² with different J values and different line widths. There was no evidence that the line broadening observed at the lower temperatures was associated with an increased multiplicity of the resonances beyond triplets. However, the line-broadening correction causes a greater uncertainty at the lower temperatures, as is reflected in the error bars in Figure 3.

The results show the temperature coefficient for the coupling constant to be -0.28 Hz/100°. To us, this was surprising. If we assume that J_{13} and J_{14} are independent of temperature (for comparison, $^3J_{\text{HH}}$ of ethanol changes about -0.04 Hz/100°)²³ and ascribe all of the observed increase in J over a 100° temperature change to an increase in the proportion of *gauche* conformer to 74%, then ΔH can be estimated as -1.9 kcal/mol and ΔS as about 6 eu. However, obtained in this way, the values

(26) Murphy, R. B.; Pollard, W. T.; Friesner, R. A. *J. Chem. Phys.* **1997**, *106*, 5073.

(27) Wei, A.; Raymond, M. K.; Roberts, J. D. *J. Am. Chem. Soc.* **1997**, *119*, 2915–2920.

of ΔH and ΔS must be regarded as maximal, because a -0.28 Hz change in the average of J_{13} and J_{14} corresponds in the Altona procedure to a change in the equilibrium percentage of *gauche* from 66% to 74%. Without other compensating changes, 74% corresponds to J_{13} being about 7.1 Hz and J_{14} being about 5.8 Hz and this combination of couplings at 300 MHz with a line width of 0.7 Hz would give a rather clear AA'BB' rather than an A₂B₂ spectrum. With a 0.7 Hz line width, we should be able to detect a change from 66% *gauche* to more than 71.5% or more of *gauche*.

Is this estimate of ΔS for the conformational equilibrium in water of <6 eu reasonable? The theoretical calculations indicate that the solvation energy of the *trans* conformation must be larger than that of the *gauche* by 10 or more kcal/mol. It is difficult to see how this could be so without a substantial difference in the entropies of solvation of the *trans* and *gauche* conformations. A larger *trans* solvation energy would seem to correspond to a greater tying down of water molecules, as is seen in the solvation of inorganic ions, where $\Delta H(\text{aq})$ values for Li⁺ and Na⁺ are -66 and -57 kcal, respectively, while the corresponding $\Delta S(\text{aq})$ values are 3 and 36 eu. Here, Professor J. D. Dunitz has suggested that there could well be an enthalpy–entropy compensation of the type he has shown to be possible for hydrogen bonding in water solutions, which would tend to minimize small enthalpy differences and lead to more nearly statistical distributions of the conformers than might otherwise be expected.²⁸

Another critical test of theoretical predictions with respect to the conformational preferences of β -alanine should be provided by changes in the solvent. Unfortunately, β -alanine is not soluble enough in nonpolar solvents such as tetrahydrofuran, chloroform, ethyl acetate, diglyme, or anhydrous dimethyl sulfoxide to allow observation of useful proton NMR signals. From simple electrostatics, we could well expect that solvents with lower dielectric constants than that of water should favor the *gauche* conformation, although there is substantial experimental evidence for the 1,2-dihaloethanes²⁹ that the opposite is true. To try to sort this out, we investigated the change in coupling constant of the triplet β -alanine proton spectrum with solvent composition in methanol and ethanol solutions containing variable amounts of deuterium oxide. At low water concentrations, the solubility of β -alanine is small, but it is still possible to obtain useful spectra. The results are shown in Figure 4, and as with temperature, the coupling constant shows the small change of -0.31 Hz with solvent dielectric constant $\epsilon = 80$ in water to $\epsilon = 30$ in deuteriomethanol and -0.42 Hz to $\epsilon = 32$ in 52% D₂O/48% ethanol. For comparison, $^3J_{\text{HH}}$ of ethanol changes by -0.09 Hz from D₂O to neat ethanol with a dielectric constant of 25.²³ If all of the -0.42 Hz change of coupling between D₂O and the D₂O–ethanol as solvent is attributed solely to a conformational equilibrium change in the average of J_{13} and J_{14} , the Altona procedure can be used to estimate that this corresponds to a change from 66% *gauche* to 78% *gauche*. However, the corresponding required change in J_{13} and J_{14} would be to 7.25 and 5.30 Hz, respectively, and these couplings with a line width of 0.7 Hz would give a very clear AA'BB' spectrum. Because we only observe triplet spectra, the change in equilibrium proportions of *gauche* is likely to be smaller than to 72% *gauche*.

The way the energies of the conformations of β -alanine were calculated for water solution as shown in Figure 2 takes into account the effects of the solvent by using a continuum solvent

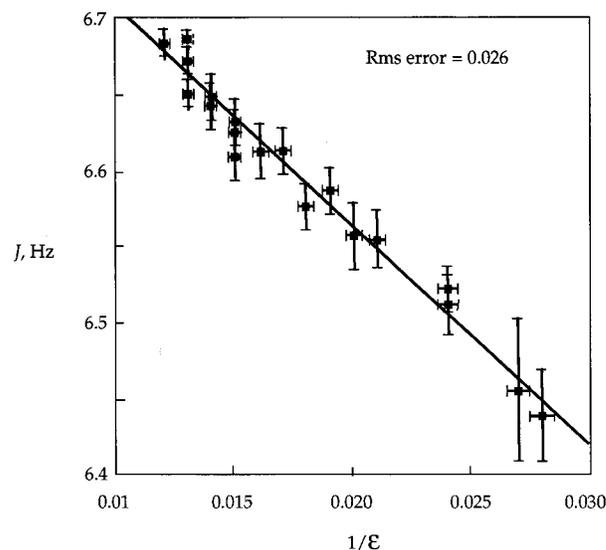
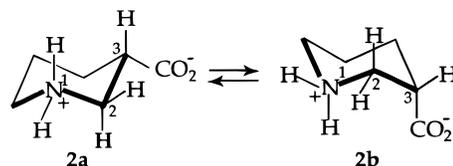


Figure 4. Variation of the $^3J_{\text{HH}}$ coupling constant of β -alanine as a function of the reciprocal of the dielectric constant in ethanol-*d*-D₂O mixtures.

description with a dielectric constant ϵ . Such a procedure will not predict a decrease in the energy of the *trans* conformer relative to the *gauche* in simple proportion to the change in dielectric constant, but some significant influence might be expected on the conformational equilibrium for the change of $\epsilon = 80$ to $\epsilon = 32$ as for 52% D₂O/48% ethanol or $\epsilon = 30$ for methanol. However, the fact is that the predicted difference with dielectric constant from 80 to 30 is quite small. Calculations (Table 2) by HF/6-31G** predict a *gauche*–*trans* energy difference of 2.38 kcal/mol when $\epsilon = 80$ and 2.75 kcal/mol at $\epsilon = 30$. With the larger basis set of LMP/2cc-pVTZ, the corresponding values are 2.91 and 3.23 kcal/mol. Despite the substantial change in ϵ , the energy difference only changes by about 0.4 kcal, which provides confidence in the physical model used for the calculations.

Although not directly applicable to solvent effects on the conformational equilibrium of the dipolar ion, it is interesting that the conformational equilibrium of the ethyl ester hydrochloride of **1** shows less than 0.5 Hz change in the simple triplet couplings over the range of solvents, methanol, dimethyl sulfoxide, trichloromethane, and tetrahydrofuran.

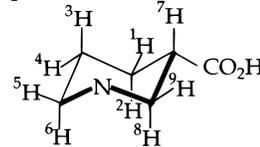
It has been suggested that a possible but improbable-sounding explanation of the pattern of our results would be to have the proton–proton couplings of β -alanine fail to follow a Karplus-type relation with the rotational angle ϕ because of the unusual +, – character of the substituent groups. This possibility is inconsistent with the fact that the coupling constants do not change appreciably with pH and concomitant formation of the conjugate acid or conjugate base of β -alanine (Table 1) where the +, – character of the substituents is not present. Furthermore, analysis of the proton–proton couplings in nipecotic acid **2a** \rightleftharpoons **2b** indicates nothing unusual. The crux of the argument



for **2** has to do with the couplings between the protons on C2 and the one on C3. These couplings of 9.15 and 3.84 Hz are as expected for **2a** with the carboxylate being predominantly

(28) Dunitz, J. D. *Chem. Biol.* **1995**, *2*, 709–712.

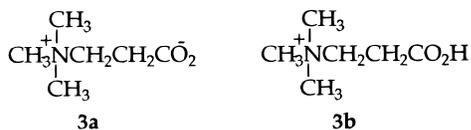
(29) Abraham, R. J.; Gatti, G. J. *Chem. Soc. B* **1969**, 961–967.

Table 3. Coupling Constants in Hertz for Nipepic Acid **2** as a Function of pH in D₂O


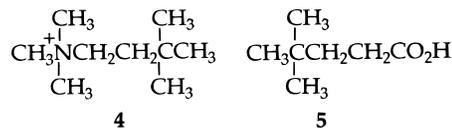
2J	$^3J_{a,a}$	$^3J_{a,e}$	$^3J_{e,e}$	4J
pH = 1.0				
-13.43 (1,2)	7.73 (2,3)	4.94 (1,3)	3.28 (1,4)	0.56 (1,5)
-13.92 (3,4)	7.73 (2,7)	4.94 (2,4)	3.28 (4,5)	0.56 (1,9)
-13.04 (5,6)	9.23 (3,6)	4.19 (3,5)		
-12.91 (8,9)	8.71 (7,8)	2.37 (4,6)		
		5.06 (1,7)		
		4.19 (7,9)		
pH = 7.4				
-13.65 (1,2)	9.15 (2,3)	5.36 (1,3)	3.85 (1,4)	0.28 (1,5)
-12.61 (3,4)	9.15 (2,7)	3.85 (2,4)	3.77 (4,5)	
-12.61 (5,6)	9.15 (3,6)	5.36 (3,5)		
-12.62 (8,9)	9.15 (7,8)	4.58 (4,6)		
		4.29 (1,7)		
		3.84 (7,9)		
pH = 11.3				
-11.42 (1,2)	7.14 (2,3)	4.42 (1,3)	3.27 (1,4)	
-11.42 (3,4)	9.22 (2,7)	4.77 (2,4)	2.79 (4,5)	
-13.98 (5,6)	12.27 (3,6)	4.23 (3,5)		
-12.25 (8,9)	10.76 (7,8)	5.02 (4,6)		
		4.42 (1,7)		
		3.41 (7,9)		

in the equatorial position. Clearly, if there were to be some unexpected strong substituent influence to give either the statistical equilibrium of conformers or negate the Karplus relationship for the dipolar form of β -alanine, it disappears in **2**. A complete analysis of all the proton-proton couplings in the very complex spectrum of **2** is given in Table 3. There seems to be a significant change in the conformational equilibrium of **2** with increasing pH as judged by the progression of J values (7,8) and (7,9) in Table 3. However, quantitation of the changes in percent of equatorial CO₂⁻ by the Altona procedure²³ was not satisfactory because the (7,8) and (7,9) couplings each gave more than 100% equatorial. In this respect, nipepic acid differs from β -alanine (compare Table 1 and Table 3).

In an attempt to throw more light on the basis for the lack of conformational preference of β -alanine, we have investigated the proton spectrum of *N,N,N*-trimethyl- β -alanine **3** in neutral and acidic aqueous solutions and also in other solvents, fortunately in which **3a** is more soluble than β -alanine.

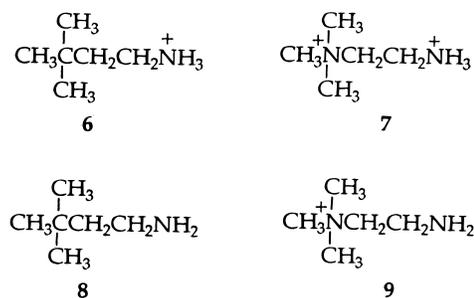


With **3a**, we expected to see a substantial steric effect, because Abraham and co-workers⁵ report that the 3,3-dimethylbutyl-*N,N,N*-trimethylammonium cation **4** exists exclusively in the *trans* conformation and Whitesides and co-workers³⁰ have shown, and we concur, that there is a smaller but real preference for *trans* (~70%) with salts of 4,4-dimethylpentanoic acid **5**. It is significant that **3a** and **3b** are isosteres of **5** and its conjugate base.



Again, we were surprised to find that *N,N,N*-trimethyl- β -alanine as **3a** or **3b** does not display strong conformational preferences. Indeed, an analysis similar to that made for β -alanine (compare Table 4 with Table 1) suggests that **3a** has no more conformational preference than does β -alanine. This calculation used an empirical electronegativity²³ of 0.81 obtained for the (CH₃)₃N⁺ group as determined from the $^3J_{HH}$ coupling constant (7.33 Hz) of the ethyl group of the (CH₃)₃⁺NCH₂CH₃ cation. This value is very close to the 0.82 used for the NH₃⁺ group so the predicted couplings in Table 1 are quite close to those of β -alanine. It is interesting that, despite the large *N,N,N*-trimethyl group, the change in the $^3J_{HH}$ coupling of **3a** was observed to be only -0.43 Hz/100° compared to -0.28 Hz/100° for β -alanine. Also solutions of **3a** in ethanol show the same triplet spectrum as in D₂O, so just as with β -alanine, the effect of changing solvent dielectric constant is small. Further, the same generally small changes with pH in $^3J_{HH}$ observed for **1** and **3a** were also exhibited by *N,N*-diethyl- β -alanine. With this substance, the dipolar ion, its conjugate base, and its conjugate acid show simple triplet spectra in D₂O with respective $^3J_{HH}$ values of 6.98, 7.3, and 6.96 Hz. In this connection, it should be noted that when a long straight-chain alkyl group such as CH₃(CH₂)₁₁ is substituted for one of the methyl groups of **3a**, the *trans* conformation is favored for both of the -CH₂-CH₂- chains directly attached to the nitrogen.³¹ This would seem to be very largely a steric effect. We were unable to obtain useful material to determine a crystal structure of **3a**. However, the chloride salt of **3b** gave satisfactory crystals which are of the *trans* conformation (see Figure 5).

The lack of correlation of the positions of conformational equilibria of **3** and **4** is particularly surprising when comparisons are made between compounds **6** and **7**, as well as **8** and **9**. Here,



there are rather close correspondences, and we can calculate from $^3J_{HH}$ couplings that **6** and **7** are (9 ± 1)%³⁰ and (13 ± 3)% *gauche*, respectively, while **8** and **9** are (23 ± 7)%³⁰ and (19 ± 2)% *gauche*, respectively. One possibility for the differences might be in the mode of solvation. The amine and ammonium groups of **6**–**9** are different from the carboxyl and carboxylate groups of **3** and **5** in that the critical atoms being solvated are much closer to the bulky methyl groups. As a result, steric hindrance to solvation of the *gauche* conformers should be greater than for the *trans* conformers with these substituents.

Further interesting comparisons to **1** and **3** are provided by taurine **10** and its *N,N,N*-trimethyl¹¹ derivative **11**. Prior studies

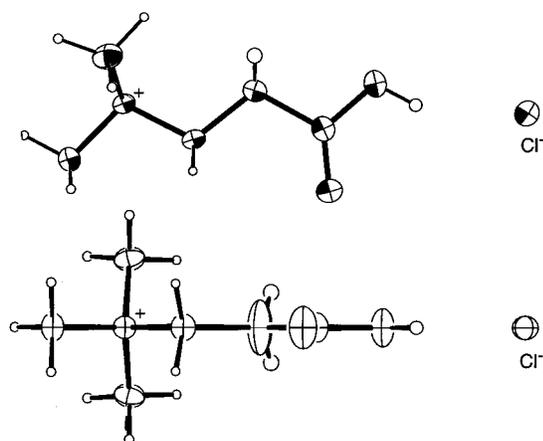
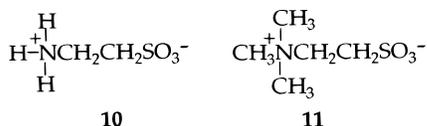
(30) Whitesides, G. M.; Sevenair, J. P.; Goetz, R. W. *J. Am. Chem. Soc.* **1967**, *89*, 1135–1144.

(31) Weers, J. G.; Rathman, J. F.; Axe, F. U.; Crichlow, C. A.; Foland, L. D.; Scheuing, D. R.; Wiersema, R. J.; Zielske, A. G. *Langmuir* **1991**, *7*, 854–867.

Table 4. Predicted Vicinal Proton-Proton Coupling Constants^a and Calculated *Gauche* Populations for Perfectly Staggered *Gauche* and *Trans* Conformations of Taurine **10** and Its Conjugate Acid and Base and for *N,N,N*-Trimethyltaurine **11** and Its Conjugate Acid

J'_t	J'_g	J''_t	J''_g	$J''_{g'}$	J_{13}	J_{14}	% <i>gauche</i> , calcd ^b
13.41	4.07	13.41	3.44	2.81	6.77 ^c 6.76 ^d	6.77 ^c 6.72 ^d	66, 67
13.51	4.05	13.51	3.46	2.88	6.81 ^c 6.62 ^d	6.81 ^c 6.25 ^d	62, 73
13.03	4.22	13.03	3.29	2.36	6.54 ^c 5.93 ^d	6.54 ^c 7.41 ^d	49, 58 ^e 92, 73 ^e
13.42	4.07	13.42	3.45	2.83	6.77 ^c 4.68 ^d	6.77 ^c 11.63 ^d	16, 8
13.53	4.05	13.53	3.47	2.89	6.82 ^c 4.70 ^d	6.82 ^c 12.66 ^d	15, 18

^a Procedure of Altona.²³ ^b The first value given is calculated from J_{13} and the second from J_{14} . ^c Average calculated values for a 1:1 mixture of *gauche* and *trans* conformations. ^d Experimental values. ^e Ambiguous assignments; see text.

**Figure 5.** Two views of the conformation of *N,N,N*-trimethyl- β -alanine chloride in a crystal. Structure determination supplied by Dr. Michael Day of the Beckman Institute, California Institute of Technology.

of **10** gave somewhat mixed messages as to what one should expect for its conformational equilibria.³²⁻³⁷ To begin with, we determined the empirical electronegativities²³ for SO_3H and

(32) Okaya, Y. *Acta Crystallogr.* **1966**, *21*, 726-735.

(33) Ohno, K.; Mandai, Y.; Matsuura, H. *J. Mol. Struct.* **1992**, *268*, 41-50.

(34) Ham, N. S. *Molecular and Quantum Pharmacology*; D. Reidel Publishing Co.: Dordrecht, Holland, 1974; pp 261-268.

(35) Braghiroli, D.; Di Bella, M. *Farmaco* **1995**, *50*, 555-557.

(36) Fugler-Domenico, L.; Russell, C. S.; Sapse, A. M. *Biol. Amines* **1989**, *6*, 289-299.

(37) Jauregui, E. A.; Ciuffo, G. M.; Estrada, M. R.; Santillan, M. B. *Acta Cient. Venez.* **1984**, *35*, 337-341.

SO_3^- from $^3J_{\text{HH}}$ of ethanesulfonic acid respectively in strong acid or alkaline solutions, 7.47 Hz, which yields $\lambda_{\text{SO}_3\text{H}} = 0.53$ and 7.44 Hz, for which $\lambda_{\text{SO}_3^-} = 0.59$. Proceeding as with **1** and **3**, we obtained the data shown in Table 4. For taurine and its conjugate acid, the pattern is virtually identical with that of β -alanine, despite the fact that the sulfonic acid group is relatively bulky. Only when we turn to the conjugate base of **10** do we see what appears to be preference for the *trans* conformer. However, the situation here is not wholly clear-cut because there is an ambiguity with the conjugate base of **10** in that we do not know for sure which of the pair of $^3J_{\text{HH}}$ couplings is actually J_{13} and which is J_{14} . Conformational ambiguity is always possible in $\text{X}-\text{CH}_2-\text{CH}_2-\text{Y}$ systems when both J_{13} and J_{14} are less than $(J''_g + J''_t)/2$, a term which in our systems is about 8.4 Hz. In the compounds encountered earlier, the J_{13} and J_{14} couplings were indistinguishable except as averages, so the ambiguities were not important. For the conjugate base of taurine, one way of assigning J_{13} and J_{14} has $J_{14} > J_{13}$, giving 49% and 58% *gauche* for perfectly staggered conformations, which averages to 53%. For $J_{13} > J_{14}$, the corresponding figures are 92% and 73% *gauche*, with an average of 80%. We regard the figures obtained with $J_{14} > J_{13}$ as intuitively more reasonable but have no other basis for making a selection.

There is no ambiguity for the two species of **11**, where the *trans* conformations are clearly preferred. With these substances, of course, we are approaching a situation somewhat more like **4** as regards steric hindrance. Thus, there are sizable tetrahedral groups at one end of the CH_2-CH_2 linkage interacting with sizable tetrahedral groups at the other end. Again, we note that, as with **3a**, when a long straight-chain alkyl group such as $\text{CH}_3(\text{CH}_2)_{11}$ is substituted for one of the methyl groups of **11**, the *trans* conformation is favored for both of the CH_2-CH_2 chains directly attached to the nitrogen.³¹

The overall conclusion that we draw is that there is still a need for better understanding of why many of the compounds studied here have small conformational preferences as compared with most of the substances studied in aqueous solution by Whitesides,³⁰ Abraham,⁵ and their co-workers. Perhaps these differences can be accounted for by the kind of quantum-mechanical calculations that we have employed here to study the conformational equilibrium of β -alanine.

Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this research. We are also deeply indebted to the Summer Undergraduate Research Fellowship Program (SURF), the Beckman Institute of the California Institute of Technology, the E. I. Du Pont Company, and Dr. and Mrs. Chester M. McCloskey for their helpful financial assistance. Helpful suggestions were supplied by Professor J. D. Dunitz. The quantum-chemistry calculations reported here were funded by DOE-BCTR (DE-FG36-93CH105 81, David Boron). The facilities of the MSC are also supported by grants from NSF (CHE 95-22179 and ASC 92-100368), Chevron Petroleum Technology Co., Saudi Aramco, Asahi Chemical, Owens-Corning, Exxon, Chevron Chemical Co., Chevron Research and Technology Co., Avery-Dennison, Hercules, BP Chemical, and the Beckman Institute.

JA974311U