mental uncertainty of 0.02 Å quoted for the cyclopropanone value, the amount of shortening which actually occurs is not well determined and may be less than 0.03 Å.

(17) R. Nelson and L. Pierce, *J. Mol. Spectrosc.*, 18, 344 (1965).

The CH parameters obtained are similar to those found in related molecules. The parameters of the ring hydrogens are the same within experimental error as those found in cyclopropanone.[15] It is interesting that in both molecules the HCH plane bisects the CCC angle.

The Rotational Isomerism of the Aromatic Amino Acids by Nuclear Magnetic Resonance[1a]

J. R. Cavanaugh

Contribution from the Eastern Utilization Research and Development Division,[1b] Philadelphia, Pennsylvania 19118. *Received September 2, 1969*

Abstract: The chemical shifts and coupling constants for the aliphatic protons of tyrosine, tryptophan, and histidine in basic aqueous solution have been obtained as a function of temperature and concentration. The tryptophan anion is nearly identical with the phenylalanine anion previously reported; that is, at low concentration the vicinal coupling constants diverge with increasing temperature while at high concentration they converge. The vicinal coupling constants for the tyrosine dianion are independent of concentration and exhibit the divergent temperature variation typical of phenylalanine at low concentration. On the other hand, those for the histidine anion are nearly temperature independent but vary with concentration in a manner intermediate between tyrosine and phenylalanine. The variations in the vicinal coupling constants may be interpreted in terms of variations with temperature and concentration of the relative energies of the three classical staggered rotamers. In all cases, the two less favorable conformations become less stable with increasing temperature. The effect of concentration is in this same direction; the magnitude of the effect increases in the series tyrosine (no effect) < histidine < tryptophan, phenylalanine. The rotamer energy variations appear to reflect both solute–solvent and solute–solute interactions.

Recent investigations[2] of the nmr spectra of phenylalanine in aqueous solution have shown rather unusual effects of temperature and concentration on the vicinal coupling constants for the α,β protons. These effects have been related to temperature and concentration-dependent changes of the relative energies of the classical staggered rotamers. The rotamer energies appear to be strongly influenced by the nature and extent of intermolecular solute–solvent interactions. In an effort to understand more about such interactions, these studies have been extended to the aromatic amino acids tyrosine, tryptophan, and histidine. We report here the results of this study as applied to the negatively charged species in aqueous solution.

Experimental Section

The nmr spectra were recorded on a Varian Associates DA-60IL spectrometer,[3] equipped with a variable temperature probe and operated at 60 MHz in the internal-lock, frequency-sweep mode. The frequency sweep was calibrated in the vicinity of each resonance peak by counting the difference between fixed and swept oscillators. Line positions were calculated as the averages of at least four recordings taken with alternating upfield and downfield sweep. Precision of the measurements was better than 0.1 Hz. The variable temperature apparatus was calibrated using the internal chemical shifts of ethylene glycol and methanol as standards for the high and low temperature scales, respectively. The reported temperatures are estimated to be accurate to ±2°.

The amino acids were obtained commercially and were stated to be chromatographically pure. The anion of tryptophan was prepared by dissolving the acid in D_2O containing a slight excess of an equivalent amount of NaOH. For the tyrosine dianion and the histidine anion the solvent contained a slight excess of twice an equivalent amount of NaOH.[4] Concentrations are reported in moles of amino acid per liter of solvent. *t*-Butyl alcohol (2% v/v) was added as an internal standard.

Results and Discussion

Analysis of the Spectra. The aliphatic protons of all the amino acids studied give rise to spectra that are similar to corresponding portions of the phenylalanine spectra. These have been discussed previously in detail.[2a] For tryptophan, the α C–H proton of the indole ring couples to the aliphatic β protons, splitting the eight β-proton transitions into doublets. Similarly, the proton adjacent to the carbon–carbon double bond in the imidazole ring of histidine couples to the aliphatic β protons. The splittings in both cases are essentially independent of temperature and concentration. On the basis of a first-order analysis, the values for the coupling constants averaged over all the measurements are as follows: tryptophan, 0.83 and 0.65 Hz for the low- and high-field β proton resonances, respectively; histidine, 0.78 and 0.64 Hz for the low- and high-field β proton resonances, respectively. The three-spin-system transitions of the aliphatic protons appropriate to the β protons were taken as the midpoints of the doublets.[5] The

(1) (a) Presented in part at the 158th National Meeting of the American Chemical Society, New York, N. Y., Sept 7–12, 1969; (b) Agricultural Research Service, U. S. Department of Agriculture.

(2) (a) J. R. Cavanaugh, *J. Amer. Chem. Soc.*, 89, 1558 (1967); (b) *ibid.*, 90, 4533 (1968).

(3) Mention of commercial products does not constitute an endorsement by the U. S. Department of Agriculture over others of a similar nature not mentioned.

(4) An excess of twice an equivalent amount of base for histidine was used to ensure that the imidazole ring would not be protonated.

(5) At low temperature, the doublet splitting was masked by an in-

Table I. Chemical Shifts[a] and Coupling Constants[b]

Concn[c]	Temp[d]	$J_{geminal}$	$J_{vicinal}$[e]		$\Delta\nu_\alpha$	$\Delta\nu_\beta$	
			Tyrosine Dianion				
0.3	15	−13.58	5.36	7.13	129.65	86.39	95.57
	33	−13.69	5.16	7.42	129.41	85.13	96.14
	50	−13.78	5.25	7.59	129.19	83.82	96.65
	70	−13.73	5.15	7.80	128.90	82.62	97.36
	95	−13.83	5.08	7.99	128.61	81.16	98.48
1	20	−13.72	5.15	7.20	129.44	86.62	95.73
	33	−13.62	5.26	7.40	129.70	85.71	96.59
	50	−13.76	5.20	7.52	129.84	84.66	97.32
	70	−13.76	5.27	7.64	129.84	83.60	98.08
	95	−13.81	5.19	7.88	129.77	82.61	98.90
2	33	−13.69	5.11	7.51	130.12	85.34	97.71
	50	−13.70	5.19	7.70	130.74	84.72	98.64
	70	−13.76	5.14	7.81	131.23	84.30	99.53
	95	−13.75	5.09	7.93	131.17	83.24	100.14
			Tryptophan Anion				
0.3	20	−14.34	5.09	7.33	138.57	104.72	115.54
	33	−14.37	5.12	7.51	139.33	104.50	116.56
	50	−14.41	5.14	7.66	139.95	103.99	117.47
	70	−14.42	5.15	7.79	140.54	103.70	118.31
	95	−14.46	5.09	7.96	140.88	103.09	119.15
1	20	−14.43	4.87	7.90	141.00	103.32	118.74
	33	−14.43	4.89	7.96	142.73	104.44	120.42
	50	−14.46	4.95	8.02	144.55	105.52	122.05
	70	−14.43	4.97	8.09	145.66	106.02	123.35
	95	−14.47	4.94	8.12	146.22	106.03	124.19
2	33	−14.33	4.65	8.46	151.00	109.25	129.66
	50	−14.46	4.74	8.44	153.12	110.70	131.16
	70	−14.39	4.79	8.39	153.70	111.17	131.91
	95	−14.47	4.82	8.40	153.61	111.01	131.80
			Histidine Anion				
0.3	20	−14.60	5.19	7.78	134.57	93.01	102.48
	33	−14.62	5.19	7.79	134.62	92.93	102.84
	50	−14.67	5.09	7.89	134.54	92.64	103.45
	70	−14.70	5.14	7.86	134.39	92.43	104.20
	95	−14.76	5.08	7.94	134.12	92.00	104.84
1	20	−14.51	4.93	8.10	136.03	92.34	104.18
	33	−14.56	4.89	8.12	136.14	92.37	104.73
	50	−14.57	4.91	8.16	136.06	92.09	105.30
	70	−14.62	4.91	8.18	135.96	91.93	105.83
	95	−14.72	4.93	8.16	136.02	91.84	106.28
2	20	−14.52	4.73	8.26	138.58	93.51	106.97
	33	−14.47	4.82	8.23	138.57	93.23	107.20
	50	−14.48	4.74	8.33	138.56	93.24	107.83
	70	−14.60	4.84	8.28	138.33	93.11	108.04
	95	−14.59	4.83	8.30	137.68	92.48	108.18

[a] In Hz at 60 MHz downfield from *t*-butyl alcohol (methyl resonance). [b] In Hz. [c] In mol/l. of solvent. [d] In °C. [e] The smaller of the two vicinal coupling constants is associated with the β-proton resonance at lower field.

results of the analyses of the spectra as described previously[2a] are presented in Table I. The reported coupling constants are estimated to be accurate to better than 0.1 Hz.

Rotational Isomerism and the Vicinal Coupling Constants. The rotational isomerism of amino acids in solution is usually represented in terms of an equilibrium mixture of the three staggered rotamers illustrated in Figure 1 for a substituted alanine. Since the internal rotation is sufficiently rapid, the observed coupling constants are weighted averages over those corresponding to the individual rotamers. The vicinal coupling constants for the rotamers are often taken to be functions of the dihedral angle (the Karplus equation)[6] and independent of the particular conformation. Consequently, the observed averaged vicinal coupling constants, $J_{1,2}$ and $J_{1,3}$, may be expressed for the staggered conformations as

$$J_{1,2} = (a + c)J_g + bJ_t$$
$$J_{1,3} = (a + b)J_g + cJ_t \quad (1)$$

where J_g and J_t are the coupling constants of protons *gauche* and *trans* to one another, respectively, and where a, b, and c are the normalized populations of rotamers

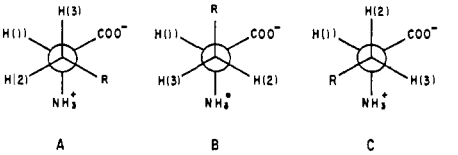

Figure 1. Newman projections of the staggered rotamers of an R-substituted alanine (dipolar ion).

crease in line width for some samples. Moreover, at high temperature the histidine β-proton doublets were gradually replaced by singlets as a result of deuterium exchange of the coupling ring proton.

(6) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959).

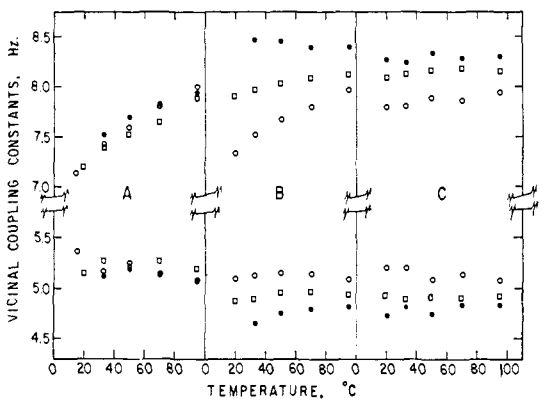

Figure 2. Observed temperature variations of the vicinal coupling constants: (A) tyrosine dianion, (B) tryptophan anion, (C) histidine anion; ○, □, and ● refer to concentrations of 0.3, 1, and 2 mol of amino acid/l. of solvent, respectively.

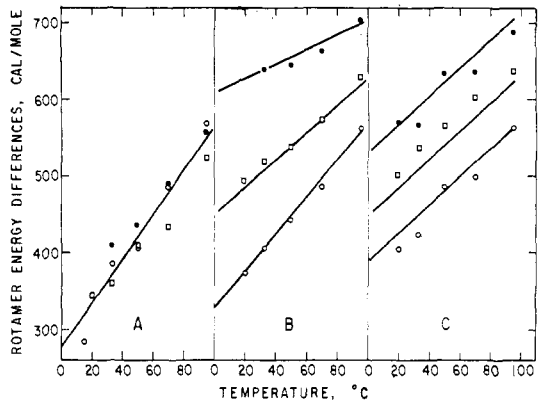

Figure 3. B rotamer free energies relative to the C rotamer ($F_B - F_C$): (A) tyrosine dianion, (B) tryptophan anion, (C) histidine anion; ○, □, and ● refer to concentrations of 0.3, 1, and 2 mol of amino acid/l. of solvent, respectively.

A, B, and C, respectively. The populations conform to a Boltzmann distribution; that is

$$a:b:c = e^{-F_A/RT}:e^{-F_B/RT}:e^{-F_C/RT} \quad (2)$$

where F_i is the rotamer free energy. If the entropy is explicitly taken into account, the Boltzmann factor may be written

$$e^{-F_i/RT} = e^{S_i/R}e^{-E_i/RT} \quad (3)$$

where S_i and E_i are the rotamer entropy and internal energy,[7] respectively. It is usually assumed that any entropy differences are small and may be neglected.

Equations 1 and 2 provide the basic framework on which nmr studies of the rotational isomerism of a variety of systems have been based.[8] In an attempt to apply this approach to the rotational isomerism of phenylalanine,[2] two anomalies were forthcoming: (1) the temperature dependence of the observed vicinal coupling constants could not be made to fit a Boltzmann-like equation with reasonable values for the coupling constants and energy parameters; and (2) the temperature behavior was strongly dependent on the concentration. The interpretation that appeared to fit the data best was that deviations from the staggered conformations were small and that the relative rotamer energies were dependent on the temperature and concentration. The two less favorable rotamers gain stability at low temperatures, this effect diminishing with increased concentration.

The temperature variations of the vicinal coupling constants for the aromatic amino acids studied here are shown graphically in Figure 2. Those for the tryptophan anion bear a striking resemblance to the corresponding curves for the phenylalanine anion. The tyrosine dianion exhibits the divergent temperature variation typical of phenylalanine at low concentration[2] but for tyrosine the results are essentially independent of concentration. The vicinal coupling constants for the histidine anion, on the other hand, are nearly constant over the temperature range but change with concentration. In no case do the coupling constants converge with increasing temperature as expected under the usual formulation represented by eq 1 and 2 with constant rotamer energies; in this sense, all the aromatic amino acids are similarly anomalous.

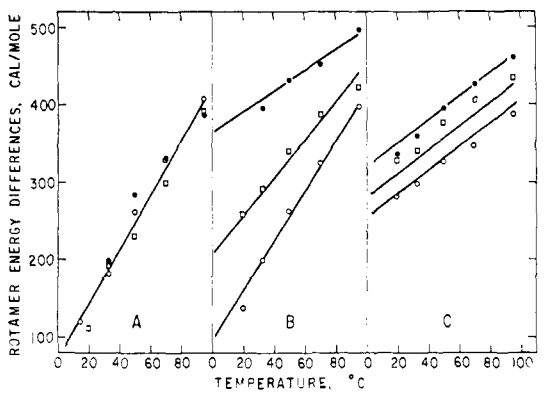

Figure 4. A rotamer free energies relative to the C rotamer ($F_A - F_C$): (A) tyrosine dianion, (B) tryptophan anion, (C) histidine anion; ○, □, and ● refer to concentrations of 0.3, 1, and 2 mol of amino acid/l. of solvent, respectively.

Therefore, the interpretation given to the phenylalanine anion data should be applicable to the other aromatic amino acids as well.[9] This implies that the anomalous variations of the vicinal coupling constants derive solely from changes in the relative rotamer energies themselves.[10] Using eq 1 and 2 and the values 2.60 and 13.56 Hz for J_g and J_t, respectively,[11] the rotamer populations and their relative energies can be calculated directly from the experimentally observed coupling constants. The results are illustrated in Figures 3 and 4.[12]

Admittedly, the rotamer energies so computed are sensitive to the values chosen for J_g and J_t and depend

(7) E, the rotamer internal energy, and H, the enthalpy, appear to be used interchangeably in the literature. Their equality depends on the implicit assumption of a negligible effect from volume differences between conformations. In view of the small contribution from even appreciable volume changes, this assumption appears to be well justified.

(8) For example: R. R. Dean and J. Lee, *Trans. Faraday Soc.*, **65**, 1 (1969); K. D. Kopple and M. Oshnishi, *J. Amer. Chem. Soc.*, **91**, 962 (1969); F. Heatley and G. Allen, *Mol. Phys.*, **16**, 77 (1969). Also *cf.* other references cited here and in ref 2.

(9) It could be argued that the temperature insensitivity of the vicinal coupling constants for histidine implies a single conformation for that species. However, because the observed values are in the same range as for the other aromatic amino acids and are not compatible with any one staggered conformation, it appears that the histidine anion data should also be interpreted in terms of an equilibrium mixture of the three staggered rotamers.

(10) *Cf.* ref 2b for details of the argument.

(11) K. G. R. Pachler, *Spectrochim. Acta*, 20, 581 (1964).

(12) The relative energies of the A and B rotamers as shown in Figures 3 and 4 of ref 2b were mislabeled and should be reversed; that is, the B rotamer has a higher relative energy than the A rotamer.

on the assumption that a single J_g is adequate to describe the system.[13] The experimental errors also add to the uncertainties concerning the values of the relative rotamer energies but these are sufficiently small to have little effect on the results. (Typical error limits for the vicinal coupling constants of ±0.05 Hz lead to uncertainties in the relative rotamer energies of ±20 cal/mol in the worst case.) The probable errors in the values assigned to J_g and J_t are much more difficult to assess. However, assigning other values to J_g and J_t has the effect of increasing or decreasing the relative rotamer energies as shown in Figures 3 and 4 but has only a small effect on the slopes of the curves shown. For example, if J_g were 2.80 instead of 2.60 Hz, the relative rotamer energies of the B rotamer for the tryptophan anion would be increased on the average by about 35 cal/mol but the slopes of the lines would be increased on the average by less than 0.1 cal/(mol deg). Therefore, the overall changes of the rotamer energies with temperature and concentration would be largely unaffected by errors in the values chosen for J_g and J_t (or by assigning more than one value to J_g in the various conformations).

The Variations of the Rotamer Energies. Figures 3 and 4 show the variations of the A and B rotamer energies relative to that of the most stable rotamer C. For a given concentration, the relative energies increase nearly linearly with increasing temperature. Moreover, for tryptophan and histidine, the relative energies display a marked increase with concentration.

The linear dependence for these curves may be expressed by

$$\Delta F = f_1(c) + f_2(c)t \qquad (4)$$

where ΔF is the rotamer free energy relative to the C rotamer, t is in °C, and the f_i are appropriate functions of the concentration c. For tyrosine, f_1 and f_2 are constants for the A and B rotamer relative energies. The f_2 functions for histidine may also be set equal to constants since the curves shown in Figures 3 and 4 for histidine are nearly parallel. f_1 for histidine and both functions for tryptophan (and phenylalanine)[2] may be approximated by linear functions of the concentration. Consequently, the relative rotamer free energies all fit an equation of the form

$$\Delta F = a_1 + a_2t + a_3c + a_4ct \qquad (5)$$

For histidine, a_4 is set equal to zero since the slopes of the curves do not vary with concentration; for tyrosine, both a_3 and a_4 are set equal to zero because of the overall concentration independence. The coefficients, a_i, may be determined by a least-squares procedure.[14] The results are given in Table II and are illustrated by the appropriate lines drawn in Figures 3 and 4.

The root mean square deviations are within acceptable limits with the possible exception of ΔF_{BC} for phenylalanine. Deviations here result from departures from linearity of the concentration dependence at the higher concentrations. The negative sign for the ct coefficients for tryptophan and phenylalanine reflect the fact that the slopes of the rotamer energy–temperature curves diminish with increasing concentration.

(13) G. M. Whitesides, J. P. Sevenair, and R. W. Goetz, *J. Amer. Chem. Soc.*, **89**, 1135 (1967).
(14) All computations were performed on an IBM-1130 computer.

Table II. Coefficients Relating the Relative Rotamer Energies[a] to the Temperature[b] and Concentration[c] Using Eq 5

	a_1	a_2	a_3	a_4	Error[d]
		B Rotamer			
Phenylalanine[e]	102	3.51	12,300	−60.7	30
Tryptophan	278	2.66	9,400	−49.0	10
Histidine[f]	362	1.83	4,800		22
Tyrosine[g]	275	2.86			24
		A Rotamer			
Phenylalanine[e]	102	3.35	6,600	−28.3	22
Tryptophan	41	3.63	9,200	−65.6	11
Histidine[f]	243	1.52	2,200		12
Tyrosine[g]	72	3.49			19

[a] In cal/mol. [b] In °C. [c] In mole-fraction units (assuming that the solvent produces 55.5 mol/l. when treated with the solute). [d] Root mean square deviation in cal/mol. [e] From the data of ref 2. [f] Using the equation, $\Delta F = a_1 + a_2t + a_3c$. [g] Using the equation, $\Delta F = a_1 + a_2t$.

In order to avoid any overinterpretation of these results, it should be pointed out that the root mean square deviations represent simply the fit of the data to the given equations and that the presentation of the results in this form is for the purpose of convenient intercomparison. The values of the coefficients obtained will be useful for establishing the general trends of the variations of the rotamer energies with temperature and concentration.

The Temperature Dependence of the Rotamer Energies. The curves in Figures 3 and 4 and the coefficients of t in Table II indicate an appreciable variation of the rotamer energies with temperature. Recently it has been pointed out[15] that a linear temperature dependence of rotamer free energies could result from entropy differences between rotamers. If it is assumed that the temperature dependence originates solely from rotamer entropy differences (that is, that ΔE in eq 3 is a constant), then the coefficients of t in Table II imply entropy differences in the range from 1.5 to 3.5 eu. These values are substantially higher than rotamer entropy differences calculated for some substituted ethanes which fall in the range 0.2–0.4 eu.[15,16] Nevertheless, it is conceivable that the contributions to the rotamer entropy from the large aromatic rings of these amino acids could change significantly with internal rotation.

Accordingly, the relative rotamer entropies were determined using the formula[15]

$$\Delta S_{12} = \frac{R}{2} \ln \frac{A_1B_1C_1}{A_2B_2C_2} \qquad (6)$$

where ΔS_{12} is the entropy difference between rotamers 1 and 2 and A_i, B_i, and C_i are the principal moments of inertia of the molecule in the ith conformation. The products of the principal moments of inertia were calculated for each molecule as a function of all internal rotations.[17] Both the carboxyl and amino groups were taken to be ionized for ease of computation. Atomic coordinates were calculated using tetrahedral angles for the aliphatic protons and the NH_3^+ group, and angles of 120° for the CO_2^- group and the ring geometry for tyrosine and phenylalanine. Representative bond dis-

(15) G. Govil and H. J. Bernstein, *J. Chem. Phys.*, 47, 2818 (1967).
(16) G. Govil and H. J. Bernstein, *ibid.*, **49**, 911 (1968).
(17) The products of the principal moments of inertia were determined by calculating the determinant of the moment of inertia tensor without resorting to a diagonalization procedure.

tances were taken from published tables.[18] The C–O bond distance for the phenolic oxygen in the tyrosine dianion was assumed to be 1.3 Å.[19] Ring geometries for histidine and tryptophan were taken from crystal structure data on imidazole[20] and 3-indolylacetic acid,[21] respectively. (Internal angles were adjusted to give ring planarity where appropriate.) The results for the three staggered rotamers averaged over the other internal rotations are shown in Table III.

Table III. Products[a] of Moments of Inertia and Relative Rotamer Entropies[b]

	Moment products ($\times 10^{-6}$) Rotamer			Relative entropies	
	A	B	C	ΔS_{CA}	ΔS_{CB}
Phenylalanine	175.6	205.7	258.7	0.387	0.229
Tryptophan	630.5	746.4	1011.0	0.469	0.301
Histidine	98.3	116.4	143.3	0.375	0.207
Tyrosine	334.6	377.5	450.2	0.295	0.175

[a] In (amu Å²)³. [b] In cal/(mol deg).

The averages were calculated assuming free rotation of the carboxyl group and the aromatic ring.[22] The sinusoidal-like variation of the moment product with the rotation of the aromatic ring is small for phenylalanine, tyrosine, and histidine, maximum departures from the mean value amounting to approximately 4, 2, and 10%, respectively. The assumption of free rotation can have little effect on the results for these compounds. The departures for tryptophan are much larger and approach nearly 45% in the worst case. However, even if the ring rotation in tryptophan were restricted in the worst possible way, that is, if the ring were fixed at an angle corresponding to the maximum moment product in one staggered conformation and at the minimum in another, the results would be in error by at most a factor of 2.

The results given in Table III are too low by an order of magnitude to explain the temperature effects. They are, however, in the correct direction; that is, the positive relative entropy of the C rotamer is consistent with its increased stability with increasing temperature. It is conceivable that solute–solvent interactions present in the aqueous system reinforce these entropy effects already inherent in the amino acid molecules. For example, the stability of the more compact rotamers (A and B), as measured by their moments of inertia, may be enhanced by the increased order of the water at the lower temperatures.

Another possible source for the temperature-dependent rotamer energies may arise from electrostatic interactions, the theory of which has been successfully applied to the study of rotational isomerism in some substituted ethanes.[23] The theory works less well with polar solvents,[16] however, and in view of the lack of understanding of the interactions in aqueous solution, an attempt to apply the theory to the present systems seems impractical at this time.

The Concentration Dependence of the Rotamer Energies. The effects of concentration on the rotamer energies as reflected in the coefficients of c in Table II vary widely with the amino acid. Tryptophan and phenylalanine show the greatest changes; histidine lies intermediate between them and the concentration-independent tyrosine. Previously,[2b] the temperature and concentration effects on the phenylalanine anion rotamer energies were correlated with changes in the dielectric constant of the aqueous solution. In view of the similarity of tryptophan to phenylalanine, an analogous relationship will hold for the tryptophan anion rotamer energies. Even the results for histidine can be correlated with the dielectric constant because the diminished concentration effects are concomitant with diminished temperature effects.[24] Tyrosine, on the other hand, shows no such relationship.

If, for all the amino acids, the rotamer energies were directly related to the dielectric constant, then it could have been argued that these effects were primarily due to solute–solvent interactions, whatever their origin.[25] Tyrosine does not fit into this interpretation and this suggests that solute–solute interactions also influence the rotamer energies. In fact, it is possible to interpret the effects of concentration on the rotamer energies primarily as a result of solute–solute interactions. In this view, the strengths of the interactions fall in the series tyrosine (ionized phenolic group) < histidine < tryptophan, phenylalanine. The nature of the solute–solute interactions is of course open to question, but the order of the series reflects in a qualitative way what might be expected of hydrophobic interactions;[26] that is, the interactions increase with decreasing polarity of the substituent.

Conclusion

The variations in the anion rotamer energies of the aromatic amino acids appear to be mediated by temperature- and concentration-dependent intermolecular interactions present in aqueous solution. Both solute–solvent and solute–solute interactions appear to be implicated but their precise mode of operation is not as yet well defined. It is tempting to try to interpret these phenomena in terms of water structure and hydrophobic interactions; recent observations, however, concerning the ambiguity of such interpretations,[27] seem to preclude further development along these lines until the basic understanding of interactions in aqueous solution con-

(18) "Tables of Interatomic Distances and Configuration in Molecules and Ions," Special Publication No. 11, The Chemical Society, London, 1958.

(19) The results are quite insensitive to the tyrosine phenolic C–O bond distance; relative rotamer entropies differ by less than 1% with the bond distance equal to 1.26 Å from those with the bond distance equal to 1.30 Å.

(20) S. Martinez-Carrera, *Acta Crystallogr.*, 20, 783 (1966).

(21) I. L. Karle, K. Britts, and P. Gum, *ibid.*, 17, 496 (1964).

(22) The moments of inertia are independent of the rotation of the NH_3^+ group because of its threefold axis of symmetry.

(23) R. J. Abraham, L. Cavalli, and K. G. R. Pachler, *Mol. Phys.*, 11, 471 (1966); R. J. Abraham and M. A. Cooper, *J. Chem. Soc., B*, 202 (1967); K. K. Deb and R. J. Abraham, *J. Mol. Spectrosc.*, 23, 393 (1967).

(24) In fact, tryptophan and histidine show a better correlation than phenylalanine with root mean square deviations of about 19 and 21 cal/mol, respectively, as compared to about 35 cal/mol for phenylalanine.

(25) The dielectric constant can also be influenced by solute–solute interactions. However, the correlations which are obtained here are based not on experimentally measured values of the dielectric constant but on a theoretical model which does not explicitly take solute–solute interactions into account.

(26) W. Kauzmann, *Advan. Protein Chem.*, 14, 1 (1959).

(27) A. Holtzer and M. F. Emerson, *J. Phys. Chem.*, 73, 26 (1969).

siderably improves. Hopefully, the study of the rotational isomerism of a variety of molecules in aqueous systems will contribute to that understanding.

Acknowledgment. The technical assistance of Messrs. T. W. Cassidy and M. Krangel is gratefully acknowledged.

Spectroscopic Studies of Alkali Metal Ions in Dimethyl Sulfoxide and 1-Methyl-2-pyrrolidone[1]

John L. Wuepper[2] and Alexander I. Popov

Contribution from the Department of Chemistry, Michigan State University, East Lansing, Michigan 48823. *Received July 24, 1969*

Abstract: The solvation of lithium and sodium ions in dimethyl sulfoxide (DMSO) and 1-methyl-2-pyrrolidone (1M2PY) has been studied by the nmr and the far-infrared spectroscopic techniques. Mole ratio study of the $NaAl(But)_4$–DMSO and the $NaAl(But)_4$–1M2PY systems in 1,4-dioxane solutions indicates that the solvation numbers of the Na^+ ion in DMSO and in 1M2PY are 6 and 4, respectively. Infrared spectra of the tetrabutylaluminate and nitrate anions in solutions of these salts as well as the influence of isotope substitution of ^6Li in the lithium salts on the vibration frequency of the far-infrared band are consistent with the view that in the above solvents the anions do not enter into the inner solvation shell of the alkali cations.

Far-infrared spectra of electrolyte solutions in various nonaqueous solvents have been recently reported by several investigators.[3-5] This relatively new technique promises to be very useful in the elucidation of the structure of electrolyte solutions.

Similar work which was carried out in our laboratory[6] has shown that solutions of alkali metal salts in dimethyl sulfoxide (DMSO) and in 1-methyl-2-pyrrolidone (1M2PY) exhibit an infrared band in the 450–100 cm^{-1} spectral region whose frequency is dependent only on the nature of the cation and on the solvent. Similar bands have been observed by Edgell and coworkers[3] in tetrahydrofuran (THF) solutions of alkali salts, but in this case the frequencies of the far-infrared bands were also dependent on the nature of the anion, thus indicating that the bands may be due to the vibrations of ion pairs, or that at least the anion entered into the inner solvation shell of the alkali cations. In contrast to THF (D = 7.4), both DMSO and 1M2PY are polar solvents with the relatively high dielectric constant values of 46.4[7] and 32[8] and the dipole moments of 3.9[9] and 4.09[10] D, respectively. It would be expected, therefore, that in these solutions the concentrations of ion pairs will be much lower than in THF.

Previous studies have shown that the lithium ion has a solvation number of 2 in DMSO[6b] and of 4 in 1M2PY.[6d] Attempts to obtain the solvation number of Na^+ ion were unsuccessful due to the limited solubility of common sodium salts in solvent mixtures. Schaschel and Day, however, have reported a nmr study of the solvation number of sodium in ethers using sodium tetrabutylaluminate as the solute.[11] This salt has high solubility in organic solvents.

This work was undertaken to study the solvation number of the sodium ion in the two solvents DMSO and 1M2PY as well as to extend the infrared spectral measurements to a more thorough study of the role of the anions in the solutions.

Experimental Section

Reagents. The sources and the purification procedures for DMSO, 1M2PY, and 1,4-dioxane have been described in previous publications.[6] The water content, as determined by the Karl Fisher titration was less than 0.005 M for all solvents.

Lithium-6 metal was obtained from Union Carbide Oak Ridge Laboratory, Oak Ridge, Tenn. The assay which was furnished with the metal showed 95.6% ^6Li and 4.4% ^7Li. In order to prepare ^6Li salts, the metal was first added to water. The resulting base solution was then neutralized with a reagent grade acid with the desired anion. The salt solutions were crystallized and dried at 200°.

The method of Schaschel and Day[11] was used to prepare sodium tetrabutylaluminate. Tri-*n*-butylaluminum was obtained from K & K laboratories. The salt was prepared by adding tri-*n*-butylaluminum to an excess of metallic sodium dispersed in *n*-heptane which was previously dried over sodium wire. After refluxing for several hours, the mixture was filtered through a fine porosity filter stick to remove excess sodium and aluminum. The solvent was then removed by vacuum evaporation. The resulting white product was recrystallized twice from *n*-heptane at approximately −80°. The final purified product melted at 65 ± 1°. The capillary tube was filled with dried deoxygenated nitrogen before the salt was introduced. All manipulations which involved $Al(But)_3$ or $NaAl(But)_4$ were performed in a drybox under a dry nitrogen atmosphere.

Measurements. Nuclear magnetic resonance measurements were performed on Varian A-60 and A-56/60D spectrometers. A Model 200 AB Hewlett-Packard audio oscillator was used as an

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(2) Socony-Mobil Fellow, 1968–1969.
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