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Conformational Analysis of Charged Flexible Molecules in Water by Application of a New Karplus Equation Combined with MM2 Computations: Conformations of Carnitine and Acetylcarnitine

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Abstract: The solution conformations of carnitine and acetylcarnitine in D_2O are estimated from high-resolution ^1H NMR coupling data. Conformations and populations of conformers are calculated from vicinal coupling constants by employing a new Karplus relationship with empirically derived substituent constants (Colucci, W. J.; Jungk, S. J.; Gandour, R. D. *Magn. Reson. Chem.* **1985**, *23*, 335–343). For this study, substituent constants are assigned for the monosubstituted ethanes: XCH_2CH_3, where X = –COOH, –OAc, –OH, $-N^+Me_3$, $-CH_2N^+Me_3$, $-CH_2COOH$. Solvent effects are accounted for by measuring the coupling constants of the monosubstituted ethanes under the same conditions employed in the measurement of the carnitine and acetylcarnitine spectra. The assignment of diasteriotopic protons of carnitine and acetylcarnitine is made by analogy with previous experimental work and comparison with the lowest energy conformation as determined by molecular mechanics (MM2) calculations parameterized with atomic charges from ab initio (3-21G) calculations. The vicinal coupling constants are then used to calculate populations of conformers, arising from rotation about the C2–C3 and C3–C4 bonds, in solution. Both compounds adopt a highly preferred g^- conformation about the N1–C4–C3–O3 torsion angle. In contrast, the C1–C2–C3–C4 torsion angle exhibits substantial rotational freedom between g^- and a. In carnitine, the anti conformer dominates, regardless of the solution pD, whereas in acetylcarnitine the g^- conformer is most prevalent. In either compound, ionization from cation to zwitterion results in a net population increase of g^-,g^- (folded) conformer over the a,g^- (extended) conformer. The relative energetics of extended and folded conformers suggest that binding of either carnitine or acetylcarnitine to the enzyme carnitine acetyltransferase occurs with the folded form.

The problem of how to determine the conformation of flexible molecules in solution has challenged researchers for decades and will likely continue to do so. The problem is even more difficult for flexible molecules that are either quite polar or charged, especially if these molecules interact with the solvent. The techniques of NMR spectroscopy[1] and computational chemistry,[2] especially when used in combination,[3] have facilitated considerable progress in conformational analysis during the past two decades. Even so the task of assigning meaningful numbers to conformations and their populations is still in the early stages of development.

In NMR spectroscopy, the Karplus equation[4] has enabled conformational analysis of simple organic molecules in solution. Extensions of this equation by Pachler[5] and others[6] have permitted broader applications. We have recently introduced[7] a Karplus equation with empirically derived substituent constants, eq 1, which makes possible an explicit accounting of solvent effects.

$$^3J(\mathrm{HCCH}) = A + B\cos\theta + C\cos 2\theta + \sum_{i=1}^{4}\Delta S_i \cos\theta\cos\phi_{\mathrm{HX}_i} \quad (1)$$

Equation 1 shows the dependence of vicinal coupling constants, ^3J(HCCH) on θ, the torsion angle between vicinal hydrogens, and ϕ_{HX_i}, the torsion angle(s) between hydrogen and substituent(s) X_i.[7] The expression is the Karplus equation[8] adjusted for the effect of substituents and their orientation. For simplicity and ease of calculation, ϕ_{HX} has been replaced by $\theta \pm 120$ as shown in Figure 1. Substituent constants, ΔS_i, have been determined from ^1H NMR data.

Integration of eq 1 for a monosubstituted ethane yields an expression, eq 2, that describes the average coupling in a freely

$$\langle {}^3J\rangle = A - 0.25\Delta S_{\mathrm{X}} \quad (2)$$

rotating ethyl group. When the value of A is known, eq 2 affords a means of rapidly assigning a substituent data set for any compound containing HCCH fragments. Since ΔS_i values are defined as $S_X - S_H$ (substituent X – hydrogen), A is the observed coupling in ethane. This equation allows for the generation of a novel

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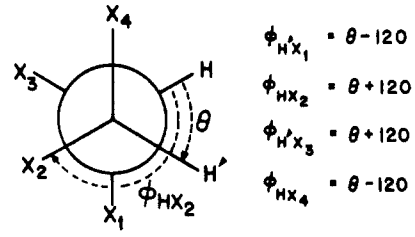

Figure 1. Newman projection showing the relationship between θ and ϕ angles.

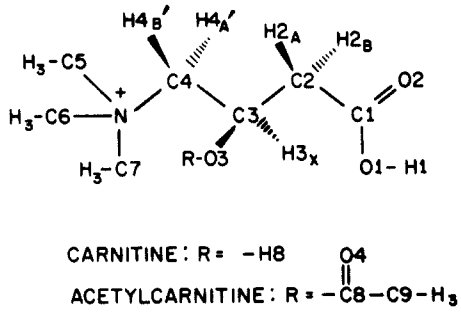

Figure 2. Name conventions for the atoms of carnitine and acetylcarnitine.

substituent data set based on the observed coupling in monosubstituted ethanes. Solvent effects on ^3J can be accounted for by measuring the coupling constant of representative monosubstituted ethanes under the same conditions in which ^3J is measured in the compound of interest.

In this report, eq 1 has been utilized to determine the solution conformation of two biologically important molecules, carnitine and acetylcarnitine (see Figure 2). Carnitine, bound to one or more translocase proteins, functions as a carrier of fatty acyl groups across the inner mitochondrial membrane.[9] In addition, a specific enzyme, carnitine acetyltransferase, catalyzes the reversible transfer of acetyl groups from acetylcarnitine to coenzyme A.[10] Chemical mechanisms for the translocase and transferase systems have been proposed based on suggested conformational preferences of carnitine and acetylcarnitine.[11-13] These chemical mechanisms have emphasized widely differing importance to the potential charge interaction between quaternary ammonium and carboxylato groups.

Both carnitine and acetylcarnitine can exist as neutral (zwitterion) or protonated (cation) forms, depending upon pH. A detailed X-ray study of zwitterion vs. hydrochloride crystal structures has revealed a minimal effect of charge state on conformation in the solid state.[13] Since the crystalline structures may not coincide with the solution structures, a detailed study of the conformations of carnitine and acetylcarnitine in solution has been undertaken. The ^{1}H NMR data for carnitine have been reported previously by Agostini et al.,[14] while the data for acetylcarnitine are reported herein.

This study is significant because it applies to these highly charged molecules in water, the new Karplus relationship in combination with molecular mechanics (MM2), modified to handle these charged species by utilizing atomic point charges determined by ab initio calculations. This combination of computational and experimental techniques provides a useful approach for elucidating conformations of charged flexible molecules in solution. As such, this study should be viewed as the initial report of the application of this combined approach toward the long-range goal of developing a method to quantitatively determine structures in solution.

Table I. Chemical Shifts (ppm), Coupling Constants (Hz), and Substituent Constants (Hz) for the Ethyl Group in Selected Monosubstituted Ethanes Measured at 200 MHz

substituent	pD	$\delta(-CH_3)^a$	$\delta(-CH_2-)^a$	^3J(HCCH)	ΔS (A = 8.37)
-COOH	2.76	1.09	2.39	7.59	3.12
	6.44	1.06	2.19	7.71	2.64
	10.05	1.05	2.18	7.66	2.84
-OAc	1.34	1.24	4.13	7.13	4.96
	7.55	1.26	4.15	7.18	4.76
	10.80	1.26	4.15	7.18	4.76
-OH	2.53	1.16	3.64	7.10	5.08
	7.40	1.17	3.64	7.08	5.16
	9.66	1.18	3.65	7.10	5.08
$-N^+Me_3$	2.41	1.36	3.39	7.33	4.16
	7.03	1.38	3.42	7.30	4.28
	10.58	1.38	3.43	7.34	4.12
$-CH_2N^+Me_3$	3.19	0.96	1.79	7.34	4.12
	6.62	0.98	1.81	7.36	4.04
	10.16	0.98	1.81	7.33	4.16
$-CH_2COOH$	2.75	0.92	1.60	7.44	3.72
	6.41	0.90	1.56	7.49	3.52
	10.34	0.90	1.56	7.56	3.24

[a] Chemical shifts are referenced to internal standard sodium 3-trimethylsilylpropionate-d_4 (TSP-d_4).

Conformational preferences obtained through MM2 studies are invaluable for assigning the diasteriotopic protons of carnitine and acetylcarnitine. The N1-C4-C3-O3 torsion angle (see Figure 2) assumes a gauche conformation in similar compounds such as choline;[15] therefore, the assignment of H4A' and H4B' is straightforward. Since the C1-C2-C3-C4 torsion angle, on the other hand, exhibits much greater conformational freedom, assignment of H2A and H2B requires either specific labeling or knowledge of the conformational preference. These modified MM2 calculations can provide this knowledge.

Experimental Section

Materials. (*R,S*)-Acetylcarnitine hydrochloride was prepared from racemic carnitine (Aldrich Chemical Co.) by "method A" of Ziegler, Bruckner, and Binon[16] and crystallized by vapor diffusion of acetone into a saturated methanolic solution.

The quaternary ammonium iodides were synthesized from their alkyl iodides (Aldrich Chemical Co.). Iodoethane (or iodopropane) (0.05 mol) was added to a mixture containing 60 mL of 20% aqueous trimethylamine (Aldrich Chemical Co.) and 20 mL of THF (5 mol excess amine). The biphasic reaction mixture was stirred vigorously at 50 °C until clear (about 30 min). The reaction mixture was then evaporated to dryness at reduced pressure and 70 °C. *N,N,N*-Trimethylethanaminium iodide was recrystallized twice from absolute ethanol (7.73 g, 80%): mp 322 °C dec, (lit.[17] mp 320-322 °C dec); ^{1}H NMR (ref to TSP-d_4 in D_2O) 3.42 (q, 2 H), 3.12 (s, 9 H), and 1.38 (tt, 3 H).[18] *N,N,N*-Trimethylpropanaminium iodide was recrystallized twice from anhydrous isopropyl alcohol (9.06 g, 77%): mp 192.0-192.5 °C (lit.[19] mp 188-189 °C); ^{1}H NMR (ref to TSP-d_4 in D_2O) 3.30 (m, 2 H), 3.13 (s, 9 H), 1.81 (m, 2 H), and 0.98 (t, 3 H).[20]

^{1}H NMR. The 200-MHz spectra were recorded on a Bruker WP-200 FT-NMR at 300 K. Extra precision was required of the spectra for compounds in the substituent data set to yield very accurate coupling constants. The instrument was configured to give a maximum digital

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resolution of 0.085 Hz/pt using a 32 K block size.

Samples of the monosubstituted ethanes (Table I) were prepared as 5% solutions in deuterium oxide. All compounds were obtained from commercial sources and used as received, with the exception of the quaternary amines which were prepared as previously described. The only detectable impurity was the residual water (HDO), which posed no problem for spectral interpretation. The pD of all samples was adjusted to acidic, neutral, or basic conditions with 20% DCl and 40% NaOD in D_2O using a Beckman Model 4500 digital pH meter equipped with an Ingold Model 6030-0 NMR tube electrode. Solution pD was calculated as meter reading +0.4 unit.

Peak positions (relative to TSP-d_4) for the ethyl fragment of each compound were determined from a digital printout. All spectra reduced to nearly first-order systems when measured at 200 MHz (ref Table I for chemical shifts and coupling constants). Coupling constants were interpreted rigorously according to the method of McGarvey and Slomp.[21]

Samples for NMR of acetylcarnitine hydrochloride were prepared by dissolving 40 mg in 0.5 mL of D_2O and adjusting the pD as described above. The spectra were recorded at both acidic and neutral pD, the former to form cation, and the latter, zwitterion. (The pK_A's of carnitine and acetylcarnitine are 3.80 and 3.60, respectively.)[22]

The 200-MHz spectra of acetylcarnitine were measured at digital resolutions of 0.085 and 0.092 Hz/pt using a 32 K block size. Peak positions (relative to *tert*-butyl alcohol, δ 1.28 ppm) were determined from a digital printout.

Spectral Simulations. Chemical shifts and coupling constants for acetylcarnitine were calculated by best fit to peak positions. These spectral simulations were performed on a Bruker ASPECT 2000 computer using the Bruker PANIC (1981) program, a minicomputer version of the LAOCOON-type program[23] used in larger computers. The root-mean-square errors for the fits were 0.034 and 0.041 for acetylcarnitine measured at pD 2.13 and 6.49, respectively.

Computational Methods

Molecular Mechanics. The molecular mechanics program (MM2)[24] was obtained from Quantum Chemical Program Exchange (No. 395). The program was modified to include a dielectric constant in the calculation of charge interaction energies according to eq 3, the classic Coulombic-type potential function used in similar applications.[25]

$$E_c = kq_iq_j/\epsilon r_{ij} \quad (3)$$

The variables q_i and q_j are the point charges centered on atoms i and j, ϵ is the effective dielectric constant, and r_{ij} is the distance between atoms i and j. The program only computes interactions between atoms 1,4 and greater. A range of dielectric constants have been evaluated but only the results at the dielectric constant of water at 25 °C, ϵ = 78.3, are presented in detail.

The use of an electrostatic function was preferred over a dipole interaction energy. Dipole values were not available for many bond types found in carnitine, whereas point electronic charges could be determined from ab initio calculations as described below.

The MM2 force field was further modified to include a Morse potential (eq 4) in place of the cubic function (eq 5) used to describe

$$E_s = \tfrac{1}{2}k_s[1 - \exp(-a\Delta R)]^2 \quad (4)$$

$$E_s = \tfrac{1}{2}k_s(\Delta R^2 + C_s\Delta R^3) \quad (5)$$

bond-stretch energies. The Morse potential is recognized by Allinger as an alternative to the cubic function.[2] In eq 4 and 5, ΔR is the difference in the observed bond length from its equilibrium value. The stretching force constant, k_s, has the same value in both equations. The exponential constant, a, is 1.00; from this a value of −2.00 is obtained for C_s, the cubic stretch constant.

Development of Force-Field Parameters. Parameters for the quaternary ammonium and carboxylato groups (shown in Table II) were chosen principally to reproduce distances and angles of the carnitine and acetylcarnitine crystal structures. Default values were used for all other parameters not cited in Table II. The quaternary nitrogen stretching, bending, and torsion angle parameters were obtained from an analysis done on the crystal structures of several quaternary amines.[26] The

Table II. Force Constants Used in the Molecular Mechanic Calculations of Carnitine and Acetylcarnitine

stretching	l_0 (Å)	k_s (mdyn/Å)
C_{sp^3}–$N^+_{sp^3}$	1.48	5.10
C_{SP^2}–O δ-	1.24	7.90
bending	θ_0 (deg)	K_b (mdyn Å/rad^2)
C_{sp^3}–$N^+_{sp^3}$–C_{sp^3}	107.7	0.630
C_{sp^3}–C_{sp^3}–O δ^-	117.9	0.500
δ^- O–C_{sp^3}–O δ^-	124.2	0.800
torsion	V_1[a]	V_2[a]	V_3[a]
O_{sp^3}–C_{sp^3}–C_{sp^3}–$N^+_{sp^3}$	−0.533	−0.267	1.133
C_{sp^3}–C_{sp^3}–$N^+_{sp^3}$–C_{sp^3}	−0.200	0.450	0.800
H–C_{sp^3}–C_{sp^2}–O δ^-	−0.167	0.000	−0.100
C_{sp^3}–C_{sp^3}–C_{sp^2}–O δ^-	−0.300	1.210	−0.350
van der Waals	r^* (Å)	ϵ (kcal/mol)	
O δ^-[b]	1.74	0.050	

[a] In kcal/(mol·deg). [b] O δ^- is the oxygen atom of the resonant carboxylate.

O_{sp^3}–C_{sp^3}–C_{sp^3}–N_{sp^3} torsion angle function was similar to related systems such as O_{sp^3}–C_{sp^3}–C_{sp^3}–O_{sp^3} and N_{sp^3}–C_{sp^3}–C_{sp^3}–N_{sp^3}. These systems show a minimum energy at approximately 60°, representing a considerable gauche effect, and a shallow minimum at 180°. The depth of the minimum well at 60° was adjusted to −0.60 kcal/mol, while the 0° barrier was set to 0.60 kcal/mol. Other values ranging from −1.0 to −0.5 and 0.4 to 0.8 kcal/mol, respectively, were investigated empirically, but these values most closely reproduced torsion angles found in the carnitine and acetylcarnitine crystal structures.

The parameters of the symmetrical carboxylate were also fitted to reproduce geometrical features of the crystal structures. The van der Waals parameter for O δ^- was the same as O_{sp^3}, while the torsion angle parameters H–C_{sp^3}–C_{sp^2}–O δ^- and C_{sp^3}–C_{sp^3}–C_{sp^2}–O δ^- were the same as their counterpart H–C_{sp^3}–C_{sp^2}=O_{sp^2} and C_{sp^3}–C_{sp^3}–C_{sp^2}=O_{sp^2} default values in the MM2 program.

Calculation of Atomic Point Charges. All charges were determined from calculations employing the GAUSSIAN 80 series of programs.[27] Charges[28] were obtained directly from the Mulliken population analysis.[29] Charges determined in this manner are subject to several uncertainties,[30] including an interdependence of charge and conformation, and a strong dependence upon the basis function.[31]

For carnitine and acetylcarnitine initial MO coefficients were determined at the STO-3G level.[32] These were subsequently used as an initial guess for calculations employing the split valence 3-21G basis set.[33] Because calculating the charges for all conformational states of carnitine and acetylcarnitine was impractical, single point calculations were performed only on coordinates determined from crystal structures.[13] However, all hydrogen positions were calculated because hydrogen atoms were poorly located by X-ray. The solid-state geometries correspond to the most stable conformations determined by molecular mechanics.

Results

Interpretation of the Acetylcarnitine Spectra. Absorptions for the protons of the C2 and C4 methylenes of acetylcarnitine illustrated in Figure 2 consisted of distinct eight-line patterns indicative of simple ABX systems. Chemical shifts, shown in Table III, for the diasteriotopic H4A′ and H4B′ protons were assigned so that the coupling constants (shown in Table IV), when manipulated by eq 1, agreed with a strong g^- conformational preference about the N1–C4–C3–O3 torsion angle. This assumption was in accordance with the observed conformational preference of similar systems such as choline and acetylcholine[34] and was

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Table III. ^{1}H NMR Chemical Shifts (ppm) for Carnitine and Acetylcarnitine Measured in Deuterium Oxide

		$-CH_2COOH$		$-CH_2N^+$		$-C(OR)Hx$	$-N(CH_3)_3$	$C(O)CH_3$
	pD	δH2A	δH2B	δH4A′	δH4B′	δH3X	$\delta(CH_3)_3$	$\delta(CH_3)$
acetylcarnitine zwitterion	6.49	2.67	2.55	3.65	3.89	5.63	3.24	2.18
acetylcarnitine cation	2.13	2.91	2.88	3.73	3.96	5.68	3.25	2.20
carnitine zwitterion[a]	5.02	2.47	2.42	3.42	3.44	4.57	3.22	
carnitine cation[a]	2.30	2.63	2.68	3.47	3.50	4.68	3.23	

[a] The data on carnitine were taken from ref 14, measured at 240 MHz.

Table IV. ^{1}H NMR Coupling Constants (Hz) for Carnitine and Acetylcarnitine Measured in Deuterium Oxide

		$-CH_2COOH$			$-CH_2N^+$		
	pD	$^2J_{AB}$	$^3J_{AX}$	$^3J_{BX}$	$^2J_{A'B'}$	$^3J_{A'X}$	$^3J_{B'X}$
acetylcarnitine zwitterion	6.49	15.46	5.64	7.68	14.45	1.13	8.89
acetylcarnitine cation	2.13	16.92	5.35	7.23	14.56	1.17	8.84
carnitine zwitterion[a]	5.02	15.65	7.26	6.04	14.22	1.92	9.13
carnitine cation[a]	2.30	16.25	7.82	5.08	14.10	1.87	9.83

[a] The data on carnitine were taken from ref 14, measured at 240 MHz.

Table V. Comparison of Torsion Angles for Minimum Energy Conformations of Acetylcarnitine and Carnitine Determined by X-ray and MM2

	torsion angle		
acetylcarnitine	C1–C2–C3–C4	N1–C4–C3–O3	C2–C3–O3–C8
crystal, zwitterion[a] monohydrate	−74.3	−83.1	−91.8
MM2 zwitterion	−70.5	−78.8	−84.8
crystal, cation hydrochloride	−71.4	−88.0	−78.8
crystal, cation hydrochloride·H_2O	−77.1	−83.7	−87.1
MM2 cation	−71.2	−79.0	−83.9
carnitine			
crystal, zwitterion	−171.6	−61.5	
MM2 zwitterion	−173.3	−68.7	
crystal, cation hydrochloride	−166.2	−66.1	
MM2 cation	−173.3	−68.7	

[a] See ref 13 for a summary of carnitine and acetylcarnitine crystal data.

further supported by both X-ray crystal data (Table V) and the MM2 calculations (Table VI).

Chemical shifts (Table III) for the diasteriotopic H2A and H2B protons were assigned in a similar fashion. Since both the X-ray data (Table V) and MM2 calculations (Table VI) suggested a preference for the g^- conformation about C1–C2–C3–C4, we assigned H2A and H2B so that calculation of populations from coupling constants gave a preference for the g^- conformation.

Carnitine Spectral Parameters. Agostini et al.[14] measured the 240-MHz spectra of carnitine in D_2O (data in Tables III and IV). The spectrum at pD 2.30 was used for the cation, while the spectrum at pD 5.02 was used for the zwitterion. The latter pD was selected because the spectrum under these conditions gave the best resolution of the nearly coincidental H2A and H2B protons, and therefore the most reliable coupling constants, while having a pD high enough to maintain the zwitterion. We determined the 200-MHz ^{1}H NMR spectra of carnitine in D_2O (not shown) and found good agreement with the 240-MHz spectra, except that at near neutral pD the C2 protons were not resolved by the lower field.

We used the parameters of Agostini et al. as interpreted, except for the spectrum at pD 5.02 where the assignments of H2A and H2B were reversed. Whereas they assigned H2B (H2A in their diagram) to the downfield signal, we assumed that these chemical shifts had crossed over at pD 4.03 where they coincided.

With their assignment, Agostini et al. found the g^- conformer as the most stable for the C1–C2–C3–C4 torsion angle of the zwitterion. Using the alternative assignment, we found the anti conformer as the most stable. Both the crystal structure of the zwitterion[13] and the MM2 calculations showed a preference for the anti conformation.

Another reason for rejecting the previous assignments for the chemical shifts of H2A and H2B in carnitine is the change in population that occurs upon ionization (see below). Because the molecule is charged and contains an ionizable group (carboxyl), the solution conformation is pH dependent. Using the assignments of Agostini et al. leads to a 20% increase in g^- conformer upon ionization; our assignment of H2A and H2B leads to a 9% increase, a value consistent with the MM2 result.

Substituent Constants. The 200-MHz data and substituent constants for the six monosubstituted ethanes, comprising the relevant substituents of carnitine and acetylcarnitine, are shown in Table I. These ΔS values are calculated from eq 2 for A = 8.37, a value determined by INDO calculations.[35] The substituent constants differ from those previously reported,[7] largely as a consequence of the value chosen for A.

Table I shows that in some cases the substituent constants are solvent dependent. Especially noteworthy are the differences in ^3J(HCCH) for propionic acid and ethanol measured in D_2O vs. $CDCl_3$. The values in $CDCl_3$ are 7.4 Hz for propionic acid and 7.01 Hz for ethanol.[7,36] Also significant are the pH effects seen for the ionizable substituents –COOH and $-CH_2COOH$. Fortunately, in the calculation of conformations and populations, these solvent effects are included when eq 1 and 2 are used together.

Calculation of Conformations. For eq 1, the values A = 8.37, B = −2.83, and C = 7.44, determined from INDO calculations,[35] are used. These values differ slightly from those determined by a best fit to conformationally rigid compounds:[7] A = 8.17, B = −1.96, and C = 6.30. Previously[7] we have discussed errors associated with attempting to fit values for the parameters A, B, and C using data from rigid compounds. Using these fitted parameters in eq 1 does not give a ^3J value small enough to fit $^3J_{A'X}$. This points out the sensitivity of eq 1 to these parameters.

In the interest of comparison with previous methods, we have included results from calculations employing the equations of Pachler[5] (eq 6) and Haasnoot et al.[6] (eq 7). The parameters

$$^3J(\mathrm{HCCH}) = 7.48 - 2.03\cos\theta + 4.60\cos 2\theta + (\Delta E_1 + \Delta E_4)(-0.74 + 0.17\cos\theta - 0.23\cos 2\theta + 0.06\sin\theta + 0.62\sin 2\theta) + (\Delta E_2 + \Delta E_3) \times (-0.74 + 0.17\cos\theta - 0.23\cos 2\theta - 0.06\sin\theta - 0.62\sin 2\theta) \quad (6)$$

$$^3J(\mathrm{HCCH}) = 13.22\cos^2\theta - 0.99\cos\theta + \sum_{i=1}^{4}\Delta E_i[0.87 - 2.46\cos^2(\xi_i\theta + 19.9|\Delta E_i|)] \quad (7)$$

(35) Maciel, G. E.; McIver, J. W.; Ostlund, N. S.; Pople, J. A. *J. Am. Chem. Soc.* **1970**, *92*, 4497–4506.

(36) The ^3J coupling for ethanol in $CDCl_3$ was redetermined.

Table VI. Summary of Carnitine and Acetylcarnitine Conformers: Energies and Populations from Molecular Mechanics Calculations

	conformer	C1–C2–C3–C4 torsion	N1–C4–C3–O3 torsion	rel total energy[a]	electrostatic energy[a]	population MM2[b]
acetylcarnitine zwitterion	g^-,g^-	−70.5	−78.8	0.000	0.000	0.53
	a,g^-	−176.4	−72.8	0.387	0.279	0.28
	g^+,g^-	62.2	−78.0	0.687	0.235	0.17
	a,a	−174.3	−148.2	2.097		0.02
	a,g^+	−164.6	67.3	3.195		<0.01
acetylcarnitine cation	g^-,g^-	−71.2	−79.0	0.000	0.000	0.44
	a,g^-	−176.2	−73.4	0.191	0.134	0.32
	g^+,g^-	63.2	−78.3	0.417	0.104	0.22
	a,a	−175.2	−146.4	1.986		0.02
	a,g^+	−163.2	68.0	3.010		<0.01
carnitine zwitterion	a,g^-	−173.3	−68.7	0.000	0.104	0.47
	g^-,g^-	−70.5	−74.4	0.087	0.000	0.40
	g^+,g^-	64.6	−68.2	0.831	0.190	0.12
	a,a	−173.4	−146.5	2.432		0.01
	a,g^+	−161.3	66.8	3.062		<0.01
carnitine cation	a,g^-	−173.3	−68.7	0.000	0.034	0.48
	g^-,g^-	−70.7	−72.6	0.182	0.000	0.35
	g^+,g^-	65.2	−68.6	0.659	0.088	0.16
	a,a	−174.1	−144.8	2.426		0.01
	a,g^+	−161.3	67.3	3.025		<0.01

[a] Relative energies (kcal/mol) were determined from MM2 calculations at dielectric 78.3. [b] Populations were determined from a Boltzmann distribution based upon the energies calculated by MM2.

Table VII. Populations of the C1–C2–C3–C4 Torsion Angle Calculated from ^{1}H NMR Coupling Constants

	C1–C2–C3–C4		populations		
	conformer[a]	torsion[b]	eq 1	eq 6	eq 7
acetylcarnitine zwitterion	a	−176.4 ± 5.0	0.36 (1)[c]	0.33 (2)	0.35 (2)
	g^-	−70.5 ± 5.0	0.55 (2)	0.57 (2)	0.59 (2)
	g^+	62.2 ± 5.0	0.09 (3)	0.10 (3)	0.06 (4)
acetylcarnitine cation	a	−176.2 ± 5.0	0.35 (1)	0.31 (1)	0.33 (2)
	g^-	−71.2 ± 5.0	0.54 (2)	0.51 (2)	0.52 (2)
	g^+	63.2 ± 5.0	0.11 (3)	0.18 (3)	0.15 (4)
carnitine zwitterion	a	−173.3 ± 5.0	0.53 (1)	0.53 (1)	0.55 (2)
	g^-	−70.5 ± 5.0	0.42 (2)	0.40 (2)	0.45 (3)
	g^+	64.6 ± 5.0	0.05 (3)	0.07 (3)	0.00 (5)
carnitine cation	a	−173.3 ± 5.0	0.60 (2)	0.60 (1)	0.62 (2)
	g^-	−70.7 ± 5.0	0.33 (2)	0.29 (2)	0.34 (3)
	g^+	65.2 ± 5.0	0.07 (3)	0.11 (3)	0.04 (5)

[a] Conformers are depicted in Figure 3. [b] The C1–C2–C3–C4 torsion angles were estimated from the MM2 calculations. [c] Standard deviations are shown in parentheses.

employed for these equations are those reported by the respective authors. Results are given in Tables VII and VIII.

C1–C2–C3–C4 Torsion Angle. As the MM2 calculations indicate, the C1–C2–C3–C4 torsion angles of both carnitine and acetylcarnitine can be treated as freely rotating systems involving three possible minimum energy staggered conformations ($\theta \sim 60$, 180, or −60°). In this case the observed coupling for either H2A or H2B with H3X can be described as a sum of populated states, eq 8, where P_i is the population of conformation i, and $^3J(\theta_i)$ is

$$\langle {}^3J \rangle = \sum P_i {}^3J(\theta_i) \qquad (8)$$

the angular dependence of ^3J(HCCH) from eq 1. For rotation about C2–C3 there are three possible conformations and therefore three corresponding populations. However, since the sum of all populations must be unity, ($\sum P_i = 1$), any one population may be expressed as a function of the other two; one is left with five unknowns: three θ's and two populations. This is matched by only two observed coupling constants: $^3J_{AX}$ and $^3J_{BX}$. As a result the three torsion angles, θ_i, must be estimated and only solutions for populations are possible.

Estimates for the three torsion angles were taken from the MM2 calculations. The three lowest energy populated states occurred for the three staggered conformations as anticipated. These torsion angles were assumed accurate to within ±5°, and thus populations were calculated for all permutations of θ_1, θ_2, and θ_3 within this 10° range.

Mean values and standard deviations for the populations calculated in this manner are shown in Table VII, while the conformers are illustrated in Figure 3. Within acceptable error, no significant differences are noted among the three equations employed.

N1–C4–C3–O3 Torsion Angle. In contrast to the significant population of each of the three conformers about C2–C3, only one conformer, g^-, about the C3–C4 bond of both carnitine and acetylcarnitine was substantially populated. MM2 calculations predicted that greater than 98% of the population was in this conformer. Thus the N1–C4–C3–O3 torsion angle could be treated as a two-conformation problem. Because the population was so skewed toward one conformer and despite the fact that there were more variables (θ_1, θ_2, and P_1) than observed coupling constants ($^3J_{A'X}$ and $^3J_{B'X}$), the mathematics approximated a single-conformation problem and unique solutions for θ_1 and P_1 were possible in theory. While in practice a unique solution for θ_1 was possible, θ_2 could not be precisely determined. Small

Table VIII. Populations of the N1–C3–C4–O3 Torsion Angle Calculated from ^{1}H NMR Coupling Constants

		N1–C4–C3–O3 torsions			populations		
	conformer	eq 1	eq 6	eq 7	eq 1	eq 6	eq 7
acetylcarnitine zwitterion	g^-	−83	−87	−81	0.98	(1.05)[b]	(1.03)
	a	[−142][a]	[−151]	[−140]	0.02	(−0.05)	(−0.03)
acetylcarnitine cation	g^-	−83	−87	−81	0.98	(1.04)	(1.02)
	a	[−146]	[−153]	[−140]	0.02	(−0.04)	(−0.02)
carnitine zwitterion	g^-	−77	−80	−75	0.91	0.95	0.95
	a	[−154]	[−154]	[−162]	0.09	0.05	0.05
carnitine cation	g^-	−74	−76	−71	0.93	0.98	0.98
	a	[−147]	[−168]	[−166]	0.07	0.02	0.02

[a] Because of large uncertainties, angles in brackets are approximate values only. [b] Populations <0 and >1 are not real but represent mathematical solutions which are presented for completeness.

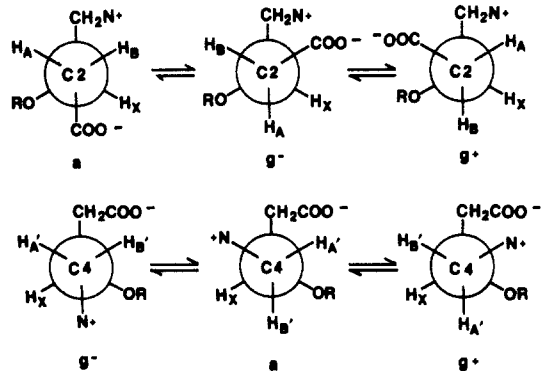

Figure 3. Rotational conformers of carnitine and acetylcarnitine.

variations (0.1°) in θ_1, which were within acceptable error, resulted in large variation in θ_2.

Solutions for the N1–C4–C3–O3 torsion angle and calculated values of the minor conformer are shown in Table VIII. Equations 6 and 7 predict larger populations of g^- than does eq 1 and in the case of acetylcarnitine both produced nonreal solutions ($P_i > 1$ or < 0). But in all fairness to these methods, a 5% error may not be unexpected, and thus these values may be taken to mean 100% g^- conformer.

Discussion

Comparison between Computational and ^{1}H NMR Methods. Is it valid to compare molecular mechanics and the ^{1}H NMR results since the former are used to produce the latter? The ^{1}H NMR assignment of the protons on C2 and C4 has been made so that the most populated conformation in water is the lowest energy conformation calculated by MM2 (as well as the conformation seen in X-ray crystal structures). However, the *relative energies* as calculated by MM2 in no way bias the *relative populations* as determined by ^{1}H NMR. As discussed above, the assignments at C2 and especially C4 are additionally supported by experimental evidence.

The results obtained from MM2 and ^{1}H NMR methods are in close agreement for structures and their populations. Both methods predict minimum energy conformers that correspond to the X-ray structures: g^-,g^- for acetylcarnitine and a,g^- for carnitine. These conformations have been described previously as "folded" and "extended" forms, respectively.[11,12]

Both MM2 and ^{1}H NMR methods indicate a significant gauche effect[13] about the N1–C4–C3–O3 torsion angle. MM2 calculations predict greater than 98% combined g^- conformers for both carnitine and acetylcarnitine. Equation 1 gives virtually the same result for acetylcarnitine regardless of pD. By contrast, the populations of g^- conformer determined for carnitine using eq 1 are slightly pD dependent and are lower than those found using MM2, eq 6, or eq 7.

The slight discrepancy between MM2 and eq 1 may be due to specific solvation effects; i.e., the hydroxyl group of carnitine may be more heavily hydrated than the acetoxyl group of acetylcarnitine. This increased hydration could reduce the gauche effect between quaternary ammonium and hydroxyl portions of the molecule. A specific hydration of hydroxy is not included in the MM2 calculations, because the solvent effect is approximated only by a dielectric term.

Although the data presented in Table VI corresponded only to calculations at the dielectric constant of water, in the event that this dielectric was arbitrarily high for some atom combinations having small R_{ij} values, the dependence of populations on dielectric constant was investigated. As Figure 4 demonstrates ϵ will have only small effects on populations down to a value of about ϵ = 40 at which point electrostatics becomes a dominant factor in determining the energy of the zwitterions.

The greater population of g^- conformers in acetylcarnitine than in carnitine, also observed in acetylcholine and choline,[34] may be due to an increased gauche effect. As the ab initio calculations suggest, O3 of acetylcarnitine carries a larger negative charge than

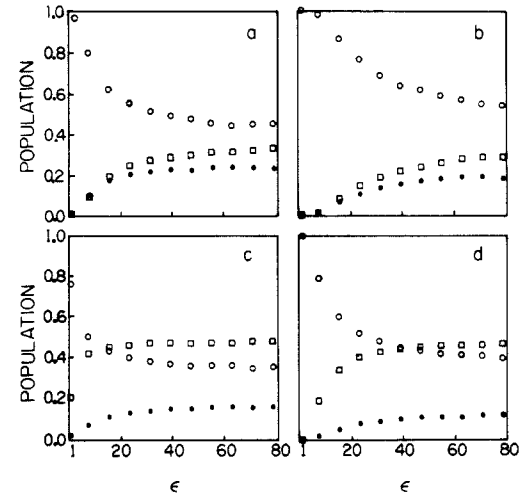

Figure 4. Sensitivity of populations calculated by MM2 to changes in dielectric constant: (a) acetylcarnitine cation, (b) acetylcarnitine zwitterion, (c) carnitine cation, (d) carnitine zwitterion (g^-,g^- conformer, O; a,g^- conformer, □; g^+,g^- conformer, ●).

that of carnitine (−0.72 vs. −0.68 and −0.73 vs. −0.66 for cations and zwitterions, respectively). Since the increased charge makes the C3–O3 bond more polar, the gauche effect should be enhanced.

Contribution of Electrostatics to Conformational Preference. How large a contribution charge stabilization (between carboxylate and quaternary ammonium ion) makes to conformational preference in both carnitine and acetylcarnitine has been a subject of controversy.[11,13] Electrostatic stabilization increases with decrease in distance between these groups and thus would favor the g^-,g^- (folded) conformation where the approximate N1–C1 distance is 4.3 Å compared to 5.1 Å in the a,g^- (extended) conformation. A comparison of crystal structures shows no shortening in N1–C1 distances nor change in conformation in going from cation to zwitterion; hence electrostatic stabilization is negligible in this state.

Is this also true in solution? Both eq 1 (see Tables VII and VIII) and MM2 (see Table VI) reveal a moderate increase in population of the g^-,g^- (folded) conformer upon transition from cation to zwitterion in carnitine. A similar result is seen for acetylcarnitine by MM2, while eq 1 suggests a somewhat diminished effect. The MM2 results show that electrostatic energy accounts for enhancement of the folded conformer. Increase in attraction between carboxylato and quaternary ammonium groups occurs for both carnitine and acetylcarnitine. The enhancement for acetylcarnitine compared to carnitine arises from relief of both electrostatic and steric repulsion between carboxylato and acetoxyl groups.

In contrast to the results of Agostini et al., in no case is the stabilization due to electrostatics large enough to cause the g^-,g^- (folded) conformer of carnitine to be more stable than the a,g^- (extended) conformer, which dominates regardless of charge state. However, the population of folded conformer at physiological pH is significant when considering which conformation is important for binding to a receptor site.

Solution Conformation: Implications for Mode of Binding to Enzyme. Both carnitine and acetylcarnitine show a large conformational bias for g^- conformations about the N1–C4–C3–O3 torsion angle in solution. Hence binding of carnitine or acetylcarnitine to carnitine acetyltransferase should occur with the N1–C4–C3–O3 torsion angle in this highly preferred g^- conformation.

In contrast, the C1–C2–C3–C4 torsion angle of both carnitine and acetylcarnitine has substantial rotational freedom and a strong preference for both g^- and a conformations. For carnitine the a conformation dominates in both cation and zwitterion, whereas in acetylcarnitine the g^- conformer is most prevalent.

What can be inferred about the conformation of bound substrate from the solution conformation? In the zwitterion, which exists at physiological pH, the g^-,g^- (folded) conformer is 38% of car-

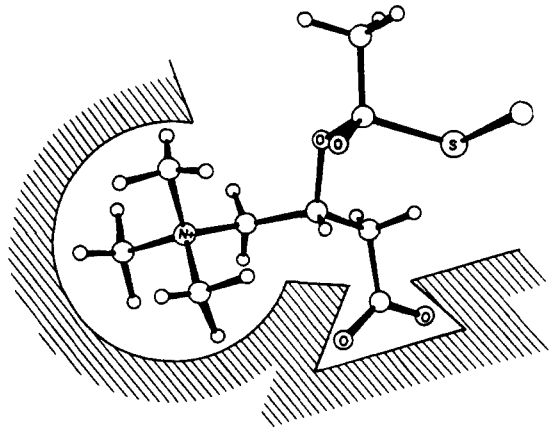

Figure 5. Proposed tetrahedral intermediate formed by acetyl transfer between O and S. Attachment of S is proposed to occur on the *re* face of the acetoxy. Molecule is proposed to bind in the folded conformation.

nitine's population[37] and 54% of acetylcarnitine's, while the a,g^- (extended) conformer is 48% of carnitine's and 35% acetylcarnitine's. We have suggested previously[13] that the folded conformer is the more favorable for binding of either carnitine or acetylcarnitine to the enzyme. Moreover, this conformer is also favored when stabilization of the enzyme-bound transition structure is considered,[13] since the a,g^- mode of binding would increase steric crowding about the acetoxy and consequently the energetics for reaction. This effect is illustrated in Figure 5, which shows a proposed enzyme bound transition structure for transferring an acetyl group between carnitine and coenzyme A or, alternatively, a sulfhydryl group on the enzyme.

Conclusion and Caveats

A Karplus relationship, eq 1, has been presented that accounts for solvent effects on ^3J by employing empirically derived substituent constants. Together with MM2 calculations (with atomic charges determined from MO calculations) and ^{1}H NMR ^3J coupling data, this equation has been used to investigate the solution conformation and population of conformers of carnitine and acetylcarnitine. Although the results are presented numerically, they should only be viewed as semiquantitative. In fact, these results are highly dependent on the parameters chosen for eq 1 as well as for the force field in MM2, especially those involving the charged atoms.

The inclusion of solvent effects on the intrinsic vicinal coupling constant is important. However, there are limitations with the present approach of using ethyl compounds. Solvation of the ethyl compound will be somewhat different than the molecule of interest. Solvation is also likely to be conformationally dependent. When water is employed as a solvent, the structure of hydrogen bonding to the substituent becomes a critical factor as do differences in substituent ionization compared to the ethyl compounds.

In summary, this paper demonstrates the combination of ^{1}H NMR coupling methods, MM2 and MO calculations (the latter for parameterization purposes only), and X-ray structural data for structure elucidation of charged flexible molecules in water. The success of the modified MM2 force field offers optimism for the future development of this combined approach. All of the equations, used in this study, that predict conformations from coupling data give similar results. Equation 1 is preferred because of its simplicity and general applicability to all molecules and conditions under which the spectra are measured. Equations 6 and 7 are theoretically satisfying because they provide some insight into the origin of the effect of substituents on coupling constants. On the other hand, eq 1 employs experimentally determined substituent constants, and its sole purpose is to reliably predict torsion angles from coupling data. In its present form eq 1 assumes complete additivity of substituent effects. As more is learned about the influence of substituents on vicinal coupling constants, the methods used in the development of eq 1 and 2 can be readily extended to include secondary effects arising from nonadditivity of substituents.

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Registry No. Acetylcarnitine zwitterion, 14992-62-2; acetylcarnitine cation, 7559-18-4; carnitine zwitterion, 461-06-3; carnitine cation, 44985-71-9; carnitine acetyltransferase, 9029-90-7.

Supplementary Material Available: Table of atomic charges calculated by 3-21G (2 pages). Ordering information is given on any current masthead page.

(37) For ^{1}H NMR, using eq 1, the determination of the population of the C1–C2–C3–C4 torsion angle is independent of the N1–C4–C3–O3 torsion angle, which is nearly fixed in a g^- conformation. To calculate the approximate population of folded and extended conformers, the populations about the two torsion angles are multiplied.