Pinamonti, and Bellavita[30] under experimental conditions similar to those prevailing in the present investigation. Although the specific reference is to hematoporphyrin, these authors reported that all porphyrins studied, which included protoporphyrin, exhibited identical behavior. Thus, it appears from the experimental findings presented here and those of Ricci, *et al.*, that this polarographic wave can indeed be attributed to the reduction of hydrogen catalyzed by the protoporphyrin ring. However, since this is only of peripheral interest to the understanding of the electron-

(30) A. Ricci, S. Pinamonti, and V. Bellavita, *Ric. Sci.*, 30, 2497 (1960).

transfer properties of the iron porphyrins, the system was not investigated in sufficient detail to elucidate the mechanism of the catalyzed reduction.

As a matter of more central significance it would be interesting to couple two or more heme–iron units by synthetic ligand chains *via* coordination sites perpendicular to the tetrapyrrole plane and determine the effect of this on the electron-transfer properties. Such low molecular weight systems might provide a valid model for conditions prevailing in the hemoproteins; *e.g.*, heme iron units could be maintained at the same distance from each other as in hemoglobin (≈ 25 A) and studied in simple environmental situations in the absence of the macromolecular matrix.

The Rotational Isomerism of Phenylalanine by Nuclear Magnetic Resonance[1a]

J. R. Cavanaugh

Contribution from the Eastern Regional Research Laboratory,[1b] Philadelphia, Pennsylvania 19118. Received October 15, 1966

Abstract: The chemical shifts and coupling constants of the aliphatic protons of the phenylalanine anion and dipolar ion and of the glycine and alanine cation, anion, and dipolar ion in aqueous solution have been obtained as a function of temperature over the range 0–100°. The temperature variations of the phenylalanine vicinal coupling constants show anomalous behavior: for the anion, the coupling constants diverge with increasing temperature; for the dipolar ion, the larger coupling is constant over the measured range. Two alternative interpretations are offered: the stability of the most favorable conformer in which the phenyl group is *trans* to the carboxyl group is considerably enhanced with increasing temperature; or, if the rotamer energies are temperature independent, the results suggest that the equilibrium positions deviate significantly from the staggered conformations and that the one which is least favorable from steric considerations is heavily populated and may even be the most stable form.

It has become increasingly apparent that nmr spectroscopy offers a powerful means for elucidating conformational equilibria of molecules in solution. Rotational isomerism about carbon–carbon single bonds has received special attention; studies of fluorine and proton coupling constants and chemical shifts have provided assessments of relative populations of the three classical staggered rotamers of substituted ethanes.[2-10] Coupled with data on the temperature dependence of the spectral parameters, investigations of this kind have yielded accurate information on rotamer

energies[3,6-11] and on potential energy barriers to internal rotation.[7,9,10]

The proton resonance spectra of a number of amino acids in aqueous solution have recently been investigated.[12-16] Conformational equilibria have been deduced from the observed chemical shifts and coupling constants at room temperature, either by assuming values for the H–H coupling in the H–C–C–H fragment for the *gauche* and *trans* conformations[13,15,16] or by assuming a specific dependence of the coupling on the dihedral angle.[14]

In this investigation, the nmr spectra of the aliphatic protons of phenylalanine [$C_6H_5CH_2CH(NH_2)COOH$] in neutral and basic aqueous solution were obtained as a function of temperature over the range 0–100°. Glycine [$CH_2(NH_2)COOH$] and alanine [$CH_3CH(NH_2)COOH$] were similarly studied in an effort to test the

(1) (a) Presented in part at the Second Middle Atlantic Meeting of the American Chemical Society, New York, N. Y., Feb 6–7, 1967. (b) Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

(2) R. J. Abraham and K. G. R. Pachler, *Mol. Phys.*, 7, 165 (1963–1964).

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(7) D. S. Thompson, R. A. Newmark, and C. H. Sederholm, *ibid.*, 37, 411 (1962).

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(14) R. J. Abraham and W. A. Thomas, *ibid.*, 3739 (1964).

(15) (a) K. G. R. Pachler, *Spectrochim. Acta*, 19, 2085 (1963); (b) 20, 581 (1964).

(16) R. B. Martin and R. Mathur, *J. Am. Chem. Soc.*, 87, 1065 (1965).

temperature independence of spectral parameters that do not depend on relative rotamer energies. The temperature variations of the chemical shifts and coupling constants should provide more complete information on the rotational isomerism of phenylalanine in solution and should enable the classical picture of staggered rotamers populated according to a Boltzmann distribution to be carefully examined.

Figure 1. Newman projections of the staggered rotamers of the phenylalanine dipolar ion.

Experimental Section

The nmr spectra were recorded on a Varian Associates DP-60 spectrometer,[17] operating at 60 Mc/sec and equipped with a variable-temperature probe. Frequency measurements, relative to t-butyl alcohol as an internal standard, were made by the usual side-band technique. Each line position was calculated as the average of at least 12 sets of recordings taken with alternating upfield and downfield sweep. Average deviations from the mean value were usually less than 0.1 cps for narrow lines (less than 0.6 cps line width at half-height) but approached 0.2 cps in some instances for the broader resonances.

Temperature measurements were made by means of a copper–constantan thermocouple situated just inside the dewar probe insert in the incoming gas stream. Calibration was performed using a similar thermocouple placed directly in a sample tube at the receiver coil. Accuracy of the temperature measurements was ±1°.

The amino acids and their esters were obtained from commercial sources and were of the highest purity. The samples were prepared as 1 M solutions as follows. The acid was dissolved in D_2O to obtain the dipolar ion; the acid in 1 N NaOH in D_2O, for the anion; and the methyl (or ethyl) ester hydrochloride in D_2O, for the cation. t-Butyl alcohol (1%) was added as the internal standard. Phenylalanine in D_2O was prepared as a saturated solution, approximately 0.2 M.

Results and Discussion

Analysis of the Spectra. The protons of the amino group undergo rapid exchange[18] under the conditions of pH encountered in this study (pH ≥ 1); no coupling is observed between the α and amino protons. Line widths for the α proton resonances of the acids in D_2O and NaOD solutions are about 0.5 cps[19] and increase slightly at lower temperatures. The α protons of glycine and alanine methyl ester hydrochlorides in D_2O solution exhibit much broader lines (widths of about 1 cps); glycine ethyl ester hydrochloride in D_2O is, in this respect, similar to the free acids.

The A_3B spectrum[20] of the aliphatic protons of alanine consists of a doublet and a quartet, the latter at lower field. For alanine methyl ester hydrochloride in D_2O and for the other alanine solutions below room temperature, the α proton resonances are sufficiently broad to mask the expected splittings of the central peaks of the quartet. Trial parameters were chosen from the spectrum and conventional iterative techniques were used to obtain a best fit between observed and calculated frequencies.

The aliphatic protons of phenylalanine give rise to an asymmetric three-spin spectrum, containing a quartet at low field identified with the α proton. No definitive assignment of the individual β protons[21] can be

made on the basis of the observed spectral parameters. The results of the three-spin system analysis[22] yield two sets of acceptable solutions, one with all coupling constants of like sign and one with the geminal coupling of opposite sign to the vicinal couplings. The latter situation prevails in many ethane-like fragments.[23] Evidence for its occurrence here is provided by the comparison, shown below, of the analyses of the 60- and 100-Mc/sec[24] spectra of the phenylalanine anion (calculated coupling constants at room temperature, in cps), which strongly favors the set with the geminal coupling of opposite sign.

	All couplings of like sign			Geminal coupling of opposite sign		
60 Mc/sec	13.27	8.93	4.12	−13.45	7.75	5.34
100 Mc/sec	13.40	8.23	4.87	−13.48	7.77	5.33

All chemical shift and coupling constant data are collected in Table I. The results of the spectral analyses are estimated to be accurate to better than 0.1 cps.

The Coupling Constants and Rotational Isomerism. It is generally assumed that the stable forms of amino acids in aqueous solution are the three staggered conformers obtained by internal rotation about the C_α–C_β bond. The Newman projections for the phenylalanine dipolar ion are shown in Figure 1.

If the internal rotation is sufficiently rapid, the observed chemical shifts and coupling constants are weighted averages over those corresponding to the three conformations. The assigned weights conform to Boltzmann distribution; that is

$$a:b:c = e^{-E_A/RT} : e^{-E_B/RT} : e^{-E_C/RT} \qquad (1)$$

where a, b, and c are the appropriate normalized weights and E_A, E_B, and E_C are the conformer energies. It is assumed that the partition functions for the three rotamers except those containing the coordinates for internal rotation are identical and that entropy differences between the rotamers are small and may be neglected.

The observed coupling constants are therefore given by

$$J_{12} = aJ_{12}{}^A + bJ_{12}{}^B + cJ_{12}{}^C$$

$$J_{13} = aJ_{13}{}^A + bJ_{13}{}^B + cJ_{13}{}^C \qquad (2)$$

$$J_{23} = J_{23}{}^A + bJ_{23}{}^B + cJ_{23}{}^C$$

where $J_{ij}{}^k$ is the coupling constant between nuclei i and j in the kth conformation.

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

(18) M. Sheinblatt and H. S. Gutowsky, J. Am. Chem. Soc., 86, 4814 (1964).

(19) A variation of the line width of the α-proton resonances of amino acids in aqueous solution with pH has been previously noted.[18] The line widths observed here for the anions and dipolar ions correspond roughly to values quoted therein.

(20) P. L. Corio, Chem. Rev., 60, 363 (1960).

(21) The β-proton resonances are unexpectedly broad, with line widths of approximately 1 cps.

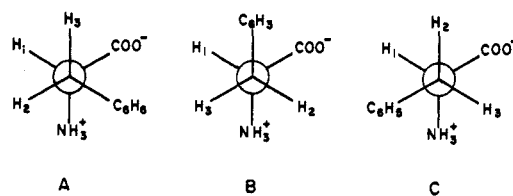(22) S. Castellano and J. S. Waugh, J. Chem. Phys., 34, 295 (1961); J. R. Cavanaugh, ibid., 39, 2378 (1963).

(23) Cf. J. W. Emsley, J. Feeney, and L. H. Sutcliffe, "High Resolution Nuclear Magnetic Resonance Spectroscopy," Vol. 1, Pergamon Press, New York, N. Y., 1965, pp 172–174.

(24) The 100-Mc/sec spectrum was obtained through the generous cooperation of Varian Associates and Mr. William Jankowski.

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

Glycine

In NaOD	Temp[c]	4	33	50	69	91
	$\Delta\nu$	116.84	116.41	116.15	115.90	116.01
In D$_2$O	Temp	4	33	50	70	91
	$\Delta\nu$	138.78	139.11	139.20	139.32	139.34
Methyl ester hydrochloride in D$_2$O	Temp	8	33	50	66	83
	$\Delta\nu$	162.34	163.17	163.55	163.91	164.27
Ethyl ester hydrochloride in D$_2$O	Temp	7	33	50	67	88
	$\Delta\nu$	160.74	161.63	161.95	162.44	162.76

Alanine

In NaOD	Temp	12	33	49	69	89
	J	7.07	6.96	6.97	7.01	7.04
	$\Delta\nu_\alpha$	124.71	124.63	124.63	124.65	124.24
	$\Delta\nu_\beta$	−1.19	−1.32	−1.31	−1.27	−1.37
In D$_2$O	Temp	3	33	48	67	85
	J	7.23	7.28	7.24	7.22	7.21
	$\Delta\nu_\alpha$	151.54	152.17	152.36	152.63	152.77
	$\Delta\nu_\beta$	13.63	13.86	14.06	14.25	14.46
Methyl ester hydrochloride in D$_2$O	Temp	8	33	51	67	87
	J	7.29	7.28	7.21	7.24	7.25
	$\Delta\nu_\alpha$	178.94	179.23	179.53	179.73	179.94
	$\Delta\nu_\beta$	19.48	19.98	20.34	20.66	21.03

Phenylalanine[d]

In NaOD	Temp	7	18	33	49	70	76	86	99
	J_{gem}	−13.39	−13.40	−13.45	−13.53	−13.53	−13.64	−13.68	−13.66
	J_{vic}	5.48	5.43	5.34	5.30	5.31	5.27	5.25	5.21
		7.39	7.52	7.75	7.83	7.91	7.99	8.03	8.07
	$\Delta\nu_\alpha$	132.53	133.16	134.25	135.09	135.77	135.96	136.16	136.30
	$\Delta\nu_\beta$	93.64	93.31	93.21	93.06	92.77	92.80	92.61	92.45
		103.14	104.46	106.02	107.26	108.66	109.19	109.72	110.40
In D$_2$O	Temp	22	33	49	67	78	87	99	
	J_{gem}	−14.51	−14.50	−14.60	−14.55	−14.66	−14.70	−14.70	
	J_{vic}	5.10	5.14	5.17	5.19	5.28	5.28	5.27	
		8.02	8.00	8.08	8.06	8.01	8.05	8.05	
	$\Delta\nu_\alpha$	163.61	164.11	164.41	164.82	165.05	165.13	165.34	
	$\Delta\nu_\beta$	111.84	112.03	112.24	112.40	112.53	112.54	112.56	
		121.75	122.36	122.83	123.52	124.00	124.24	124.79	

[a] In cps downfield from t-butyl alcohol. [b] In cps. [c] In °C. [d] The smaller of the two vicinal coupling constants is associated with the β proton resonance at lower field.

It is further assumed that the coupling constants of protons *gauche*, *trans*, and geminal to one another can be described by a single set, namely, J_g, J_t, and J_{gem} respectively, regardless of the specific conformation. Equations 2 then become

$$J_{12} = (1 - b)J_g + bJ_t$$

$$J_{13} = (1 - c)J_g + cJ_t \qquad (3)$$

$J_{23} = J_{gem}$ (independent of rotamer populations)

It is now seen that the ambiguity in the assignment of the vicinal coupling constants reduces to an interchange of b and c. Conformer C, with the bulkiest groups, $-C_6H_5$ and $-COO^-$, in the *trans* position, is favored from steric considerations. Accordingly, since $J_g < J_t$, $c > b$ implies $J_{12} < J_{13}$.

If J_g and J_t were known, eq 3 would suffice to evaluate the relative rotamer populations from spectral measurements at a single temperature. A fair volume of evidence has been collected to suggest that J_g and J_t for amino acid fragments can be accurately assessed; rotamer populations and energies have been estimated in this fashion for a number of amino acids in basic solution.[15b]

The temperature variations of the vicinal coupling constants provide a means of testing this model. Using the results at room temperature (sample temperature of

33°) and the values $J_g = 2.60$ cps and $J_t = 13.56$ cps,[15b] the calculated rotamer energies for phenylalanine (cal/mole) are as shown below; they are in excellent agreement with those reported previously.[15b]

	Dipolar ion This study	Anion This study	Anion From ref 15b
$E_A - E_C$	350	320	340
$E_B - E_C$	460	380	360

The temperature variations of the coupling constants are readily evaluated from eq 1 and 2 and may be compared with the experimental points in Figure 2. The lack of agreement, especially for the anion, is strikingly apparent.

The predicted variation is intuitively expected. As the temperature increases, the rotamer populations would tend to equalize; J_{12} and J_{13} would converge to a common value. At sufficiently high temperatures, $a \approx b \approx c \approx \frac{1}{3}$ and $J_{12} \approx J_{13}$. The actual behavior is quite different: for the anion, the coupling constants diverge with increasing temperature; for the dipolar ion, the larger coupling is approximately constant over the measured range.

The calculated curves in Figure 2 assume that (1) J_g and J_t have temperature-independent values of 2.60 and 13.56 cps, respectively; (2) the rotational isomerism of phenylalanine can be represented in terms of an equi-
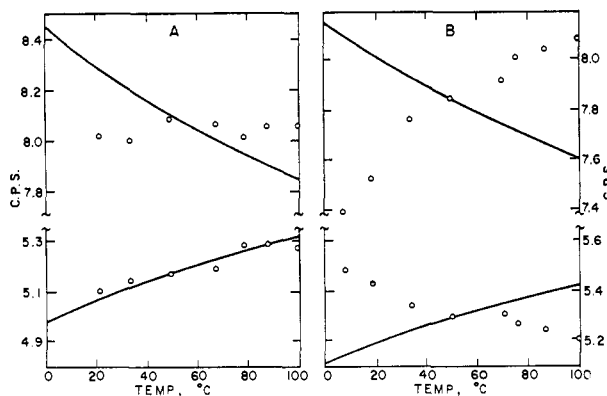
Figure 2. Observed and calculated temperature variation of the phenylalanine vicinal coupling constants assuming three staggered conformations, $J_g = 2.60$ cps and $J_t = 13.56$ cps: (A) dipolar ion, calculated curves adjusted to pass through the values 8.05 and 5.21 cps at $60°$; (B) anion, calculated curves adjusted to pass through the points 7.84 and 5.30 cps at $50°$.
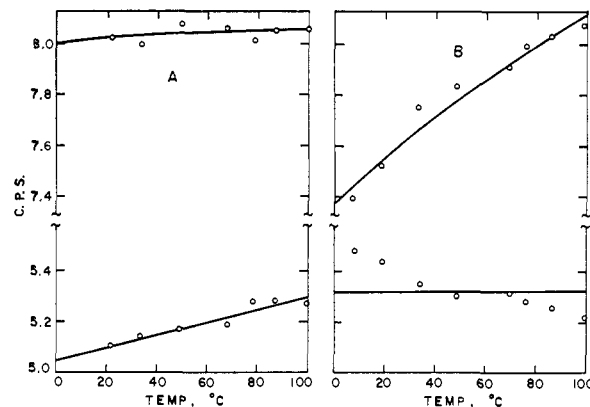

Figure 3. Observed and calculated temperature variation of the phenylalanine vicinal coupling constants assuming three staggered conformations and the values for $E_A - E_C$, $E_B - E_C$, J_g, and J_t given in Table II: (A) dipolar ion, (B) anion.

librium between the three staggered rotamers of Figure 1; and (3) the rotamer energies are temperature independent. The disparity between observed and calculated values shows that at least one of these assumptions has broken down.

J_g and J_t. **Temperature Independence and Numerical Values.** Since the coupling constant is an average over the vibrational states of the nuclei in a given conformation, an observable temperature variation is possible when the angular dependence of the coupling constant leads to different averaged values for the accessible vibrational states. Temperature variations are known to occur for fluorine–fluorine and fluorine–proton couplings.[25] The calculated variation for proton–proton couplings, however, is small;[26] for vicinal *gauche* and *trans* couplings, the predicted variations from 300 to 400°K are a 3% increase and a 2% decrease, respectively. For molecules for which the potential energy barrier to internal rotation has three-fold symmetry and for which an averaged proton–proton vicinal coupling is observed, the effects cancel out. The coupling should then be approximately temperature independent as verified for ethyl nitrate.[26a] This suggestion is also borne out by the alanine data; average deviations from the mean of the values obtained at different temperatures are 0.024, 0.024, and 0.026 cps for the alanine cation, dipolar ion, and anion, respectively.

It is possible that the experimental error could mask a small effect. For acetaldehyde, for example, a careful study[27] revealed a temperature variation of the coupling constant of about 0.15 cps over a 150° range.

It appears safe to assume that any variation of the coupling constants, J_g and J_t, will be small. Moreover, the correction[26a] is in the wrong direction; that is, when the curves in Figure 2 are calculated assuming a 3% increase in J_g and a 2% decrease in J_t from 30 to 100°, the new curves are in poorer agreement with the experimental points. J_{12} remains essentially the same

but the correction causes the predicted J_{13} to decrease slightly more rapidly with temperature.

In order to ascertain whether other values for J_g and J_t would lead to a better agreement with experiment, a "direct-search" procedure[28] was utilized in which J_g, J_t, $E_A - E_C$, and $E_B - E_C$ were independently varied in order to best fit the calculated and observed points using eq 1 and 3. The values selected were those for which the root-mean-square deviation attained a minimum. The results are shown in Figure 3 and Table II.

Table II. Results Obtained by "Direct-Search" Computation Using Eq 1 and 3 and Varying $E_A - E_C$, $E_B - E_C$, J_g, and J_t

	Anion	Dipolar ion
$E_A{}^a$	0	0
E_B	10^5	1590
E_C	790	171
a^b	0.796	0.547
b	0.000	0.040
c	0.204	0.413
$J_g{}^c$	5.32	4.82
J_t	16.22	12.60

[a] Rotamer energies in cal/mole. [b] Populations given for $33°$. [c] Coupling constants in cps.

While reasonable agreement can be obtained in this manner, especially for the dipolar ion, the values for the parameters listed in Table II are physically unappealing: the most stable conformer, A, places the phenyl group *gauche* to both the $-CO_2^-$ and $-NH_3^+$ (or $-NH_2$) groups; the B conformer at room temperature contributes only 4% for the dipolar ion and not at all for the anion; and the predicted *gauche* couplings are considerably larger than observed in similar systems.

Deviations from the Staggered Conformations. Equations 3 assume that the conformations are the classical staggered ones and that $J_{vicinal}$ is a function solely of the dihedral angle. The latter assumption is embodied

(25) W. S. Brey, Jr., and K. C. Ramey, *J. Chem. Phys.*, **39**, 844 (1963); K. C. Ramey and W. S. Brey, Jr., *ibid.*, **40**, 2349 (1964).

(26) (a) J. C. Schug, P. E. McMahon, and H. S. Gutowsky, *ibid.*, **33**, 843 (1960); (b) H. S. Gutowsky, V. D. Mochel, and B. G. Somers, *ibid.*, **36**, 1153 (1962).

(27) J. G. Powles and J. H. Strange, *Mol. Phys.*, **5**, 329 (1962).

(28) R. Hooke and T. A. Jeeves, *J. Assoc. Computing Mach.*, **8**, 212 (1961). The computations were carried out on IBM-7040 and IBM-1130 computers.
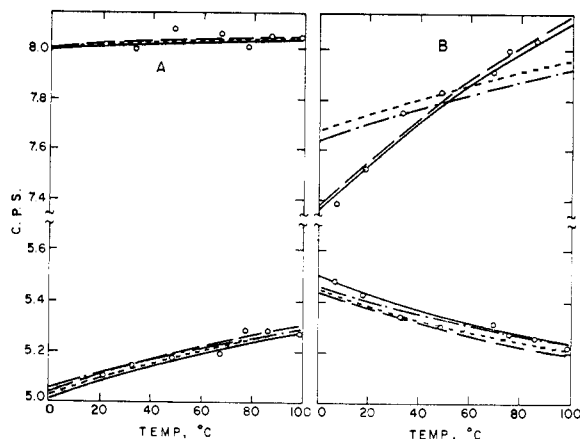
Figure 4. Observed and calculated temperature variation of the phenylalanine vicinal coupling constants assuming deviations from the staggered conformations: (A) dipolar ion, (B) anion; calculated curves from eq 1, 2, and 4. - - - - and — · — are for $J_{12} < J_{13}$ and $J_{12} > J_{13}$, respectively, with $K_1 = 10.40$, $K_2 = 13.56$, and the values of the other parameters listed in Table III. ——— and - - - - are for $J_{12} < J_{13}$ and $J_{12} > J_{13}$, respectively, with the values of the parameters listed in Table IV.

in the Karplus equation[29] which may be written as

$$J_{\mathrm{vic}} = \begin{cases} K_1 \cos^2 \varphi + C & 0 \le \varphi \le \pi/2 \\ K_2 \cos^2 \varphi + C & \pi/2 \le \varphi \le \pi \end{cases} \quad (4)$$

where K_1, K_2, and C are constants and φ is the dihedral angle. Different values of K_1 and K_2 reflect an asymmetry in the function about $\pi/2$. Despite the fact that the original valence-bond treatment underestimated K_1 and K_2, the functional form has been supported by a fair number of experiments.[30]

Accordingly, the temperature variation of the coupling constants may be calculated by allowing the stable conformations to depart from the staggered positions while assuming the validity of the Karplus equation. For the "direct-search" computation, the following parameters were selected: $E_A - E_C$, $E_B - E_C$, α_A, α_B, and α_C, where α_i is the angle of deviation from the ith staggered conformation; positive values indicate a counterclockwise rotation of the $-CH_2C_6H_5$ end of the molecule. The J values were determined by eq 4 with the constant C set equal to zero[31] and with $K_1 = 10.40$ and $K_2 = 13.56$.[15b] The averaged coupling constants were evaluated using eq 2. Since there is now no *a priori* criterion for assigning the smaller observed coupling to J_{12} or J_{13}, both were tried. The results are shown in Table III and Figure 4.

Both assignments yield essentially the same agreement. While the agreement is excellent for the dipolar ion, the calculated curves for the anion deviate significantly from the observed ones although they do exhibit a divergent behavior. Rotamer A is heavily populated in all cases, as is either B or C for the anion, depending on the assignment. For the dipolar ion, the B and C rotamers are nearly equally populated for both. The predicted deviations from the staggered conformations are appreciable, amounting to nearly 30° in several instances.

(29) M. Karplus, *J. Chem. Phys.*, 30, 11 (1959).
(30) *Cf.* ref 5 and 14, and references cited therein.
(31) C is generally assumed to be small. In view of the increased complexity of including it as an additional parameter, we follow several authors[5,14,18] in setting it equal to zero.

Table III. Results Obtained with "Direct-Search" Computation, Using Eq 1, 2, and 4 with $K_1 = 10.40$ and $K_2 = 13.56$

| | Anion | | Dipolar ion | |
	$J_{12} > J_{13}$	$J_{12} < J_{13}$	$J_{12} > J_{13}$	$J_{12} < J_{13}$
$E_A{}^a$	60	87	0	0
E_B	776	0	144	243
E_C	0	794	249	252
a^b	0.414	0.405	0.408	0.429
b	0.128	0.468	0.322	0.288
c	0.458	0.127	0.271	0.283
$\alpha_A{}^c$	−23	22	−18	28
α_B	3	−28	−16	−9
α_C	26	−3	19	12

^{a} Rotamer energies are in cal/mole. ^{b} Populations given for 33°. ^{c} α values in degrees.

Table IV. Results Obtained with "Direct-Search" Computation Using Eq 1, 2, and 4 and Varying K_1 and K_2

| | Anion | | Dipolar ion | |
	$J_{12} > J_{13}$	$J_{12} < J_{13}$	$J_{12} > J_{13}$	$J_{12} < J_{13}$
$E_A{}^a$	0	0	0	0
E_B	460	326	129	262
E_C	486	719	247	200
a^b	0.521	0.529	0.404	0.422
b	0.245	0.309	0.327	0.274
c	0.234	0.162	0.269	0.304
$\alpha_A{}^c$	12	−13	−10	18
α_B	28	−56	9	−5
α_C	52	−12	11	3
K_1	16.8	15.2	10.4	11.1
K_2	15.8	14.9	16.1	14.5

^{a} Rotamer energies are in cal/mole. ^{b} Populations given for 33°. ^{c} α Values are in degrees.

K_1 and K_2 may also be varied. The results are given in Table IV and included in Figure 4.

The parametric values obtained for the dipolar ion are only slightly changed; the angular deviations are somewhat reduced at the expense of altering the constants of the Karplus equation. The agreement for the anion is considerably improved and represents the only fully satisfactory one achieved. In order to accomplish this, however, the values for K_1 and K_2 are quite large, as are the angular deviations, approaching nearly 60° or the eclipsed conformation for one of the rotamers.

Consequently, in order to obtain agreement with experiment, strong departures from the classical picture are required: the A rotamer is substantially populated and may even be the most stable form; significant deviations from the staggered conformations occur with one of the stable forms of the anion nearly in the eclipsed position; K_1 and K_2 for the anion are considerably larger than had been previously estimated. Because the effect of bond angle distortion and a more precise evaluation of the angular dependence of the coupling constants have been neglected in this treatment, the numerical values must be regarded as approximate; the results given in Tables III and IV should be viewed as indications of possible behavior.

Temperature Variation of the Rotamer Energies. The observed temperature variation of the vicinal coupling constants may also be interpreted by assuming that deviations from the staggered positions are small and that the rotamer energies themselves change with temperature. Potential energies of rotational isomers are known to be sensitive to changes in intermolecular

environment, for example, changes in state or solvent.[32] The observed vicinal coupling constants for the phenylalanine anion exhibit a significant concentration dependence. The results[33] shown below indicate a divergence of the coupling constants with increasing concentration.

	0.17 M	0.5 M	1 M	3 M
J_{vic}, cps	5.41	5.33	5.34	4.38
	7.26	7.34	7.75	8.82

On the basis of eq 3, these values suggest that increasing the concentration enhances the relative stability of the C rotamer, that is, lowers the relative value of E_C.

A similar interpretation can be advanced to explain the temperature variation of the vicinal coupling constants. With $J_g = 2.60$ cps and $J_t = 13.56$ cps, eq 1 and 3 lead to the rotamer populations and energies shown in Table V as a function of temperature. As the temperature increases, the C rotamer becomes increasingly more stable; the expected decrease in the C population by virtue of the Boltzmann factor is masked by the relative lowering of the C rotamer energy. For the dipolar ion, the two effects are approximately equal; for the anion, the relative decrease in the C rotamer energy far outweighs the effects of the Boltzmann factor.

Table V. Calculated Rotamer Energies (in cal/mole) and Populations as a Function of Temperature

Temp, °C	E_A	E_B	E_C	a	b	c
			Anion			
7	210	282	0	0.300	0.263	0.437
49	347	423	0	0.277	0.246	0.477
99	474	547	0	0.263	0.238	0.499
			Dipolar Ion			
22	340	453	0	0.277	0.228	0.495
67	423	504	0	0.266	0.236	0.498
99	482	525	0	0.259	0.244	0.497

However, this interpretation also involves several difficulties. The concentration effects seem to imply that the increased intermolecular association coincident with the higher concentration is responsible for the enhanced stability of the C rotamer. Intermolecular associations usually diminish with increasing temperature; that is, raising the temperature often has the same effect as decreasing the concentration. It would appear that the C rotamer should become more stable at the lower, rather than the higher, temperature.

Secondly, despite their sensitivity to changes in the intermolecular environment, rotamer energies are generally temperature independent. The temperature variation of many physical properties of rotamer mixtures has been satisfactorily explained solely on the basis of the Boltzmann distribution.[32] However, it is conceivable that some aqueous solutions constitute exceptions. Phenylalanine contains highly polar hydrophilic groups as well as a large hydrophobic benzene ring. The rotamer energies are dependent both on

intermolecular and intramolecular parameters. If the solute–solvent interactions vary sufficiently with temperature, these variations would be reflected in observable changes in the rotamer energies. In view of the complexity of the interactions in aqueous solutions and the over-all lack of understanding of the nature of such interactions, an interpretation in terms of temperature-dependent rotamer energies cannot be excluded.[34]

The Geminal Couplings. The Chemical Shifts. The geminal couplings, as shown in Table I, vary slightly over the temperature range; extreme points for the anion and dipolar ion differ by about 0.3 and 0.2 cps, respectively. Since geminal proton–proton couplings are predicted to be temperature independent,[26b] the variations appear to result from population changes among conformations with different geminal couplings. Although the differences are small, this suggests the presence of some bond-angle distortion and the lack of constant bond angles for all conformations.

Amino acids display interesting variations in their chemical shifts with the ionization of the COOH group and the protonation of the NH₂ group.[35] The changes with temperature, on the other hand, are small; for glycine and alanine, they amount to a few cycles per second over the temperature range. The chemical shifts of the phenylalanine anion and dipolar ion likewise exhibit only a minor temperature dependence, with the exception of a 7-cps variation for one of the β proton resonances of the anion. It is to be noted that this variation increases the internal chemical shift of the β protons with increasing temperature. This appears to lend some support to the interpretation that one of the conformations has enhanced stability at the higher temperatures, as the magnetic nonequivalence of the two β protons is increasing rather than decreasing with temperature.[36] In view of the presently imposing difficulties of attempting to interpret small changes in the chemical shift, however, an assessment of the individual rotamer contribution is impractical at this time.

Conclusion

The temperature variations of the vicinal coupling constants challenge the generally accepted interpretation of the rotational isomerism of the phenylalanine anion and dipolar ion in solution. Two choices appear open: either the rotamer energies are temperature dependent, in which case the most likely conformation from steric considerations gains enhanced stability as a result of changes brought about in the structure of the aqueous solutions with increasing temperature; or, if the rotamer energies are held constant, strong departures from the classical picture of three staggered rotamers are required in order to obtain agreement with the experimental data. From the information so

(32) S. Mizushima, "Structure of Molecules and Internal Rotation," Academic Press Inc., New York, N. Y., 1954, Chapters 2 and 3.

(33) These results were obtained on the previously cited spectrometer converted to the DA-60-IL. They are accurate to about ±0.15 cps.

(34) To be thermodynamically correct, it would be the rotamer free energies which are temperature dependent. Whether this variation is due to changes in the rotamer potential energies *via* specific temperature-dependent interactions with the aqueous solution or stems from entropy effects resulting from such interactions is an open question.

(35) These have been reported and discussed previously.[12,13,16]

(36) A similar effect has been reported for the methylene protons of 2,3,5-trimethyl-2,3,5-tricyanohexane in several solvents: J. J. McLeskey, III, Ph.D. Dissertation, Duke University, 1965, part II.

far obtained, there appear to be no compelling reasons to choose either alternative. Nevertheless, the results presented here provide a clear indication of anomalous

behavior. When similar studies are extended to other amino acids, a fuller understanding of the underlying causes will hopefully emerge.

Electronic States of the Azoalkanes

M. B. Robin, Robert R. Hart, and N. A. Kuebler

*Contribution from Bell Telephone Laboratories, Inc., Murray Hill, New Jersey.
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Abstract: The electronic spectra of *cis*- and *trans*-HN=NH have been calculated in a Gaussian-type orbital basis with limited configuration interaction, and their states have been correlated with those of the isoelectronic molecule, ethylene. Comparison of the theoretical results with the electronic spectra of *cis*- and *trans*-azoalkanes, RN=NR, observed in the region 3–10 ev, shows that the weak band in these compounds at 3.5 ev is virtually 100% $n_+ \rightarrow \pi^*$, corresponding to the lowest Berry transition of ethylene; the second component, $n_- \rightarrow \pi^*$, is predicted to come 4 and 7 ev higher in the *cis* and *trans* compounds, respectively. Inasmuch as the N → V transition is predicted to come at 11 ev, all of the transitions observed in the 5–10-ev region, weak and strong alike, are assigned as involving σ-type orbitals in either the originating or terminating molecular orbitals, or both. The interpretation of the spectra is complicated by the fact that the compounds all show effects in the 5–9-ev region due to the substitution of one alkyl group for another, although the alkyl groups themselves do not absorb in this region, effects with which the calculations on HN=NH cannot cope.

It is rather interesting to contrast the wide popularity of the azoalkanes, RN=NR, among photochemists with the almost complete lack of interest in these molecules shown by spectroscopists. Not all azo compounds have been so mistreated, for the larger azo dyes, containing phenyl and/or naphthyl groups, have been studied extensively both experimentally and theoretically; it is only the azoalkanes, the parent molecules of this class of compounds, that have been ignored. Our current interest in the azoalkanes stems originally from the rather surprising conclusions drawn from an earlier spectroscopic study of a closely related system, the alkylolefins.[1]

As discussed in that study, in the alkylolefins there is a weak singlet–singlet transition about 1 ev *below* the N → V (singlet $\pi \rightarrow \pi^*$) transition, which has been the object of numerous investigations in the past 30 years. The assignment of this low-lying "mystery band" is puzzling since, in a conventional theory with π–σ separation, there are no other singlet–singlet excitations below the $\pi \rightarrow \pi^*$ transition. Our work on this mystery band led to its assignment as a $\pi \rightarrow$ CH σ^* transition ($^1A_g \rightarrow {}^1B_{2u}$ in ethylene) with significant s-type Rydberg character in the terminating orbital.[1] In an earlier study of the olefin spectra, Berry[2] concluded that the mystery-band excitation is CH $\sigma \rightarrow \pi^*$ ($^1A_g \rightarrow {}^1B_{3g}$) at the orbital level, just the reverse of the one quoted above.

The presence of a mystery band at 6 ev in ethylene immediately suggests that such a band might be found in this spectral region in a wide variety of other π-electron systems. Because most investigators now agree that either the originating or the terminating molecular orbital (MO) of the mystery-band transition involves σ-type orbitals, it seems clear that assignments

of bands in the 5–6-ev region made using a π-electron model, which neglects explicit mention of the σ orbitals, may, in fact, be in error. There is already one example of an erroneous spectral assignment arising from this neglect of the σ orbitals in the molecular orbital description of the electronic states of an unsaturated molecule.[3] It is our aim in this paper to investigate the importance of σ orbitals in the low-lying states of azoalkanes through an experimental study of the azoalkane spectra, the assignment of these spectra aided by calculations embracing all of the electrons and by the correlation between the olefin and azoalkane excited states involving $\pi \rightarrow \pi^*$, $\pi \rightarrow \sigma^*$, $\sigma \rightarrow \pi^*$, and $\sigma \rightarrow \sigma^*$ excitations.

Experimental Section

If two *trans* (*cis*) protons of an ethylene molecule are imagined to be pushed into the carbon nuclei, then the isoelectronic molecule *trans*- (*cis*-) diimide, HN=NH, results. Diimide is itself of great current interest, but for other, more chemical reasons.[4] Our interest in this molecule resides in the comparison between its spectrum and that of ethylene. Actually, diimide is very reactive, and our experiments were performed instead with the *trans*-azoalkanes $CH_3N=NCH_3$, $C_2H_5N=NC_2H_5$, $(CH_3)_2CHN=NCH(CH_3)_2$, and $(CH_3)_3CN=NC(CH_3)_3$, and with the *cis*-azoalkane, 3-methyl-1-pyrazoline
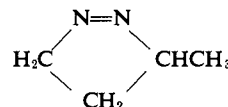
The first three materials were obtained from Merck Sharp and Dohme, Montreal, Canada, and were stated to be 99% pure. Both

(1) M. B. Robin, R. R. Hart, and N. A. Kuebler, *J. Chem. Phys.*, **44**, 1803 (1966).

(2) R. S. Berry, *ibid.*, **38**, 1934 (1963).

(3) Numerous π-electron calculations have led to the conclusion that the 6-ev band of norbornadiene is the symmetry-forbidden N → V₁, $\pi \rightarrow \pi^*$ transition. Vibronic analysis, however, shows that the band is a symmetry-allowed one and is most likely an olefin mystery band involving σ orbitals as well as π: M. B. Robin and N. A. Kuebler, *J. Chem. Phys.*, **44**, 2664 (1966).

(4) S. Hünig, H. R. Müller, and W. Thier, *Angew. Chem. Intern. Ed. Engl.*, **4**, 271 (1965).