Analysis of Proton Magnetic Resonance Spectra of Cysteine and Histidine and Derivatives. Conformational Equilibria[1]

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Chemical shifts and spin–spin coupling constants are reported for the three spin systems observed for cysteine and histidine and derivatives as a function of charge on the molecule. Charge plays a major role in determining chemical shifts in these compounds possessing several acidic groups. Diamagnetic metal ion complexes yield a shift further upfield than the free ligand of the same net charge, a difference particularly notable for the nickel ion complexes of cysteine. Spin–spin coupling constants have been interpreted to indicate that, of the three rotamers with a staggered conformation, the favored one possesses bulky carboxylate and sulfhydryl or imidazole groups trans with the amino group gauche to either of the latter groups although one alternative possibility cannot be definitely excluded. In thiazolidine-4-carboxylic acid and metal ion chelates of cysteine and histidine, the rotamer with the amino and sulfhydryl or imidazole groups trans is geometrically impossible. The similarity of the coupling constants obtained in the cysteine derivatives to these obtained for cysteine is taken to indicate that this rotamer is not favored in cysteine. Unlike their methyl esters which yield five-line spectra, cysteine and histidine exhibit ABX spectra in basic solutions. Though the ionized carboxylate group seems to play a role, the major factor in effecting a large chemical shift difference between the two β-hydrogens appears to be an asymmetric amino group. Rotamer populations are related to the chemical shift between the two β-hydrogens as well as to coupling constants.

Studies of conformational equilibria about single bonds in substituted ethanes have been performed on many halogenated ethanes by several methods,[2] including nuclear magnetic resonance spectroscopy. Spin–spin coupling and chemical shift studies of n.m.r. spectra provide a means for assessing populations of the three classical staggered forms of ethanes.[3] The rotamer populations of an important group of compounds, the amino acids and their derivatives, have received little investigation. Because side-chain conformation in proteins have been receiving detailed analysis by X-ray diffraction, we set out to examine rotamer populations in selected amino acids by proton magnetic resonance spectroscopy.

Chemical shifts in proton magnetic resonance spectra of amino acids observed in aqueous solutions of various pH values[4] and in trifluoroacetic acid[5] have been reported in the literature. Few detailed spin–spin coupling analyses were advanced in these studies. Several amino acids with a $>CH-CH_2-$ structure may be viewed as substituted ethanes; a detailed analysis of spin–spin splitting and chemical shift parameters leads to information concerning their rotamer populations. While our study was in progress, there appeared a spin–spin splitting analysis of some amino acids[6–8] and an interpretation in terms of rotamer populations.[9] Rather than attempting an over-all analysis of several amino acids, our research concentrated on two: cysteine and histidine, and their derivatives. This concentration permits an assessment of the relative effects of size and charge in determining conformational preferences. In addition, our analysis of the parameters is sufficiently different from that previously presented[9] that we advance an alternative approach more suitable for some of the cases reported here.

Experimental

Materials. L-Cysteine, S-methyl-L-cysteine, L-cysteine methyl ester hydrochloride, L-histidine, L-histidine methyl ester hydrochloride, glycyl-L-histidine hydrochloride, and acetyl-L-histidine were commercial products. Thiazolidine-4-carboxylic acid was synthesized from L-cysteine and formaldehyde,[10] m.p. 196–198° dec.; lit.[10] m.p. 196–197° dec. These reagents were made up to approximately 1 *M* concentrations in D_2O. Acid and base were added as DCl and NaOD, respectively. The chlorides of nickel, zinc, cadmium, and cuprous ions were dehydrated in an oven at 110°.

Methods. All spectra were recorded with a Varian Associates A60 spectrometer. Calculations were made on data obtained on the 100-c.p.s. sweep width scale. Some of our derived parameters were checked on computer program Freqint III as adapted for a Honeywell 800 computer at NIH.

Frequencies are reported in cycles per second with benzene in a sealed capillary as external reference. All frequencies are upfield from benzene (except protons of the imidazole ring of histidine and derivatives not recorded in this paper). No susceptibility corrections have been made on metal-free solutions. An estimate was made of the susceptibility corrections of solutions containing metal ions with respect to the metal-free solution containing the same concentration

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Table I. Proton Magnetic Resonance Parameters[a]

Compound	Charge	ν_X	ν_A	ν_B	$-J_{AB}$	J_{AX}	J_{BX}
Cysteine	+	121.5	197.5			5.2	
	+−	151.5	208.0			5.1	
	−1	169.5	209.4	218.4	13.9	3.9	8.2
	−1.5	182.9	211.0	227.2	13.2	3.6	8.7
	−2	195.4	213.2	235.5	12.8	3.3	9.5
Cysteine methyl ester	+1	118.5	197.4			5.2	
	0	152.5	209.7			5.3	
	−1	170.0	220.6			5.2	
S-methylcysteine	+	124.5	197.4			6.4	
	+−	152.7	205.0	209.5	15.0	2.3	9.7
	−	181.7	218.1	224.1	14.0	3.7	8.9
Thiazolidine-4-carboxylic acid	+[b]	99.5	176.3			6.4	
	−[c]	161.3	189.0	216.3	10.2	6.8	8.1
$^1/_2Zn^{II}$(cysteine)$_2$	−1	179.7	216.3	224.6	13.1	3.5	8.2
$^1/_2Ni^{II}$(cysteine)$_2$	−1	196.0	258.8			7.3	
$^1/_2Cu^{I}$(cysteine)$_2$	−0.5	159.2	199.2	207.6	13.5	2.8	9.4
Histidine	++	116.5	176.9			6.9	
	++−	140.0	185.7			6.7	
	+−	153.0	202.9			6.5	
	−	182.9	216.4	226.4	14.9	4.5	8.7
Histidine methyl ester	++	113.0	176.0			6.8	
	0	162.0	212.4			6.2	
Acetylhistidine	+	102.7	191.7			7.4	
	−	125.5	206.1	214.7	15.0	3.4	9.9
Glycylhistidine	++−	115.8	194.7	200.4	16.0	3.1	10.5
	+−				15.2	2.9	10.5
	−	122.5	205.2	211.6	15.2	3.0	10.6
$^1/_2Zn^{II}$(histidine)$_2$	0	154.9	206.6			4.1	
$^1/_2Cd^{II}$(histidine)$_2$	0	160.0	212.2			4.7	

[a] With respect to benzene as external reference in c.p.s. at a frequency of 60 Mc.p.s. [b] For the pair of protons on C-2, the average value of two peaks separated by 1.2 c.p.s. is 116.2 c.p.s. [c] For the pair of protons on C-2, ν = 124.1 and 143.5 and J = 9.4 c.p.s.

of ligand at the same net charge. This estimate was reached by measuring the shift of ligand peaks with respect to tetramethylammonium chloride (TMA) in a sealed capillary used as an external reference and in a different portion of the same solution as an internal reference. Only those ligand peaks not masked by TMA were employed in the comparison. The same tubes and capillary were used in all comparisons. For cysteine solutions and histidine with 2 equiv. of DCl, the internal TMA peak appears downfield from the external peak while the reverse is true of metal-free solutions of neutral histidine. For both ligands, addition of metal ions alters the internal TMA reference to lower field than in a metal-free solution. The net result is that for the cysteine solutions of Table I, 4.2 c.p.s. has been added to the observed chemical shifts for the zinc complex and 3.5 c.p.s. added for the nickel complex. Thus the pronounced upfield chemical shifts for the nickel complex are not due to neglect of susceptibility corrections. No corrections were attempted on the unstable cuprous–cysteinate complex. For the zinc and cadmium complexes of histidine, 2.9 and 3.0 c.p.s., respectively, have been added to the observed chemical shifts with respect to external benzene to yield the relative susceptibility corrected values of Table I. These relative corrections are close to those obtained by an entirely different method in solutions of similar mixtures.[11]

Results

Almost all results were obtained on three spin systems. Two of the hydrogens bound to the same carbon are designated A and B, and the separate hydrogen as C or X. Three general types of spectra were obtained: ABX with up to twelve lines, deceptively simple ABX with seven or eight lines, and A_2C with five lines.

ABX spectra were analyzed in a prescribed manner.[12] For these spectra, values of both J_{AX} and J_{BX} as well as J_{AB} are recorded in Table I. The highest field proton is taken as the B proton. The spectrum obtained from a solution of cysteine with −2 charge has a pronounced ABC character. Our calculated parameters for this spectrum were fed into a computer program which gave close agreement of calculated and observed spectra. Various sign combinations for J_{AX} and J_{BX} calculated for the solution with −1 net charge on cysteine were also subjected to computer analysis and only the computed intensities for J_{AX} and J_{BX} with like signs agreed with the observed spectrum. Like signs for vicinal coupling constants appear to be a general result for these systems.[7] Though the geminal and vicinal coupling constants appear to possess opposite signs,[6,7] our spectra are sufficiently ABX and our analysis not fine enough for this difference to be of consequence.

Only the average of J_{AX} and J_{BX} coupling constants can be calculated for ABX spectra with eight or fewer lines. Spectra for protonated thiazolidine-4-carboxylic acid, protonated S-methylcysteine, protonated N-acetylhistidine, and neutral and positively charged histidine are of a deceptively simple appearance and were analyzed on this basis.[13] Other spectra of Table I with only average values recorded for J_{AX} and J_{BX} exhibited five lines and were analyzed as

(11) N. C. Li, R. L. Scruggs, and E. D. Becker, *J. Am. Chem. Soc.*, **84**, 4650 (1962).

(12) J. A. Pople, W. G. Schneider, and H. J. Bernstein, "High Resolution Nuclear Magnetic Resonance," McGraw-Hill Book Co., Inc., New York, N. Y., 1959.

(13) R. J. Abraham and H. J. Bernstein, *Can. J. Chem.*, **39**, 216 (1961).

A_2C spectra. Frequencies of A_2C spectra were calculated by the standard method.[12] The average coupling constant, however, was determined simply and directly by the equation

$$3J = \nu_1 - \nu_4 + \nu_6 - \nu_8$$

where the subscripts refer to the nine possible lines in A_2C spectra. This equation may be derived from Table 6-8, p. 125 of ref. 12. The same spectra when analyzed as A_2C or a deceptively simple spectra gave average coupling constants differing by 0.3 c.p.s. or less and chemical shifts differing by less than 1 c.p.s.

Most spectra exhibit sharp peaks. Some broadening occurs in histidine spectra of intermediate charge.[8] Spectra for the cuprous and nickel ion complexes of cysteine recorded in Table I exhibit broad peaks. A solution containing cysteine and nickel ion in a 4:3 ratio with 8 equiv. of base[14] yields a spectrum with a broad C part and a sharp remainder. Parameters are about the same as those recorded in Table I for the 2:1 cysteine–nickel ion complex. Observation of any peaks in this kind of spectra demonstrates that these nickel ion complexes are diamagnetic. The HDO peak is sharp in all results recorded in Table I.

Discussion

For a given amino acid and its derivatives, the main parameter determining chemical shifts would appear to be charge on the molecule. Comparison of cysteine, S-methylcysteine, cysteine methyl ester, and even zinc(cysteine)$_2$ reveals that the chemical shifts for each of three protons for all four compounds are similar if they are compared at the same over-all net charge per cysteine molecule. Where significant differences appear, they may frequently be rationalized by considering the local sites of protonation for a given charged species. The rationalizations are complicated, however, by the competitive nature of the ionizations from sulfhydryl and ammonium groups. In cysteine the sulfhydryl ionization is slightly favored, while the ammonium ionization is favored in cysteine methyl ester.[15]

The frequency of the lowest field or α-hydrogen exhibits a pronounced dependence on charge as it is adjacent to two ionizing groups. Of the two β-hydrogens, the one at highest field (denoted H_B), is the more dependent on charge. As the pH is decreased from alkaline solutions, the difference $\nu_B - \nu_A$ decreases and finally disappears near zero net charge on the cysteine molecule. A detailed presentation of chemical shifts of cysteine with pH has been made.[8]

Similar statements concerning the predominant role of charge in determining chemical shifts may be made for histidine and its derivatives, including the zinc and cadmium complexes. Detailed plots of chemical shifts *vs.* pH for histidine have been presented.[8,16] Not unexpectedly, acetylhistidine and glycylhistidine are exceptional. In glycylhistidine the ammonium group is now considerably removed from the hydrogens whose shifts are being measured.

Chemical shift correlations of amino acids have also been treated by a molecular orbital method, but the results for aqueous solutions indicate much too great a contribution from uncharged, neutral rather than dipolar ion forms.[17]

The diamagnetic nickel–cysteine 1:2 complex yields abnormally high upfield shifts, especially for the highest field hydrogens. Even after susceptibility corrections, these high field hydrogens are more than 40 c.p.s. higher than uncomplexed cysteine of the same charge. The X hydrogens are shifted about 25 c.p.s. upfield in the nickel complex. A similar upfield shift is observed in a solution containing cysteine and nickel ion in a 4:3 ratio with excess base and in the diamagnetic nickel–tetraglycine complex.[18] In all cases of diamagnetic nickel complexes, the HDO line does not exhibit broadening. These relatively large upfield shifts with diamagnetic nickel complexes are in marked contrast to the smaller upfield shifts with the zinc and cadmium complexes when compared with ligands of the same net charge as the complex.

The geminal coupling constants J_{AB} increase as substrate is protonated. A similar trend has been observed in these and other amino acids,[6-8] as well as in malic acid.[19]

In order to discuss vicinal coupling constants, it is necessary to consider the three classical staggered forms of ethanes. As drawn for cysteine these are shown in Figure 1. Symbols *t*, *g*, and *h* will also be used to

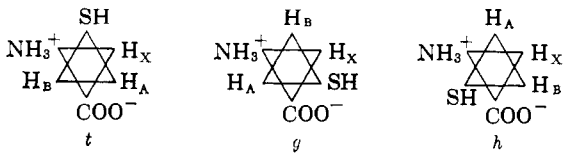

Figure 1. Three staggered rotamers of cysteine.

designate the mole fractions of a *trans* and two *gauche* rotamers, respectively. In the *trans* rotamer the bulky sulfhydryl and carboxylate groups are placed *trans*. The *gauche* rotamer *h* places the three bulkiest groups nearest to each other.

The labeling of the H_A and H_B protons in Figure 1 involves a commitment to the designation of the highest field hydrogen H_B to the position *trans* to H_X in the *t* rotamer. This assignment is made by analogy with results obtained on malic acid, which differs from cysteine in only two respects: replacement of the sulfhydryl and ammonium groups of the latter by carboxylic acid and hydroxy groups, respectively. Many features of the proton magnetic resonance solution spectra of both compounds are similar throughout the whole range of charge on the molecules, and especially in basic solutions where the charge distributions are identical.[19] A monodeuterio-L-malic acid has been prepared by specific enzymatic hydration of fumaric acid.[20] Though this malic acid was originally

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(20) T. C. Farrar, H. S. Gutowsky, R. A. Alberty, and W. G. Miller, *ibid.*, **79**, 3978 (1957).

thought to be a *threo* derivative due to *cis* hydration on the basis of a wide-line p.m.r. experiment performed on the solid acid with assumed *trans*-carboxylic acid groups,[20] it now appears that enzymatic hydration of fumaric acid occurs in a *trans* manner so that the deuterated malic acid is *erythro*.[21] In solution the monodeuterated malic acid exhibits the same highest field hydrogen as the normal acid, as well as the higher coupling constant with the α-hydrogen.[19] Thus the high-field proton we have designated H_B must be as labeled in Figure 1. This labeling for cysteine depends upon its similarity with malic acid and seems to be the best choice at this time.

Assuming the rapid exchange of all oxygen-, nitrogen-, and sulfur-bound hydrogens and the rapid interconversions of the three rotamers, the experimental coupling constants may be represented as

$$J_{AX} = tJ_G + gJ_T + hJ_G \quad (1)$$

$$J_{BX} = tJ_T + gJ_G + hJ_G \quad (2)$$

These equations assume that J_T and J_G have the same values in all three rotamers. Though this assumption is unlikely as deviations from the dihedral angles of 60 and 180° almost certainly occur, we shall make this assumption.

Since $t + g + h = 1$, the mole fractions of each rotamer may be estimated if appropriate values of J_G and J_T can be found. From the results of a detailed analysis of hydroxyprolines,[22] values of 2.60 and 13.56 c.p.s. have been employed for J_G and J_T, respectively, in analysis of rotamer distributions of amino acids.[9] The important quantity $2J_G + J_T = 18.76$ on this basis, and the average value of J is 6.25 c.p.s. Rather than follow this procedure, we pursue a different approach employing other assumptions less restrictive in the interpretation of some of our results.

First, however, four general equations that follow from eq. 1 and 2 are presented. The difference

$$J_{BX} - J_{AX} = (t - g)(J_T - J_G) \quad (3)$$

so that $J_{BX} > J_{AX}$ implies $t > g$ because $J_T > J_G$. If hydrogens A and B are reversed in Figure 1, the opposite conclusion prevails: $g > t$. The ratio of t/g is given by

$$t/g = (J_{BX} - J_G)/(J_{AX} - J_G)$$

Again, if the labeling of H_A and H_B is reversed, the right-hand side yields g/t.

The quantity $2J_G + J_T$ occurs frequently and a general expression is

$$2J_G + J_T = \frac{J_{BX}(1 - 3g) + J_{AX}(3t - 1)}{t - g}$$

In solutions where the spectra are such that only an average value of J_{AX} and J_{BX} may be deduced

$$2J_{AV} = J_{BX} + J_{AX} = J_G(1 + h) + J_T(1 - h)$$

Even with assumed values of J_G and J_T, only the mole fraction h may be determined; the ratio of t/g is indeterminate without further assumptions or information. Thus average values of J may range from J_G to $(J_G + J_T)/2$, with a value of $(2J_G + J_T)/3$ at $h = 1/3$.

By considering three limiting cases in the rotamer distribution, it is possible to achieve some simplifications in eq. 1 and 2. The three cases are (I) $g = h$; (II) $h = t$; and (III) $g = t$. Substitution of these limitations into eq. 1 and 2 yields for the designated cases

$$\text{I.} \quad 2J_{AX} + J_{BX} = 2J_G + J_T = \text{constant}$$

$$\text{II.} \quad J_{AX} + 2J_{BX} = 2J_G + J_T = \text{constant}$$

$$\text{III.} \quad J_{AX} = J_{BX}$$

Substituted ethanes of the A_2B_2 type necessarily have $g = h$, and our conclusions concerning case I are similar to those discussed for the less substituted ethanes,[23] though expressed in a different form.

When $J_{AX} \simeq J_{BX}$, case III with $g = t$ is a satisfactory approximation. Of the amino acids studied, this case seems to be best satisfied by serine.[6,7] The halogenated ethane $CF_2BrCFBrCl$ exhibits nearly equal values of fluorine coupling constants J_{AX} and J_{BX} over a 240° temperature range, indicating that the rotamers without two pairs of *gauche* fluorines are of about equal stability throughout. The same conclusion has also been reached by a more complicated analysis.[3]

Inspection of the values of J_{AX} and J_{BX} for cysteine in Table I reveals that $2J_{AX} + J_{BX} = 16.0 \pm 0.1$ over a change of a full unit of charge, so that evidently case I applies and $g = h$. Since this number, also equal to $2J_G + J_T$, is much less than that (18.76) indicated above, the previously recommended values of J_G and J_T do not seem applicable to cysteine. Deviations from the 60 and 180° dihedral angles may be reponsible. We must also assume values of J_G and J_T to estimate mole fractions, but in this case the value of one determines that of the other. If we choose $J_T = 12.0$, then $J_G = 2.0$ c.p.s., and for cysteine with a double negative charge $g = h = 0.12$ and $t = 0.75$. Similar values of J_{AX} and J_{BX} have been observed in the most basic solution of malic acid[19] so that perhaps similar numerical results also apply to it. (If the H_A and H_B assignments in Figure 1 are reversed, then $t = h = 0.12$ and $g = 0.75$.)

The conclusion that rotamer t is predominant for cysteine and malic acid is a direct consequence of the assignments of the H_A and H_B hydrogens in the diagram and the well-established[22] fact that $J_T > J_G$ for such compounds. The greater thermodynamic stability of rotamer t over rotamers g and h in basic solutions is the result expected for favored *trans* positions of the bulkiest and identically charged groups. Other workers have begun with this assumption while we assumed an analogy between the basic forms of malic acid and cysteine.

Histidine differs from cysteine only in the replacement of the sulfhydryl function of the latter by an imidazole group. High populations of the *trans* rotamers are also indicated for histidine and derivatives by arguments analogous to those presented above.

If it is assumed that $2J_{AX} + J_{BX} \simeq 16.0$ persists into acid solutions of cysteine where only an average coupling constant is listed in Table I, then $J_{AX} = J_{BX}$ within experimental error and all three rotamers are

(21) O. Gawron and T. P. Fondy, *J. Am. Chem. Soc.*, **81**, 6333 (1959); **83**, 3634 (1961); F. A. L. Anet, *ibid.*, **82**, 994 (1960).

(22) R. J. Abraham and K. A. McLauchlan, *Mol. Phys.*, **5**, 513 (1962).

(23) R. J. Abraham and K. G. R. Pachler, *ibid.*, 7, 165 (1964).

equally populated in acid solutions where the carboxylic acid group is in its acid form. A similar average coupling constant of about 5 c.p.s. is also obtained from solutions of cysteine in trifluoroacetic acid.[5] Since cysteine methyl ester exhibits nearly identical average coupling constants for all charged species, evidently all three rotamers possess similar stabilities throughout the entire pH range. Thus the bulky ester group is not as important in determining preferred rotamer conformation as is a negatively charged carboxylate group. This conclusion is confirmed by the appearance of only an A_2X spectra in the most basic solutions of histidine methyl ester, while histidine exhibits ABX spectra even in neutral solutions. Steric effects appear less important in determining conformation than charge and its consequences for dipole moment and interaction with solvent. Intramolecular hydrogen bonding may also be important in determining rotamer stabilities but has not been considered here.

Rotamer *g* is not possible for the ring structures including all the chelate compounds listed in Table I. (The 1:2 cuprous–cysteine complex probably consists of a linearly hybridized cuprous ion linked only to two sulfhydryl groups.) Thiazolidine-4-carboxylic acid and the cysteine chelates of zinc and nickel ions exhibit coupling constants differing little from that of the parent compound, and structures analogous to rotamer *t* in the parent compound are indicated for the conformation about the α- and β-carbons. The similarity of the coupling constants also indicates that rotamer *g* is not favored in cysteine. The zinc and cadmium ion complexes of histidine display relatively low average coupling constants, and a preponderance of the structure with a conformation about the α- and β-carbons similar to rotamer *h* appears to be indicated.

Further insights into rotamer distributions may be obtained by explicit consideration of observed chemical shifts in terms of shifts for each nucleus in each of the three rotamers.

$$\nu_A = t\nu_t^A + g\nu_g^A + h\nu_h^A \quad (4)$$

$$\nu_B = t\nu_t^B + g\nu_g^B + h\nu_h^B \quad (5)$$

In general, magnetic nonequivalence due to molecular asymmetry of A and B protons implies that

$$\Delta\nu_{AB} = \nu_B - \nu_A \neq 0$$

even when rotamer populations are equal.[24] For the cases presented in this paper, we have concluded from the spin–spin coupling analysis that rotamer populations are equal in acid solutions. Since $\Delta\nu = 0$ in acid solutions, evidently the magnetic nonequivalence, if any, is small and beyond the powers of our instrument to discern. We apply the result of insignificant molecular asymmetry in acid solutions to the entire pH range of solutions studied.

Accepting that molecular asymmetry is not a factor, we can differentiate chemical shifts on the basis of position of H_A or H_B in the rotamers without regard to whether the hydrogen is A or B.

$$\nu_t^A = \nu_h^B = \nu_2$$

$$\nu_g^A = \nu_t^B = \nu_3$$

$$\nu_h^A = \nu_g^B = \nu_1$$

(24) H. S. Gutowsky, *J. Chem. Phys.*, 37, 2196 (1962).

The positions where hydrogens A and B may appear are numbered clockwise beginning at the top of Figure 1. Substitution of these equations into eq. 4 and 5 yields

$$\Delta\nu_{AB} = t(\nu_3 - \nu_2) + g(\nu_1 - \nu_3) + h(\nu_2 - \nu_1) \quad (6)$$

which is zero, in general, only when $t = g = h$.

Inspection of Table I reveals that chemical shift differences $\Delta\nu_{AB}$ greater than about 10 c.p.s. appear to require a basic amino group. As deprotonation of the substituted ammonium ion occurs on increasing pH to yield an asymmetric amino nitrogen, the chemical shift difference $\Delta\nu_{AB}$ in cysteine, histidine, aspartic acid,[8] and asparagine increases. Both β-hydrogens are in identical positions with respect to the amino group in rotamer *g*. Then to a first approximation, $\nu_1 = \nu_3$.

For cysteine the conclusion of the spin–spin coupling analysis is that $g = h$. With this equality eq. 6 becomes

$$\Delta\nu_{AB} = (t - g)(\nu_3 - \nu_2) \quad (7)$$

Equation 7 does not contain the difference of the frequency terms that were taken as approximately equal in the previous paragraph so that the excess fraction of rotamer *t* over *g* may also be determined in principle from eq. 7. Since we have previously concluded that $t > g$ and the chemical shift of H_B by definition lies at higher field than H_A, ν_3 (and ν_1) appears at higher field than ν_2. Thus the positions *gauche* to the amino group in Figure 1 are at highest field.

Equation 7, which expresses the chemical shift difference for cysteine, may be combined with eq. 3 for the coupling constant difference to yield

$$\frac{\nu_B - \nu_A}{J_{BX} - J_{AX}} = \frac{\nu_3 - \nu_2}{J_T - J_G} \quad (8)$$

The left-hand side of eq. 8 contains experimental quantities and the right-hand side should be dependent upon pH but possibly independent of temperature. Detailed temperature studies are required to test eq. 8 for cysteine and A_2B_2 systems.

The increasing chemical shift difference $\Delta\nu_{AB}$ on ionization of ammonium hydrogens might be related to the unexplained decrease in the frequencies of C–H stretching vibrations observed in the Raman spectra of aqueous solutions of amino acids and other amines on loss of a proton from a substituted ammonium group.[25] The frequency decrease of the stretching vibrations might be due to changes in the populations of rotamers upon proton transfer. Alanine is exceptional in exhibiting no frequency decrease in the Raman spectra, and none would be expected since all three rotamers are equally probable. The observation of a frequency decrease for glycine demands, however, a further explanation, and perhaps conformational equilibria about the C–N single bond also play a role. Only for the asymmetric basic amino form could these rotamers possess unequal stabilities.

If the H_A and H_B designations of Figure 1 are reversed or if the chemical shift difference $\Delta\nu_{AB}$ is ascribed to the carboxylate group in an analysis similar

(25) S. A. S. Ghazanfar, J. T. Edsall, and D. V, Myers, *J. Am. Chem. Soc.*, **86**, 559 (1964); M. Takeda, R. E. S. Iavazzo, D. Garfinkel, D. H. Scheinberg, and J. T. Edsall, *ibid.*, **80**, 3813 (1958); D. Garfinkel and J. T. Edsall, *ibid.*, **80**, 3807, 3823 (1958); E. L. Elson and J. T. Edsall, *Biochemistry*, **1**, 1 (1962).

to that presented for the amino group, then the corresponding eq. 7 contains the difference of frequency terms which, with an approximation analogous to that made above, would be zero for all charged species. However, if both the designations H_A and H_B of Figure 1 are reversed and the chemical shift difference ascribed to the unequal influence of the magnetically anisotropic carboxylate group on the three β-hydrogen positions, then an equation similar to 7 would yield a nonzero result for the chemical shift difference arising from unequal rotamer populations. It can be argued that an ionized carboxylate group is the major factor responsible for an ABX-type spectra for the compounds listed in Table I. The methyl esters of cysteine and histidine do not yield this type of spectra even in basic solutions. According to the kind of analysis presented here, a major role for the carboxylate group in yielding a nonzero result for $\Delta\nu_{AB}$ implies that the population of rotamer g exceeds that of rotamer t.

As pointed out in the spin–spin coupling analysis, reversal of the H_A and H_B designations in Figure 1 yields $g > t$. All the results in this paper may be rationalized on this basis almost as well or even better than those presented here for $t > g$. The predominance of rotamer t for malic acid appears established, but the analogous rotamer for cysteine may not be sufficiently similar for the analogy employed in this paper to be valid. It is conceivable that solvation effects could stabilize rotamer g in cysteine, so that the preponderance of rotamer t, though likely on the basis of the assumptions made in this paper, is not definitely established. Studies on the four substituted ethanes, valine, and threonines in this and other laboratories[6,8] reveal coupling constants between the α- and β-hydrogens from 3.6 to 5.1 c.p.s. These low values require that rotamers with the α- and β-hydrogens in *gauche* positions be favored with lesser amounts of the rotamer with the α- and β-hydrogens *trans*.[8] Thus the four substituent groups in these amino acids evidently lie in adjacent *gauche* positions in the favored rotamers.

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A Scheme for Strain Energy Minimization. Application to the Cycloalkanes[1]

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A simple direct method for strain energy minimization which is independent of the specific molecular geometry of a given case has been developed. The required molecular parameters have been estimated from the available thermodynamic, structural, and spectral data. The method has been applied to cyclooctane, cyclodecane, and cyclododecane.

I. Introduction

The increasing interest in compounds which are thermochemically destabilized by bond angle deformation and nonbonded interactions makes it necessary to develop a simple, direct computational scheme for obtaining the minimum energy conformation of a molecule. Simultaneously, it might be possible to estimate the value of the minimum energy. A number of attempts in this direction have been made,[2,3] but in general they have been related to the specific geometry of the compound. Thus, Westheimer and Mayer[4] in their very successful treatment of the rate of racemization of *ortho*-substituted biphenyls based the calculation on the use of a set of internal coordinates (*i.e.*, bond lengths, bond angles, and torsional angles). In one of the most recent strain energy calculations, Hendrickson[2] also used a set of internal coordinates.

The use of internal coordinates is desirable in those cases in which an analytical solution to the energy minimization problem is possible.[3,4] With more complex cases, these coordinates, although directly related to the structure of the compound, are inconvenient. First, the interrelationships between the internal coordinates are complex, and it is difficult to determine the relationship between a change in one coordinate and the resultant change in all the others. With the relatively flexible cycloalkanes, a change in any one coordinate can be accommodated by any of a large number of alterations in the other internal coordinates, and one does not know which choice to make. Further, the transformation from internal coordinates to Cartesian coordinates is inconvenient. However, the latter (or its equivalent in terms of the internal coordinates) is required if one is to calculate complete sets of nonbonded distances. These considerations have led us to develop an alternate computational scheme.

II. Method of Calculation

We have chosen to work with the Cartesian coordinates of the atoms. The effect of a change in coordinates of any given atom is easily obtained since

(1) This work was supported by the Army Research Office (Durham) and the Petroleum Research Fund.

(2) J. B. Hendrickson, *J. Am. Chem. Soc.*, **83**, 4537 (1961); **84**, 3355 (1962), and references given therein.

(3) F. H. Westheimer in "Steric Effects in Organic Chemistry," M. S. Newman, Ed., John Wiley and Sons, Inc., New York, N. Y., 1956.

(4) F. H. Westheimer and J. E. Mayer, *J. Chem. Phys.*, **14**, 733 (1946); F. H. Westheimer, *ibid.*, **15**, 252 (1947); M. Rieger and F. H. Westheimer, *J. Am. Chem. Soc.*, **72**, 19 (1950).