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Co-oligopeptides Containing Two Aromatic Residues Spaced by Glycyl Residues. 11. A Conformational Study of Tryptophan- and Glycine-Containing Oligopeptides Based on the Temperature Dependence of ^{1}H NMR Spectra

Antonio Baici,† Vincenzo Rizzo,‡ Peter Skrabal, and Pier Luigi Luisi*

Contribution from the Technisch-Chemisches Laboratorium ETH, 8092 Zürich, Switzerland.
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Abstract: Proton magnetic resonance spectra were recorded in the temperature interval from +51 to −64 °C at 360 MHz with alkaline CD_3OD solutions of the following compounds: H-Trp-OH, H-Trp-Gly-OH, H-Gly-Trp-OH and H-Gly-Trp-Gly-OH (which contain one tryptophyl residue); H-Gly-Trp-Gly-Trp-Gly-OH and H-Gly-Trp-$(Gly)_2$-Trp-Gly-OH (which contain two tryptophyl residues spaced by glycyl residue(s)); H-Trp-Trp-OH, H-Trp-Trp-Gly-OH, H-Gly-Trp-Trp-OH, and H-Gly-Trp-Trp-Gly-OH (which contain two adjacent tryptophyl residues). The following parameters have been derived from the experimental spectra and their dependence on temperature is discussed: (1) chemical shifts of the aromatic protons; (2) chemical shifts and coupling constants of the $C_\alpha H$–$C_\beta H_2$ fragment of the tryptophyl residue; (3) anisochronism of the glycine C_α protons (also in view of the possible presence of hydrogen-bonded structures of the peptide backbone). On the basis of these parameters, the conformational properties of the aromatic side chains (in terms of rotamer populations) and of the backbone (in terms of possible hydrogen-bonded conformers) are discussed. A thermodynamic analysis of the rotamer populations around the χ_1 torsion angle of the tryptophyl side chain in the investigated compounds has been carried out. The enthalpy differences between the three classical rotamers G^+, G^-, and T are found to be linearly related to the respective entropy differences. For the favored conformer of H-Trp-Trp-O^- at low temperature, the detailed geometry is proposed, which is in agreement with the previously described spectroscopic properties of this molecule.

Introduction

This paper is part of a general study on the conformational properties of peptides containing one or two aromatic amino acid residues and glycyl residues. Previous CD studies[1,2] have shown that sequences containing two adjacent aromatic residues (in particular the pair -Trp-Trp-) may possess some degree of conformational rigidity.[3] A ^{1}H NMR study at room temperature[4] enabled us to describe the conformational equilibrium of the aromatic side chains. However, only subtle

† Forschungslaboratorium der Rheumaklinik, Universitätsspital, 8091 Zürich, Switzerland.
‡ Institute of Molecular Biology, University of Oregon, Eugene, Oregon 94703.

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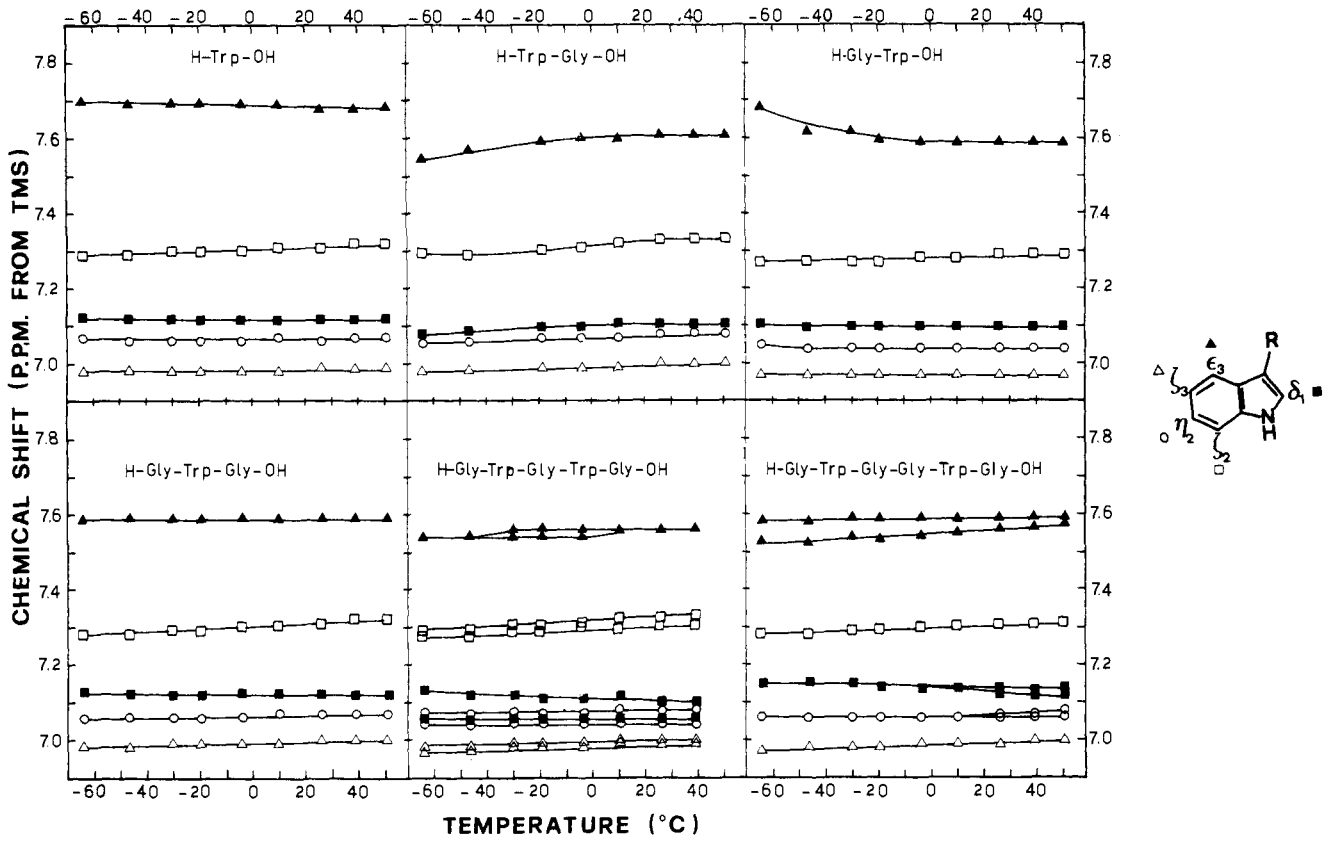

Figure 1. Temperature dependence of the chemical shifts of the aromatic protons in peptides containing one Trp or two Trp's separated by one or two Gly's. The symbols refer to the assignment shown at the right of the figure.

differences were found between sequences containing two adjacent or nonadjacent tryptophyl residues at room temperature. In particular these studies did not answer the question as to whether an intramolecular interaction between the two aromatic moieties does affect the molecular conformation in peptides containing the sequence -Trp-Trp-. Our approach in solving this question and the more general one of the conformation of the peptides in alkaline methanol solution proceeds along the following lines: (a) a temperature ^{1}H NMR study over the interval +51 to −64 °C in order to determine the most stable conformations of the side chains and to possibly locate the more rigid part of the molecules; (b) a low-temperature CD study (down to −90 °C) in an attempt to separate empirically the contributions of backbone and side chain; (c) using various approaches,[5,6] the calculation of the part of CD spectra which arises from chromophoric interactions between the two indoles in the sequence -Trp-Trp-. These three techniques should converge in defining for each compound the most stable conformations in terms of side chain as well as backbone torsion angles. This paper is concerned with part (a). Parts (b) and (c) are now in progress and will be discussed in forthcoming papers. The present study is limited to the resonance signals arising from C-bonded protons, all experiments being carried out in alkaline CD_3OD. Methanol was chosen because, in addition to its good solvation properties, it would enable us to extend the conformational study into the low-temperature region and because methanol and water solutions in some cases exhibit CD spectra similar to each other.[2] Alkaline conditions were chosen to ensure that all peptides were present as anions, and because previous CD data[2] had shown that in some cases in basic solution an interesting restriction of the conformational equilibrium, which was not observed in acid solution, is present. On the other hand, solvent exchange in alkaline methanol solution precludes the observation of signals from -NH protons, which are necessary in assessing the conformational properties of the backbone. A study of acid solutions is in progress, but of course the data are not of direct relevance to the present study, since the conformational equilibrium may change with the ionization state. The compounds investigated are H-Trp-OH, H-Trp-Gly-OH, H-Gly-Trp-OH, H-Gly-Trp-Gly-OH (which contain one Trp residue), H-Gly-Trp-Gly-Trp-Gly-OH and H-Gly-Trp-$(Gly)_2$-Trp-Gly-OH (which contain two Trp's separated by Gly residue(s)); H-Trp-Trp-OH, H-Trp-Trp-Gly-OH, H-Gly-Trp-Trp-OH, and H-Gly-Trp-Trp-Gly-OH (which contain two adjacent Trp's). The assignments of resonance signals have been discussed previously.[4]

Experimental Section

L-Tryptophan was a Serva p.A. product. The synthesis and the characterization of the peptides, including questions pertaining to their optical purity, have been described earlier.[7] All asymmetric C atoms of the amino acid residues have the L-configuration.

^{1}H NMR spectra were recorded with 0.02 M solutions of the peptides in CD_3OD/NaOD on a Bruker HXS-360 spectrometer operating in the Fourier transform mode with a digital resolution of 0.2 Hz/point. The chemical shifts relative to internal Me_4Si are given in the figures as ppm ± 0.01. CD_3OD (99.5% isotopic purity) and NaOD (40% in D_2O) were Ciba-Geigy products. The solutions contained 0.1 M NaOD and 0.3 M D_2O (0.4% NaOD, 0.6% D_2O w/v).

The temperature of the samples was measured with an accuracy of ±2 °C. Computations of the ABX systems were done at the ETH Rechenzentrum using the LAOCN 3 program.[8] The root mean square error of computed spectra was 0.3 Hz or less. Only for H-Gly-*Trp*-$(Gly)_2$-Trp-Gly-OH[9] at temperatures below 26 °C was the number of observed transitions insufficient to obtain significant parameters for the ABX spectra.

Results and Discussion

Chemical Shifts of the Aromatic Protons. As shown in Figure 1, the chemical shifts of the aromatic protons for peptides

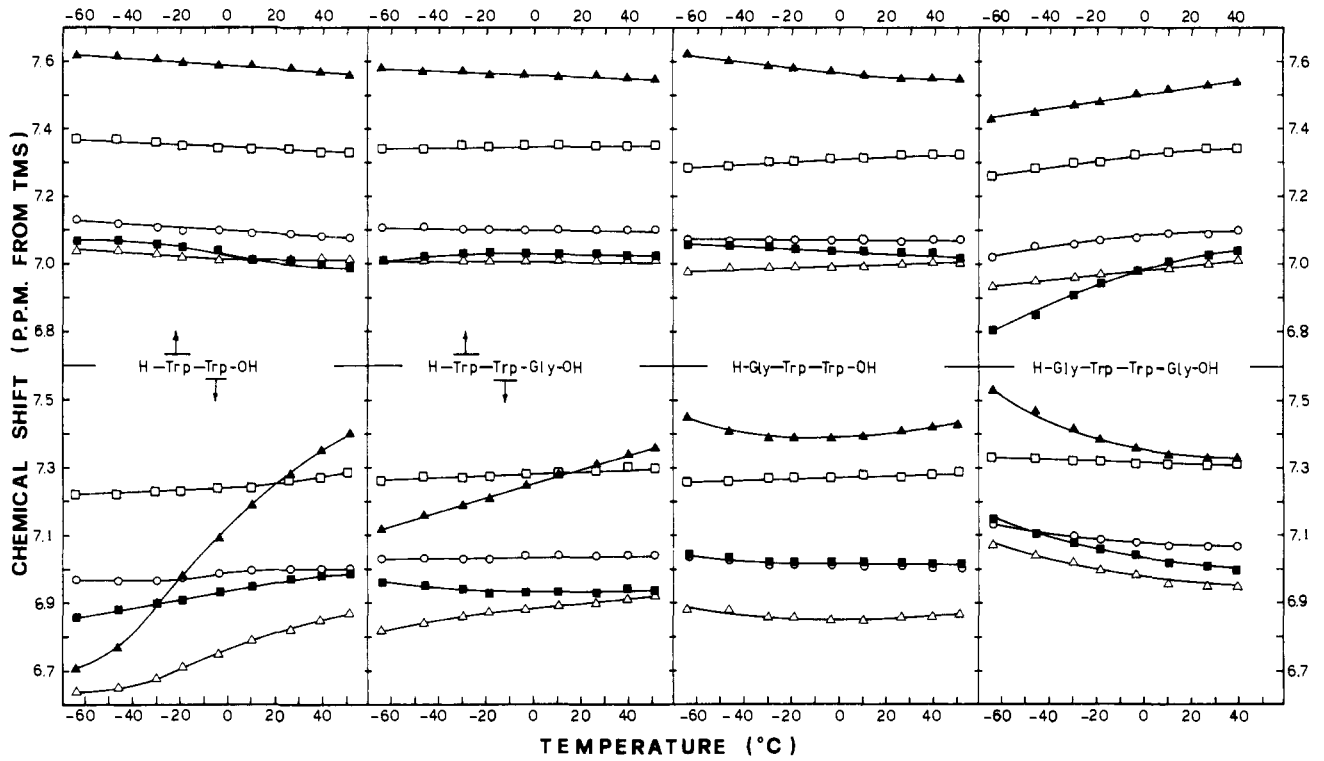

Figure 2. Temperature dependence of the chemical shifts of the aromatic protons in peptides containing the -Trp-Trp-sequence. Symbols are the same as in Figure 1. For H-Gly-Trp-Trp-OH and H-Gly-Trp-Trp-Gly-OH, the assignment to either aromatic ring cannot be done with certainty. For the latter compound, the group of signals which shifts upfield with decreasing temperature is likely to belong to Trp(2); see Discussion.

containing one Trp, or two Trp's separated by one or two Gly, are practically temperature independent. Furthermore, the chemical shift of a given proton has about the same value, regardless of the structure of the peptide. The following average values and standard deviations are obtained from the data of Figure 1: $\delta_{H_{\delta 1}} = 7.11 \pm 0.03$; $\delta_{H_{\epsilon 3}} = 7.59 \pm 0.05$; $\delta_{H_{\zeta 3}} = 6.98 \pm 0.01$; $\delta_{H_{\eta 2}} = 7.06 \pm 0.01$; $\delta_{H_{\zeta 2}} = 7.30 \pm 0.02$. The lack of sensitivity of the above chemical shifts to structure changes is surprising because in the investigated temperature range the peptides display a wide range of rotamer populations around the torsion angle χ_1 (see Figures 7 and 8). The reported standard deviations can be taken as an indication of the magnitude of both structural and conformational effects on the chemical shifts of the aromatic protons in a Trp side chain.

Figure 2 reports the chemical shifts of the aromatic protons of peptides containing two adjacent Trp residues. Comparison of the spectrum of H-Trp-Trp-OH with the spectrum of H-Trp-(δ_1,ϵ_3,ζ_3,η_2,-d_5)Trp-OH permitted an unequivocal assignment of the aromatic resonances.[4] In the case of H-Trp-Trp-Gly-OH the assignment is suggested by the analogy with H-Trp-Trp-OH (upfield shift of $H_{\epsilon 3}$ of Trp(2)[10] with decreasing temperature). For the other two compounds the assignment of the signals to the two aromatic moieties cannot be done at present. As can be seen the peptides containing the -Trp-Trp- sequence differ from H-Gly-Trp-Gly-Trp-Gly-OH and H-Gly-Trp-$(Gly)_2$-Trp-Gly-OH by large deviations from the average values reported above for the chemical shifts of the aromatic protons. Striking is the case of H-Trp-*Trp*-OH, where at −64 °C $H_{\epsilon 3}$ is shifted upfield by 0.9 ppm with respect to the reference average value. Because of the observation that the dependence of the chemical shifts of the aromatic protons on the backbone chemical structure and χ_1 rotamer populations is small, these large effects must be attributed to the ring current of the neighboring aromatic side chain.

Chemical Shifts of the Gly C_α Protons. In the previous study,[4] the C_α protons of glycyl residues at the N side of a tryptophyl residue showed an upfield shift with respect to those of H-Gly-Gly-Gly-OH. This fact was attributed to a preferential orientation of the aromatic side chain toward its amino side. In this work we direct our attention to the chemical-shift differences of geminal C_α protons of Gly residues (anisochronism = $\Delta\alpha$), which is observed in all but one of the cases investigated (H-Gly-Trp-Trp-OH). Data are reported in Figure 3. There is good evidence in the literature[11] indicating that this anisochronism is sensitive to the conformation around the torsion angles ϕ and ψ of the Gly residue itself. In particular, large values for $\Delta\alpha$ have been observed for Gly-containing peptides, whose peptide backbones are involved in intramolecular hydrogen bonding (e.g., in β or γ turn).[12,13] However, as has been pointed out by Gutowsky[14] and by Binsch and co-workers,[15] any observed time-averaged anisochronism is the sum of an intrinsic and a conformational term.[16] In principle, the relative magnitudes and signs of these two terms should be known, which is impossible in our case. Nevertheless, since the intrinsic term depends only on the nature of the substituent, we can compare peptides having the same substituent attached to the glycine C_α carbon. In addition, since there are indications that the intrinsic terms of anisochronism are only slightly temperature dependent,[15] we can qualitatively discuss differences of the temperature dependence of the anisochronisms among the various peptides in terms of conformational differences. For this comparison, it is useful to consider the dipeptides as reference compounds, since a dipeptide cannot form any intramolecularly H-bonded structure (except for the unfavored C_5 one[17]). Thus, among the peptides having a C-terminal glycyl residue, the relatively large anisochronism and its temperature dependence observed for H-Gly-Trp-Trp-Gly-OH vis-à-vis H-Try-Gly-OH can be attributed to a local conformational rigidity of the carbon terminal: the hypothesis that this end group is involved in a hydrogen-bonded structure of the backbone appears very likely, the statistical weight of this conformation increasing with decreasing temperature. Similar suggestions can be made for H-Trp-Trp-Gly-OH and H-Gly-Trp-Gly-OH.

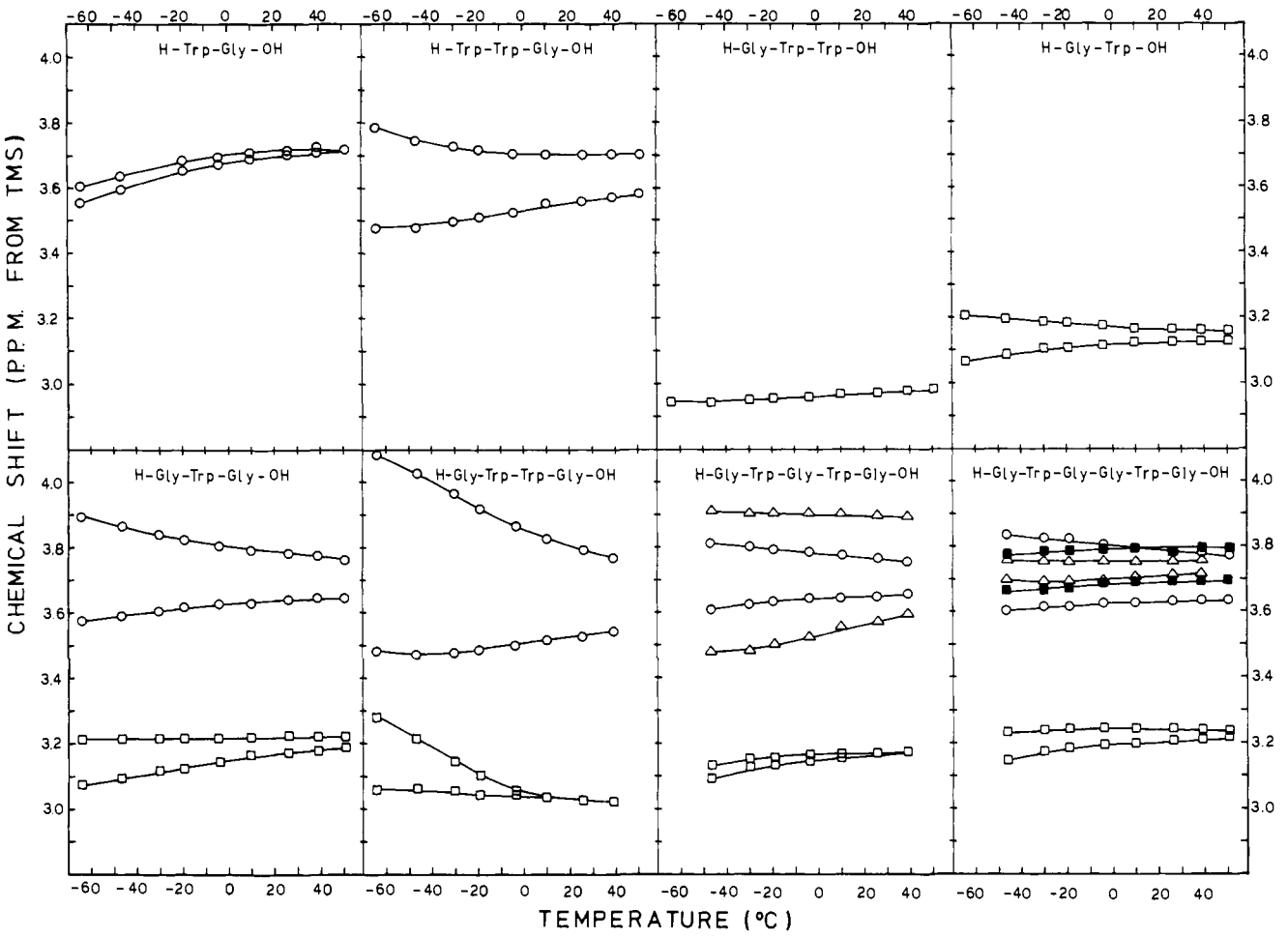

Figure 3. Temperature dependence of the chemical shifts of Gly-C_α protons. Circles refer to C-terminal glycyl residues, empty squares to N-terminal, triangles or filled squares to internal glycyl residues.

All of the N-terminal Gly residues show a small or no anisochronism, with generally a small temperature dependence, and differences with respect to the reference compound H-Gly-Trp-OH are really negligible, thus indicating that the amino terminal of the peptide chain is generally more flexible. Owing to the lack of knowledge of the respective intrinsic term, the large anisochronism of the internal Gly residue protons in the pentapeptide eludes as yet a reliable interpretation. It is, however, tempting to ascribe it to a rigidity of the backbone, and preliminary CD studies at low temperature seem to confirm this view.

The geminal coupling constant J_{AB} between the two Gly C_α protons has been shown to depend on the conformation of the backbone. In particular, for an internal Gly residue the dependence of J_{AB} on the torsion angles ϕ and ψ has been computed.[18] For the peptides reported in Figure 3, J_{AB} was found to be temperature independent within the experimental error (±0.2 Hz). Within this limit the values of J_{AB} for N-terminal or internal Gly (16.8 Hz) and for C-terminal Gly (17.2 Hz) were also found to be independent of the peptide sequence.

Chemical Shifts of the Aliphatic Protons of the Tryptophyl Residues. Figure 4 reports the temperature dependence of the chemical shifts of the ABX proton systems constituted by the $C_\alpha H$-$C_\beta H_2$ fragment of the tryptophyl residues. The assignment of ABX systems to the respective Trp residues in peptides containing two tryptophyl residues has been discussed in the previous paper.[4]

The assignment of the prochiral methylene protons H_R and H_S to the resonance signals A and B is instrumental in determining unequivocally the rotamer populations t and g^- of the side chain (see Figure 5). It was obtained in the cases of H-Trp-OH and H-Trp-*Trp*-OH by comparing their spectra with those of H-(αL,βS)(α,β-d_2)Trp-OH and H-Trp-(αL,βS)-(α,β-d_2)Trp-OH.[4] In particular, in the case of H-Trp-OH, the H_R proton absorbs at a higher field than H_S, whereas the opposite assignment is valid for H-Trp-*Trp*-OH at temperatures below 39 °C.

The assignment obtained for H-Trp-OH has been extended to all other tryptophyl residues by considering the case of H-Trp-*Trp*-OH to be an exception owing to the particular conformational properties of this molecule (see Conclusions). As previously noted,[4] the rotamer populations obtained with this assignment indicate preferential orientations of the aromatic side chains which are in agreement with the upfield shifts observed for the C_α protons of an adjacent glycyl residue with respect to reference compound H-Gly-Gly-Gly-OH.

A comparison of data reported in Figure 4 with the χ_1 rotamer populations presented in the next section shows that a correlation is present between the anisochronism of the H_R and H_S protons ($\Delta\beta$) and the populations g^-, g^+, and t. For instance, $\Delta\beta$ decreases with decreasing temperature if the rotamer populations converge (see the case of H-Trp-Gly-OH in Figures 4 and 6); on the contrary $\Delta\beta$ increases with decreasing temperature if one population becomes predominant at low temperature (see the case of H-Gly-Trp-Gly-OH in Figures 4 and 6). This correlation can be posed in a quantitative form. The best results are obtained by performing separate multilinear regression analyses on the N-terminal tryptophyl residues (case 1) and on all the other tryptophyl residues (case 2) excluding H-Trp-*Trp*-OH, which shows by far the largest deviations. The correlation coefficients are 0.9906 (1) and 0.8928 (2), respectively. The best fitting equations are

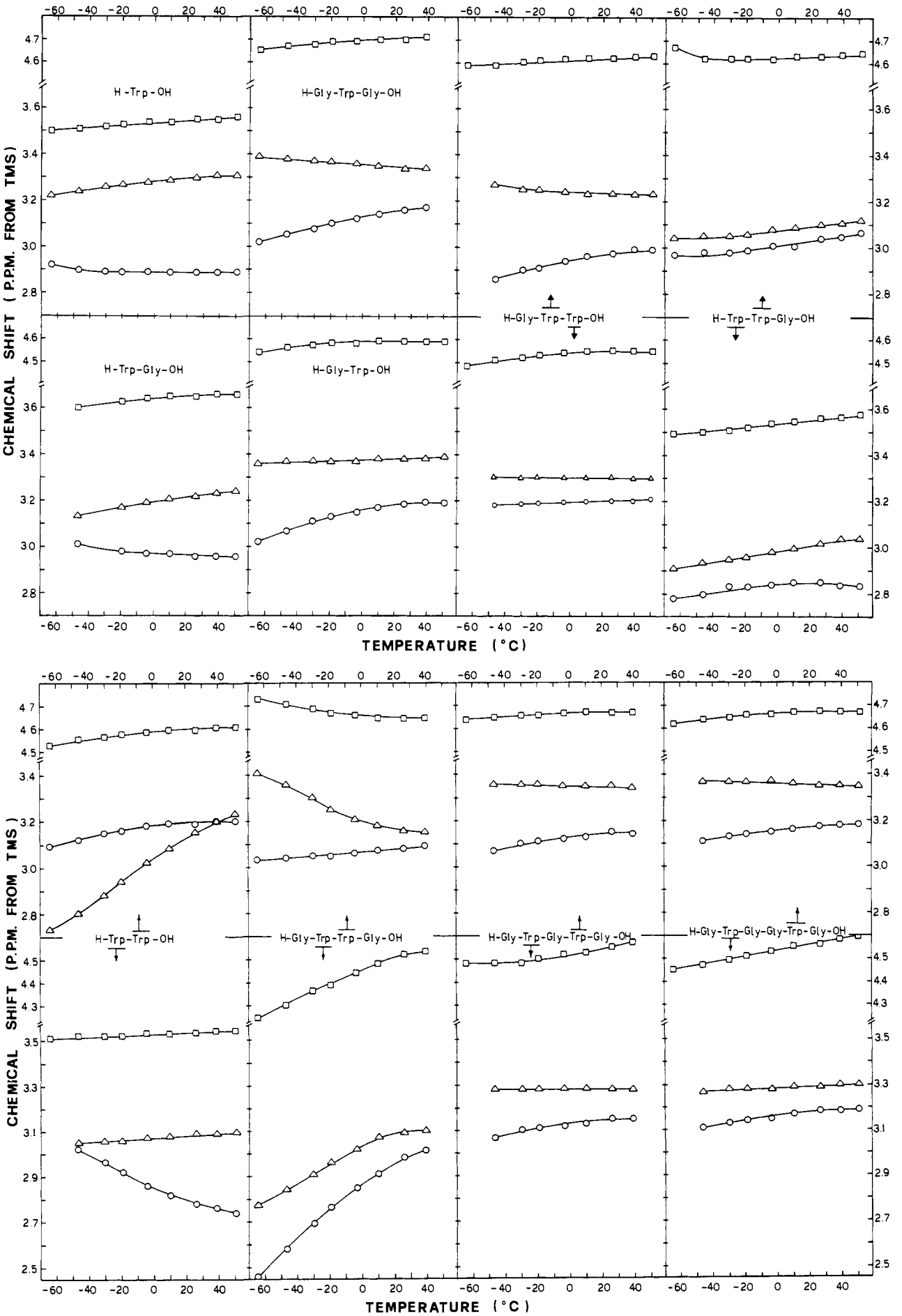

Figure 4. Temperature dependence of the ABX proton systems constituted by the $C_\alpha H$-$C_\beta H_2$ fragment of tryptophyl residues: $C_\alpha H$ = □, $C_\beta H_R$ = ○, $C_\alpha H_S$ = △.

Table I. Thermodynamic Parameters Obtained from the Temperature Dependence of the Rotamer Populations[a,b]

compd	ΔH_1	ΔH_2	ΔS_1	ΔS_2
H-Trp-OH	−0.9 (0.2)	+0.3 (0.2)	−5.1 (0.9)	−1.7 (0.9)
H-Trp-Gly-OH	−1.2 (0.5)	−0.9 (0.3)	−5.6 (1.7)	−4.2 (1.1)
H-*Trp*-Trp-OH	−2.1 (0.2)	−0.7 (0.7)	−8.2 (0.9)	−4.6 (2.7)
H-*Trp*-Trp-Gly-OH	−0.7 (0.2)	−0.06(0.2)	−3.1 (0.8)	−0.8 (0.6)
H-Gly-Trp-OH	+1.1 (0.3)	+1.5 (0.5)	+3.5 (1.2)	+3.1 (1.9)
H-Trp-*Trp*-OH	−0.4 (0.4)	+2.0 (1.3)	−0.6 (1.7)	+6.2 (5.0)
H-Gly-Trp-*Trp*-OH	−0.3 (0.1)	+0.1 (0.5)	0.0 (0.3)	−0.7 (0.3)
H-Gly-Trp-Gly-OH	+3.6 (1.2)	+1.1 (0.1)	+9.8 (4.9)	+2.5 (0.5)
H-Gly-*Trp*-Trp-OH	+3.3 (1.6)	+1.0 (0.2)	+8.7 (5.9)	+1.2 (0.6)
H-Trp-*Trp*-Gly-OH	−0.3 (0.3)	−0.4 (0.2)	−0.7 (1.2)	−0.5 (0.6)
H-Gly-*Trp*-Trp-Gly-OH	+2.6 (0.7)	+1.2 (0.4)	+6.6 (2.6)	+3.5 (1.5)
H-Gly-Trp-*Trp*-Gly-OH	+6.1 (2.1)	+1.6 (0.3)	+18.4 (7.7)	+4.1 (1.1)
H-Gly-*Trp*-Gly-Trp-Gly-OH	+0.6 (0.2)	+0.5 (0.2)	−0.7 (0.7)	+1.4 (0.7)
H-Gly-Trp-Gly-*Trp*-Gly-OH	+0.01 (0.7)	+1.0 (0.4)	−4.0 (2.8)	+2.0 (1.6)
H-Gly-Trp-$(Gly)_2$-*Trp*-Gly-OH	+2.0 (0.7)	+0.9 (0.3)	+2.7 (1.3)	+1.7 (1.0)

[a] $\Delta H_1 = H(G^+) - H(G^-)$; $\Delta H_2 = H(T) - H(G^-)$ in kcal/mol. $\Delta S_1 = S(G^+) - S(G^-)$; $\Delta S_2 = S(T) - S(G^-)$ in eu. [b] In parentheses is indicated the 95% confidence interval.

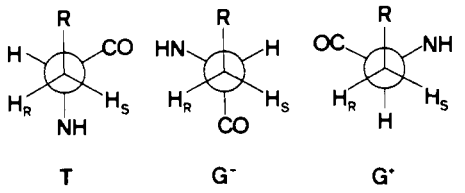

Figure 5. The three staggered rotamers around the torsion angle χ_1 R = indol-3-yl.

$$\Delta\beta = -0.44g^+ + 0.83g^- - 0.23t \quad \text{(case 1)}$$

$$\Delta\beta = -0.03g^+ + 0.44g^- - 0.20t \quad \text{(case 2)}$$

To these coefficients no simple physical meaning can presently be attributed. Studies are in progress to see to what extent the same equations apply to other classes of aromatic peptides.

Several factors can be responsible for the relatively poor correlation coefficient obtained in case 2: experimental errors in the rotamer populations, dependence of $\Delta\beta$ on other conformational variables which are not taken into account in eq 2, or, finally, some wrong assignments of the H_R and H_S protons. The lack of fit observed in the case of H-Trp-*Trp*-OH can be explained by assuming a contribution to the anisochronism $\Delta\beta$ arising from a specific ring current effect on one of the two C_β protons in the proposed preferential conformation of this molecule (see Conclusions). Ring current effects of the adjacent tryptophan side chain can also explain the upfield shift observed with decreasing temperature for the ABX protons of H-Gly-*Trp*-Trp-Gly-OH (see Figure 4). This effect suggests a restriction of the conformational flexibility with decreasing temperature also in the case of H-Gly-Trp-Trp-Gly-OH.

Rotamer Populations around the Torsion Angle χ_1. A Thermodynamic Analysis. Using Feeney's approach[19] and on the basis of the assignment discussed in the previous section, the populations of the three staggered rotamers G^+, G^-, and T (see Figure 5) around the torsion angle χ_1 have been calculated from the vicinal coupling constants J_{AX} and J_{BX}. The temperature dependence of these populations has been interpreted, using two equilibrium constants defined as the ratios $K_1 = g^+/g^-$ and $K_2 = t/g^-$. Standard, two-parameter thermodynamic analysis (ΔH and ΔS constant with temperature) has been applied in order to obtain a best fit with the experimental data. The best values obtained for ΔH_1, ΔH_2, ΔS_1, and ΔS_2 are collected in Table I. Using these values, the populations g^-, g^+, and t have been recalculated as a function of the temperature and are shown in Figures 6-8 as curves fitting the

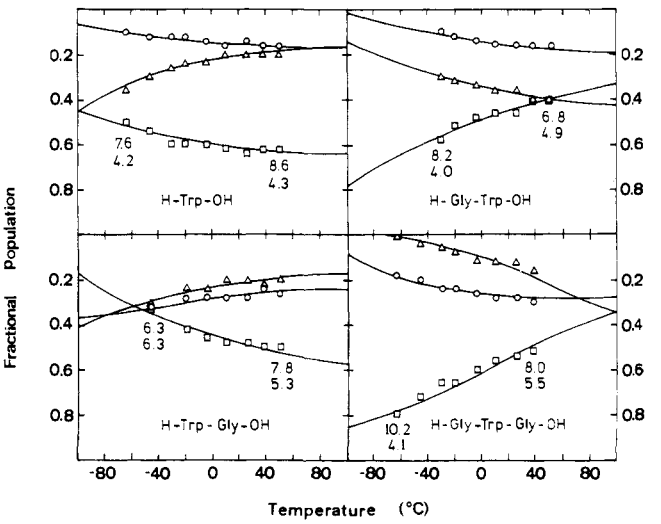

Figure 6. Temperature dependence of the χ_1 rotamer populations for the tryptophan side chains of H-Trp-OH, H-Gly-Trp-OH, H-Trp-Gly-OH, and H-Gly-Trp-Gly-OH. G^- = □, G^+ = △, T = ○. Curves have been calculated using the thermodynamic parameters reported in Table I. For each compound, J_{AX} (upper) and J_{BX} are given (Hz) at the measured temperature extremes. The values at room temperature have been given in ref 4.

experimental points. Whereas the fitting with the experimental data is good using a two-parameter thermodynamic treatment, a one-parameter model ($\Delta S \approx 0$) cannot fit the data at all. This fact has already been noted by Cavanaugh[20,21] in the case of aromatic amino acids in alkaline aqueous solutions and has been attributed to temperature-dependent intermolecular effects (association). Our data have been obtained for solutions at least by one order of magnitude more diluted, so that association is not to be expected. Extrapolation of Cavanaugh's data[21] for the H-Trp-OH anion in water at the concentration used in our study (0.02 M) indicates that indeed the contributions of association can be disregarded.

As Cavanaugh noted,[20] large entropy differences between the three rotamers cannot be expected for an isolated amino acid molecule. If one disregards association phenomena, one possible alternative explanation for the large values found in our studies for ΔS_1 and ΔS_2 would be interactions with solvent molecules, dependent on the rotameric state of the side chain[21-23] (and possibly of other parts of the molecule). In this regard, it is interesting to consider Figure 9, in which the data of Table I are plotted to show the relation between ΔH and ΔS values. The linearity observed in this case is analogous to that

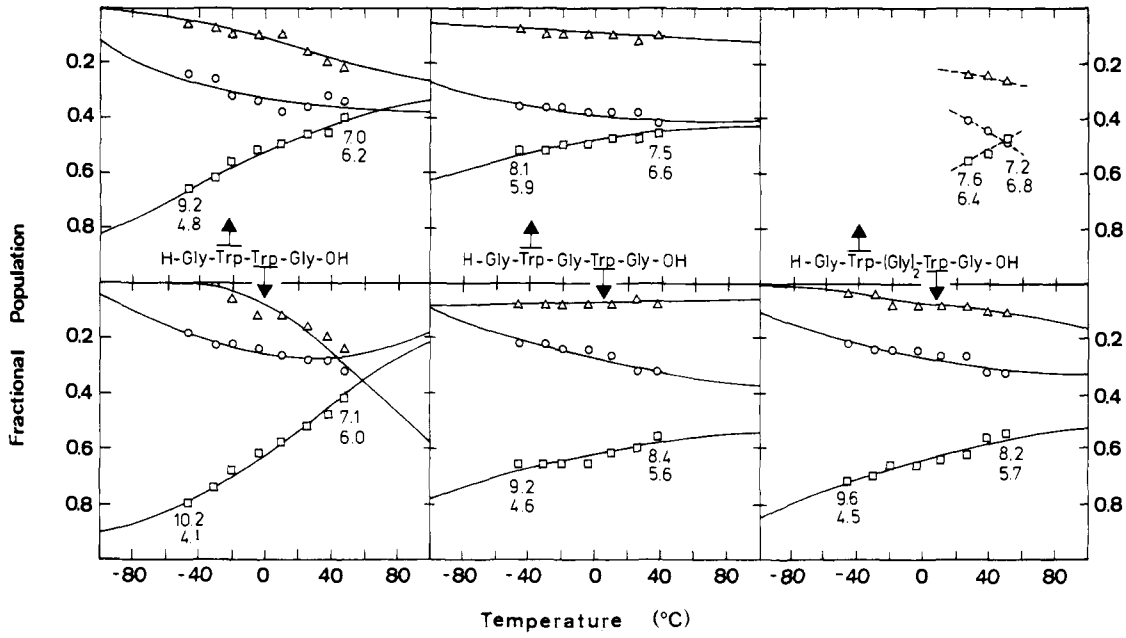

Figure 7. Temperature dependence of the χ_1 rotamer populations for the tryptophan side chains of H-Gly-Trp-Trp-Gly-OH, H-Gly-Trp-Gly-Trp-Gly-OH, and H-Gly-Trp-$(Gly)_2$-Trp-Gly-OH. See caption to Figure 6.

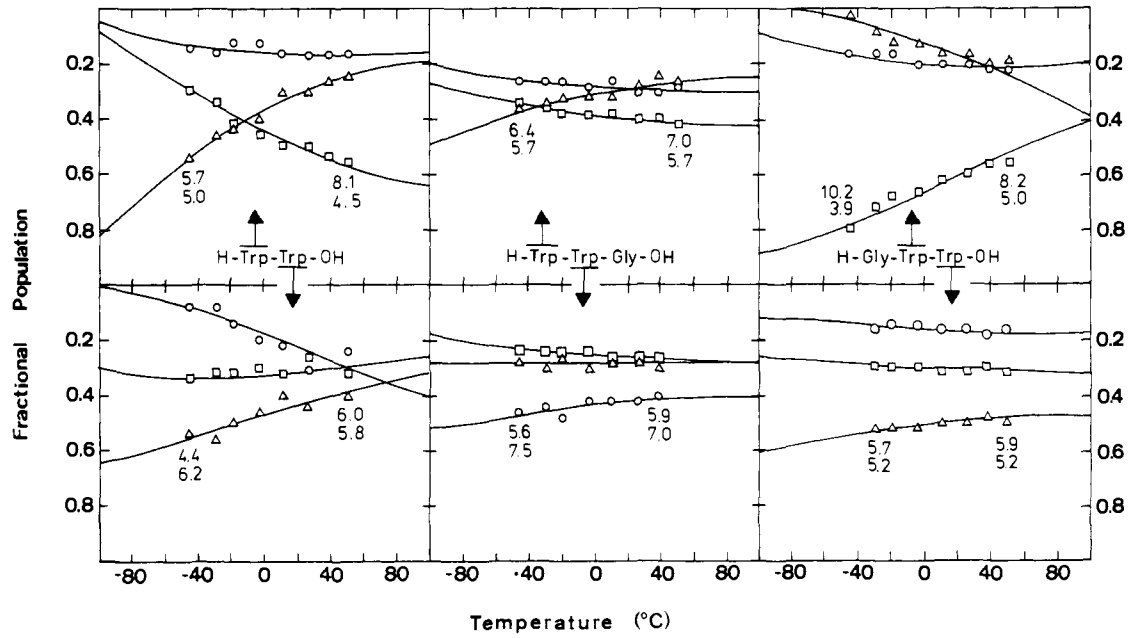

Figure 8. Temperature dependence of the χ_1 rotamer populations for the tryptophan side chains of H-Trp-Trp-OH, H-Trp-Trp-Gly-OH, and H-Gly-Trp-Trp-OH. See caption to Figure 6.

observed for many processes in aqueous solutions and generally attributed to solvation effects.[24]

An inspection of the data reported in Table I as well as of Figures 6-8 indicates that certain regularities are present. For all of the N-terminal Trp's (first four rows in Table I), G^+ is the favored rotamer by enthalpic effects, but G^- is the more populated one at room temperature, since it is stabilized by the entropic term. For an internal or C-terminal Trp, G^- is the enthalpically favored rotamer and at room temperature it is also the most populated one despite a generally unfavorable entropic contribution. In exception to this rule are the cases of H-Trp-*Trp*-OH, H-Trp-*Trp*-Gly-OH, and H-Gly-Trp-*Trp*-OH, where the rotamer G^- is destabilized probably for steric reasons owing to the presence of the adjacent bulky side chain. A special comment is required for the case of H-Gly-Trp-*Trp*-Gly-OH, where G^- is the favored rotamer, even in the presence of another N-adjacent Trp.[25] One possible explanation is that the conformational equilibrium of the whole molecule is shifting at low temperature toward one single conformer having no steric interaction between the two side chains in the G^- rotameric state. This explanation agrees with the very high values of ΔH_1 and ΔS_1 observed in this case. These values could indeed reflect a conformational change of a large part of the molecule and not only of one single side chain.

Conclusions

The conformational properties of the tryptophan side chain are determined by the torsion angles χ_1 and χ_2. Experimental[4,26] and theoretical[27] observations indicate that the χ_2 torsion angle can adopt two values corresponding on an average to +90 and −90°. In crystals[28-34] of tryptophan derivatives

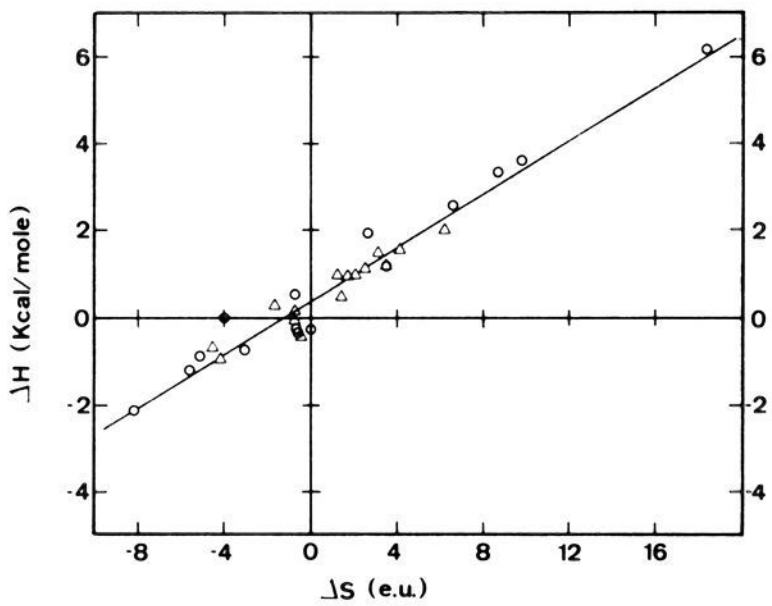

Figure 9. Correlation between enthalpy and entropy differences among the three χ_1 rotamers. The straight line obtained from a least-squares analysis is shown. Circles refer to differences between the thermodynamic functions of rotamers G^+ and G^- (ΔH_1, ΔS_1); triangles refer to differences between the thermodynamic functions of rotamers T and G^- (ΔH_2, ΔS_2).

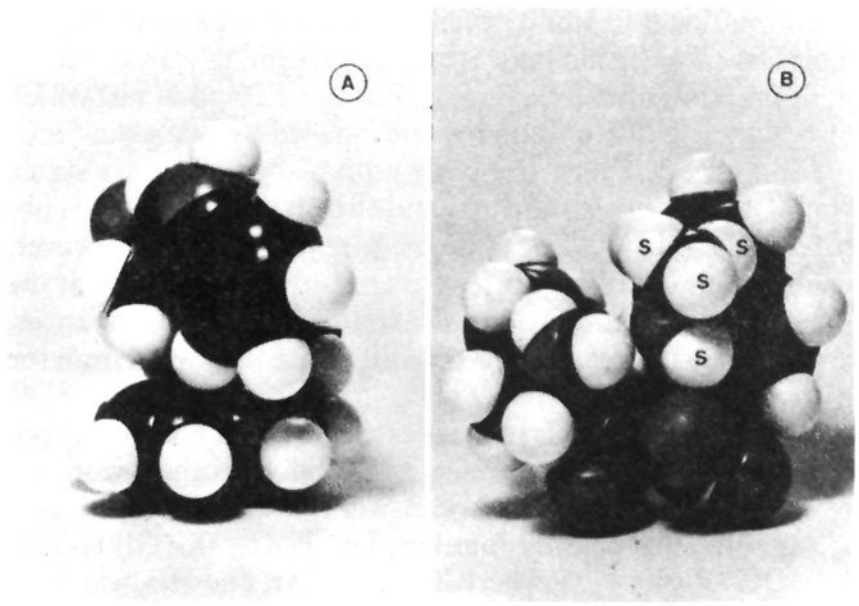

Figure 10. Proposed model for the most stable conformation of the H-Trp-Trp-O^- anion at low temperature. The two aromatic groups are close and perpendicular to each other (A). The backbone conformation is optimally stabilized by binding one solvent molecule CD_3OD (B). The deuterium atoms CD_3OD are labeled with an S. The backbone conformation is approximately described by $\phi(1)$ −60 (or +120°), $\psi(1)$ −30°, $\phi(2)$ −80°, $\psi(2)$ +180 (or 0°); for the nomenclature, see ref 35.

and peptides, the G^- rotamer of χ_1 is invariably found associated with $\chi_2 = 90 \pm 30°$. Taking these restrictions into account, data for the χ_1 rotamer equilibrium (reported in Figures 6–8) are a fair representation of the conformational mobility of the entire side chain. In a previous paper,[4] we have suggested that the interaction backbone–side chain plays an important role in determining the orientation of the latter. In particular, all our data on peptides containing one single tryptophyl residue were consistent with a preferential orientation of the indolyl group toward the N side of the tryptophyl residue, suggesting a dipolar interaction between the aromatic moiety and the positive end of the backbone dipole moment. The thermodynamic data obtained in the present work confirm this picture, but they also suggest that solvation effects play an important role in determining the relative conformational stability. Typical is the case of H-Trp-OH, where the rotamer G^- is the most populated at room temperature because of entropic effects (due probably to solvation), but with decreasing temperature, g^+ increases at the expense of g^-. We suppose that dipolar interactions with the backbone in part contribute to the enthalpic term which favors G^+.

For the investigated peptides, definition of the most stable molecular conformations at low temperature requires information about the torsion angles ϕ and ψ, information which we have not yet obtained. Data presented in this work permit us, nevertheless, to propose the whole conformation in the case of H-Trp-Trp-OH, and to make some reasonable guesses as to the backbone conformation of several peptides.

A proposed structure for the most stable conformer of H-Trp-Trp-OH at low temperature is shown in Figure 10. Both torsion angles χ_1 have been adjusted to +60°, as suggested by the temperature dependence of rotamer populations (see Figure 8). The χ_2 torsion angles of residues 1 and 2 have been fixed to −90 and +90°, respectively, and the planes of the two indoles lie close and perpendicular to each other. In Figure 10, the corresponding backbone conformation is approximately described by the following set of torsion angles:[35] $\phi(1) = -60$ (or +120°), $\psi(1) = -30°$, $\phi(2) = -80°$, $\psi(2) = +180$ (or 0°). Such a structure agrees with the large upfield shift of $H_{\epsilon 3}$ of Trp(2) and with the smaller ones observed for $H_{\zeta 3}$ and $C_\beta H_S$ of the same residue, on the basis of literature data for the ring current effect of indole[36] and assuming a ca. 60% population for this conformer at −64 °C, as suggested by the temperature dependence of the rotamer populations around χ_1. A standard stacked structure with both aromatic moieties close and parallel, as proposed for *cyclo*-Trp-Trp,[37] would certainly not agree with our data on H-Trp-Trp-OH, since for such a structure ring current effects are to be expected on the aromatic protons of both side chains. Finally, the backbone conformation proposed for H-Trp-Trp-OH appears optimally stabilized by interactions with an OH group of the solvent. We have already obtained evidence from previous CD studies[2] that in this case an OH group of the solvent is of importance for the conformational equilibrium of the molecule.

As indicated by the temperature dependence of rotamer populations and by the chemical shifts of the aromatic protons, the conformation proposed for H-Trp-Trp-OH cannot be extended to the other peptides containing the -Trp-Trp- sequence. This fact shows the importance of the backbone–side chain interaction in determining the molecular conformation.

As already mentioned, for all other peptides we are not able to propose a molecular conformation; only some reasonable guesses are possible in some cases. Thus, in the tripeptide H-Gly-Trp-Gly-OH, the anisochronism of the C-terminal Gly-C_α protons can be tentatively explained by assuming the presence of H-bonded backbone conformations. These can be achieved in a C_7 structure[38] with one H bond between the two peptide groups or in a $\tilde{\gamma}$-turn,[39] with a further H bond between the terminal NH_2 and COO^- groups. This would restrict also the conformational freedom of the N-terminal glycyl residue in H-Gly-Trp-Gly-OH, which would be in keeping with the moderate anisochronism of its C_α protons. The absence of anisochronism for the N-terminal glycyl residue in H-Gly-Trp-Trp-OH may suggest in this case a different conformational equilibrium.

In the case of H-Gly-Trp-Trp-Gly-OH, the large value of anisochronism observed for the C_α protons of Gly(4) suggests the predominance at low temperature of a partially H-bonded structure of the backbone. Either a type I β turn[40] or a γ turn[39] is a candidate for such a structure. Both conformations are consistent with the ring current effect of Trp(3) side chain observed on the C_α and C_β protons of Trp(2) (see Figure 4). For both conformations one would predict a moderate upfield shift for the H_{δ_1} of Trp(2). A 0.3-ppm upfield shift is in fact observed for one of the H_{δ_1} signals of the tetrapeptide (see Figure 2). On this basis, we can tentatively assign the two sets of aromatic protons. For the hexapeptide H-Gly-Trp-$(Gly)_2$-Trp-Gly-OH, the moderate anisochronism of the Gly C_α protons observed at low temperature may suggest—with the caveat of the undetermined intrinsic term—a relatively high

conformational flexibility even in the low-temperature region. This last observation may appear surprising in view of recent data presented for enkephalins. For these compounds, which also possess two aromatic residues spaced by two glycyl residues, a type I β turn has been proposed as the most stable conformation in solution[41,42] and this structure has been observed in the crystalline state by X-ray analysis.[43] However, enkephalins contain another bulky residue (Leu, Met) at the C end, which could change the conformational preferences. Another possible reason for this difference may arise from the different solvent systems.

A general question is to what extent the data obtained in our work for tryptophyl residues are valid for other aromatic residues. We expect to partly answer this question by investigation of other peptide families, i.e., H-Gly-X-$(Gly)_n$-Trp-Gly-OH (n = 0, 1, 2), where X = Phe, Tyr, and His, which we have already prepared and characterized.

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NMR Studies of Enolate Anions. 6. A ^{13}C NMR Study of Alkali Metal Chelation by 3-Alkylacetylacetonates[1]

Morton Raban* and Demitris P. Haritos

Contribution from the Department of Chemistry, Wayne State University, Detroit, Michigan 48202. *Received November 13, 1978*

Abstract: The low-temperature ^{13}C NMR spectra of the sodium salts of 2,4-pentanedione, 2,4-hexanedione, 3-methyl-2,4-pentanedione, and 3-ethyl-2,4-pentanedione are presented. At the temperatures at which spectra were measured torsion about carbon–carbon partial double bonds is slow on the NMR time scale and resonances of *Z,Z*, *E,Z*, and *Z,E* diastereomers or topomers are distinguishable. Addition of lithium iodide (configurational titration) was used to assign resonances and to study chelation of lithium ion by enolate anions. The effects of steric bulk on chelation are described and the effects of gegenion and stereochemistry on ^{13}C NMR chemical shifts are discussed. The ^{13}C NMR spectra of 2,4-pentanedione, 2,4-hexanedione, 3-methyl-2,4-pentanedione, and 3-ethyl-2,4-pentanedione are also reported and the enol chemical shifts compared with those of the enolates.

Introduction

Reactions of resonance-stabilized carbanions, notably enolate anions, are among the most important class of reactions in synthetic and mechanistic organic chemistry.[2] Although numerous authors[3] have suggested that ion pairing and the stereochemistry of β-keto enolates have an important effect on their regiospecificity, stereoselectivity, and rates of alkylation, it has been possible, only recently, to study these properties directly. The solid-state structures of several alkali metal salts of β-dicarbonyl compounds have been reported recently[4-7] and low-temperature ^1H NMR spectroscopy has been shown to be useful for directly studying stereochemistry and associ-

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