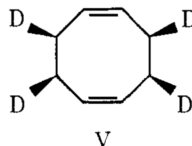
I in solution) corresponding to $\Delta G^{\ddagger}$ values of 4.4 ± 0.1 (at 96 K) and 4.9 ± 0.1 kcal mol^{-1} (at 105 K).[1] They interpreted these as originating from twist-boat/twist-boat interconversion processes and concluded that the coalescence at lower temperature is due to either the twist or the boat process, the one at higher temperature either to the combined twist and boat processes or to the chair process. In principle, it can be decided from the study of suitably deuterated species of I (e.g., V) what



V

the nature of the low-temperature coalescence is,[1] but it is not possible to decide experimentally whether the high-temperature coalescence originates from the chair process or from a combination of the twist and boat processes. Our results (Table I) suggest that the low-temperature coalescence is caused by the twist process (calculated $\Delta G^{\ddagger}_{100}$ = 4.15 kcal mol^{-1}), while for the high-temperature coalescence both the combined twist and boat processes [calculated $\Delta G^{\ddagger}_{100}$ (for boat process) = 5.73 kcal mol^{-1}] and the chair process (calculated $\Delta G^{\ddagger}_{100}$ = 5.91 kcal mol^{-1}) have to be taken into consideration. Allinger and Sprague favor the boat process for explaining the low-temperature coalescence.[2]

We want to conclude our discussion with a comment on terminology. Conformational interconversions of the kind described here are often referred to as proceeding along pseudorotational paths despite the fact that substantial energy barriers of several kilocalories have to be overcome. We wish to advocate that the term pseudorotation should be reserved for cyclic interconversion processes with no barrier (or at most with barriers *very* close to zero), i.e., with a constant potential along the interconversion path, as originally intended by Pitzer et al.[19] Cyclic interconversions with barriers similar to those discussed here should rather be termed *hindered* pseudorotational processes.[20]

Acknowledgment. Financial support of the Deutsche Forschungsgemeinschaft is gratefully acknowledged.

References and Notes

(1) F. A. L. Anet and L. Kozerski, *J. Am. Chem. Soc.*, **95**, 3407 (1973). Note Added in Proof: For a short account of recent conformational calculations on I by these authors, see F. A. L. Anet and R. Anet in "Dynamic Nuclear Magnetic Resonance Spectroscopy", F. A. Cotton and L. M. Jackman, Ed., Academic Press, New York, N.Y., 1975, p 571. We thank Dr. Anet, a referee of this paper for pointing out this reference, and for informing us that "on the whole" their results agree with ours.
(2) N. L. Allinger and J. T. Sprague, *Tetrahedron*, **31**, 21 (1975). The discrepancies between Allinger's and Sprague's and our results are probably mainly due to the fact that the H···H nonbonded function of the former authors is too strongly repulsive, as has also been observed by others: T. Clark, T. McO. Knox, H. Mackle, and M. A. McKervey, *Chem. Commun.*, 666 (1975); D. N. J. White, private communication.
(3) O. Ermer, *Tetrahedron*, **31**, 1849 (1975).
(4) O. Ermer and S. Lifson, *J. Am. Chem. Soc.*, **95**, 4121 (1973).
(5) L. Hedberg and K. Hedberg, see *Perspect. Struct. Chem.*, **4**, 60 (1971).
(6) R. K. Mackenzie, D. D. MacNicol, H. H. Mills, R. A. Raphael, F. B. Wilson, and J. A. Zabkiewicz, *J. Chem. Soc., Perkin Trans. 2*, 1632 (1972).
(7) L. Leiserowitz, private communication; we thank Dr. Leiserowitz for kindly making available his unpublished structural results which appear in Figure 1; see also B. S. Green, M. Lahav, and G. M. J. Schmidt, *J. Chem. Soc. B*, 1552 (1971).
(8) W. Baker, R. Banks, D. R. Lyon, and F. G. Mann, *J. Chem. Soc.*, 27 (1945).
(9) O. Ermer and J. D. Dunitz, unpublished results.
(10) H. Pelzer and E. Wigner, *Z. Phys. Chem., Abt. B*, **15**, 445 (1932).
(11) J. W. McIver and A. Komornicki, *J. Am. Chem. Soc.*, **94**, 2625 (1972). Note Added in Proof: R. E. Stanton and J. W. McIver, *J. Am. Chem. Soc.*, **97**, 3632 (1975), have recently applied symmetry considerations to the characterization of transition states in the course of quantum-chemical calculations.
(12) H. B. Bürgi, *Inorg. Chem.*, **12**, 2321 (1973); H. B. Bürgi, J. D. Dunitz, and E. Shefter, *J. Am. Chem. Soc.*, **95**, 5065 (1973); H. B. Bürgi, J. D. Dunitz, J. M. Lehn, and G. Wipff, *Tetrahedron*, **30**, 1563 (1974).
(13) H. B. Bürgi, *Angew. Chem., Int. Ed. Engl.*, **14**, 460 (1975), and C. Altona, H. J. Geise, and C. Romers, *Tetrahedron*, **24**, 13 (1968), have analyzed the pseudorotational path of cyclopentane in this way.
(14) J. A. Ibers and R. G. Snyder, *Acta Crystallogr.*, **15**, 923 (1962); W. C. Baird and J. H. van den Hende, *J. Am. Chem. Soc.*, **85**, 1009 (1963); M. D. Glick and L. F. Dahl, *J. Organomet. Chem.*, **3**, 200 (1965); H. Dierks and H. Dietrich, *Z. Kristallogr., Kristallgeom. Kristallphys., Kristallchem.*, **122**, 1 (1965); J. Coetzer and G. Gafner, *Acta Crystallogr.*, Sect. B., **26**, 985 (1970); S. Koda, A. Takenaka, and T. Watanabe, *Bull. Chem. Soc. Jpn*, **44**, 653 (1971).
(15) D. N. J. White and J. D. Dunitz, *Isr. J. Chem.*, **10**, 249 (1972).
(16) J. D. Dunitz and F. K. Winkler, *Acta Crystallogr.*, Sect. B, **31**, 251 (1975).
(17) K. Kraft and G. Koltzenburg, *Tetrahedron Lett.*, 4357 (1967).
(18) J. D. Dunitz and J. Waser, *J. Am. Chem. Soc.*, **94**, 5645 (1972).
(19) J. E. Kilpatrick, K. S. Pitzer, and R. Spitzer, *J. Am. Chem. Soc.*, **69**, 2483 (1947).
(20) We have recently described a pseudorotational degree of freedom as corresponding to a one-dimensional partial inflection point with zero slope.[3] This definition is, however, incomplete: The conditions for such an inflection point are necessary, yet not sufficient conditions for a pseudorotational degree of freedom.

Conformations of Glycyl-*trans*-4-fluoro-L-prolyl-L-tryptophan in Aqueous Solution[1]

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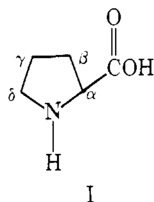
Contribution from the Department of Chemistry, University of California, Santa Barbara, California 93106. *Received August 26, 1975*

Abstract: High-resolution proton and fluorine magnetic resonance spectra of the title tripeptide have been analyzed. Two conformational forms are indicated by the data; these most likely arise from restricted rotation about the glycyl-4-fluoroprolyl peptide bond. Consideration of the vicinal coupling constants suggests that the proline ring in each isomer is in an envelope conformation with C_δ the puckered atom.

Proline (I) is the only common cyclic α-imino acid found in globular proteins, and its appearance in the amino acid sequence of a protein often signals an abrupt change in tertiary structure.[2] Because of this the conformational attributes of the pyrrolidine ring in proline and proline-containing peptides have been of continuing interest. Of the techniques that have been applied in the study of this ring system, x-ray and neutron diffraction have probably been most revealing, and these studies have shown that in linear peptides with proline as a central residue, C_γ is the most mobile, appearing above or



I

below the mean plane through the remaining four atoms of the ring.[4-7] In cyclic peptides containing a prolyl group, the situation is more variable with C_β often being the ring atom puckered out of the plane.[8,9] Proton[10] and carbon[11] magnetic resonance spectroscopy have been applied productively to a number of proline-containing systems and generally confirm the structural conclusions made on the basis of the crystalline state structures. It is particularly notable that carbon relaxation rate data indicated that C_β, C_γ, and C_δ are more mobile than C_α in the proline residues of such peptides and of these, C_γ tends to be the most active.[11]

In a previous paper we reported that fluorine substitution on the proline ring has a helpful effect on the nature of the proline magnetic resonance spectrum of the molecule, dispersing the spectrum sufficiently that a complete analysis of the seven spin ½ system defined by the proton and fluorine atoms attached to the pyrrolidine ring is possible.[12] The vicinal proton–proton and proton–fluorine coupling constants so obtained can be used via the Karplus equation[13] to define the dominant conformation of the ring. We have now prepared glycyl-4-fluoro-L-prolyl-L-tryptophan and have carried out similar work to define the conformation of the fluorinated five-membered ring in a peptide environment.

Experimental Section

***trans*-4-Fluoro-L-proline** was prepared as described previously.[12] **Glycine** (Eastman) and **L-tryptophan** (Mann) were commercial samples.

Glycyl-*trans*-4-fluoro-L-prolyl-L-tryptophan was synthesized by the following series of reactions. To a solution of *trans*-4-fluoro-L-proline methyl ester hydrochloride (0.92 g, 5 mmol) in 7 ml of dichloromethane was added 0.8 ml (8 mmol) of triethylamine with cooling at 0°. A solution of carbobenzyloxyglycine[14] (1.05 g, 5 mmol) in 7 ml of dichloromethane was added to the amino acid solution. Dicyclohexylcarbodiimide (Aldrich) (1.03 g, 5 mmol) was added and the mixture was stirred overnight at room temperature. Dicyclohexylurea was removed by filtration and the filtrate washed with 1 M HCl, water, 1 M $NaHCO_3$ solution, and, lastly, dried over anhydrous sodium sulfate. The solvent was evaporated in vacuo, affording 1.3 g (77%) of crude carbobenzyloxyglycyl-*trans*-4-fluoroproline methyl ester hydrochloride. An ^1H NMR spectrum of this material was consistent with the expected structure.

To this product was added 13 ml of methanol and 1.3 ml of 4 M NaOH solution. The resulting solution was allowed to stand 17 h at room temperature, whereupon 30 ml of water was added and the mixture was extracted with ether. The ether layer was discarded. The aqueous layer was acidified with concentrated HCl and extracted twice with ethyl acetate; the combined extracts were washed with saturated NaCl solution and dried over anhydrous sodium sulfate. Evaporation of the solvent yielded 0.9 g (74%) of the Cbz-dipeptide as an oil. Again, the ^1H NMR spectrum was that expected.

Tryptophan methyl ester hydrochloride[15] was coupled to carbobenzyloxyglycyl-*trans*-4-fluoroproline by the same procedure involving dicyclohexylcarbodiimide as described above, giving the blocked tripeptide methyl ester in 49% yield. Removal of the methyl ester function by the same method as mentioned earlier proceeded in 78% yield. The Cbz-tripeptide moved as a single spot on TLC (Eastman Chromogram silica gel plates, eluting solvent consisting of 1-butanol (63%), glacial acetic acid (23%), and water (14%)) and gave the proper ^1H NMR spectrum.

Carbobenzyloxyglycyl-*trans*-4-fluoro-L-prolyl-L-tryptophan (0.2 g, 0.4 mmol) was dissolved in 14 ml of methanol; about 100 mg of 10% Pd on charcoal (Matheson Coleman and Bell) was added and hydrogen gas was bubbled through the mixture for 1 h at room temperature. The suspension was filtered through heavy paper and the filter cake washed several times with hot methanol. Upon evaporation of the filtrate and washing, a nearly quantitative yield of crude tripeptide was realized. TLC showed a ninhydrin-positive spot at R_f 0.51, which may be compared to the position observed with glycyl-L-prolyl-L-tryptophan (R_f 0.50). Unfortunately, TLC also revealed an impurity in the sample (R_f 0.72), which proved very difficult to remove. By slowly evaporating methanol from the dissolved (impure) tripeptide, a small amount of the tripeptide of adequate purity was obtained when only the first-formed precipitate was collected. The proton and fluorine NMR spectra of this material, as discussed in the body of this paper, are uniquely consistent with the expected structure of the tripeptide. The (saturated) solution used to obtain these spectra was 0.09 M tripeptide in "100%" deuterium oxide (Diaprep) at an apparent pH of 2.7; the acidity was adjusted with 38% DCl in D_2O (Stohler). TLC was used to monitor the stability of the tripeptide sample over the course of the NMR experiments; no evidence for sample decomposition was found.

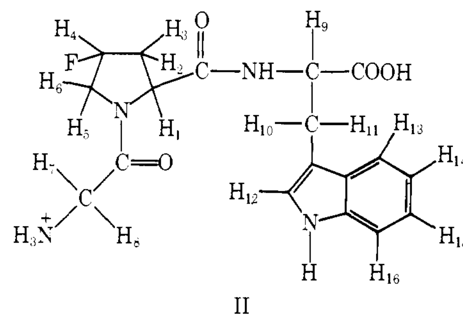
Proton spectra at 100 MHz and fluorine-19 spectra at 94.1 MHz were obtained at ambient temperature as part of an instrument demonstration by Dr. L. F. Johnson using a Varian Associates XL-100 spectrometer interfaced to a Nicolet Technology Corp. TT-100A Fourier transform accessory. Sample tubes were 12 mm and the instrument was locked to the deuterium resonance of the solvent. Spectra were signal averaged for 100–200 transients; 16K data tables were used.

Proton spectra at 360 MHz were obtained with the same sample on the Bruker HX-360 spectrometer at the Stanford Magnetic Resonance Laboratory by Mr. R. Nunlist of Bruker Scientific and Dr. N. Jardetzky of Stanford University.

Computer calculations were performed with a local version of the LAOCN3 program[12,16] using an IBM 360/75. An extended version of this program was written which allows one to prepare composite spectra from a number of separate spin systems. Examples of the output from this program appear below.

Results

There are 16 carbon-bound, nonexchangeable protons in the make-up of glycyl-*trans*-4-fluoro-L-prolyl-L-tryptophan (II). The system we have adopted for numbering these is indicated in the structure shown.



II

Preliminary examination of the 100-MHz proton spectrum of II (Figures 1 and 3) was discouraging and, except for the regions assignable to protons H_{12}–H_{16} of the tryptophan residue, it appeared that a complete analysis of the spectrum at this frequency would not be feasible. The proton spectra at 360 MHz brightened our outlook considerably. These high-field spectra (Figures 2 and 3) showed that only protons H_1 and H_9, likely concealed or partially concealed under the HOD solvent peak, could not be accounted for completely. The spectrum can be broken down into four subspectra corresponding to the ABCDEFX spin system of the fluoroproline ring, the two glycine protons, the aliphatic protons of the tryptophan, and the aromatic protons of this latter residue. Further complexity is introduced, however, because the resonances from each of these groups of nuclei appear twice. The relative intensities in each set were found to be in the ratio 83:17; this doubling of each spectral element most likely is due to the existence of rotational isomers arising from restricted rotation of the glycine–fluoroproline peptide

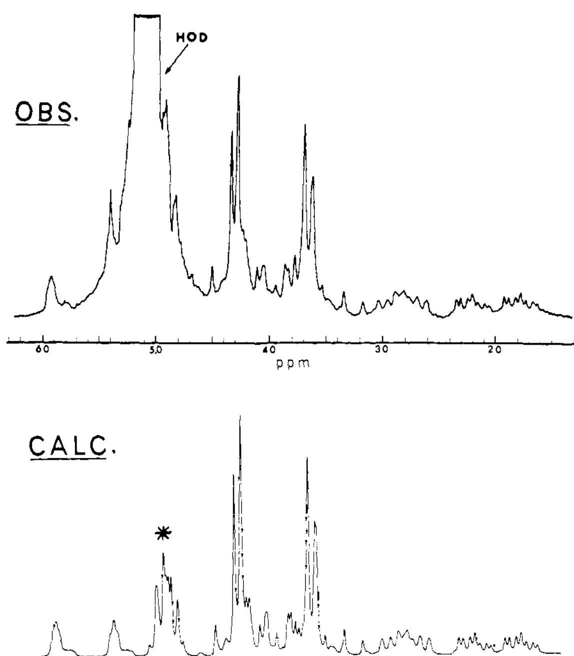


Figure 1. The upfield ^{1}H NMR spectrum of glycyl-4-fluoro-L-prolyl-L-tryptophan at 100 MHz. The calculated spectrum was obtained using the parameters in Table I and is a composite of spectra.

bond.[11,17,18] Figures 2 and 3 show how the signals from the four groups of nuclei, each in a major and minor manifestation, combine to make up the observed 360-MHz spectra.

Table I gives the chemical shifts and coupling constants generated by the above analysis. As indicated in Figures 1 and 3, these parameters led to computed 100-MHz proton spectra in good agreement with experiment. The fluorine-19 spectrum at 94.1 MHz was also well reproduced. However, the experimental spectra at all frequencies did not have the degree of resolution necessary to accurately define many of the small coupling constants. This situation was especially acute for spectra from the fluoroproline ring and, in this case, the coupling constants expected to be larger than ~0.5 Hz[12] were adjusted by trial-and-error until optimum agreement between computed and experimental line shapes was obtained. Coupling constants smaller than this were generally set equal to their value in the free imino acid.[11] As a result of poor resolution, the coupling constants in Table I are probably not more accurate than 0.2–0.3 Hz. Since our major concern was the magnitude of the large vicinal coupling constants, errors of this magnitude were acceptable, however.

In analyzing the 360-MHz spectra, the resonance positions of H_3 and H_{14} of the major isomer were each initially assigned an arbitrary chemical shift of zero and the shifts of nearby signals were referred to these. No internal reference other than HOD was used; the shifts of H_3 and H_{14} relative to HOD were estimated, thus defining the approximate chemical shifts of all nuclei relative to HOD. It was assumed that the HOD signal is 5.05 ppm downfield from Me_4Si. (This is the average of the positions of HOD in the spectra of *cis*- and *trans*-4-fluoroproline obtained at pD 5.9, as reported previously.[12] No correction was made for the pH dependence of this shift.) Thus, the chemical shift *differences* within the upfield or downfield groups of peaks are probably accurate to 0.3 Hz, but the positions relative to Me_4Si given in Table I may be uncertain by perhaps 0.2 ppm. The chemical shifts for protons H_1 and H_9 were not obtainable with accuracy, since these signals were hidden under the solvent peak; it was shown that placing the shifts of these nuclei anywhere within the envelope of the solvent resonance did not affect the appearance of the signals from nuclei coupled to these protons.

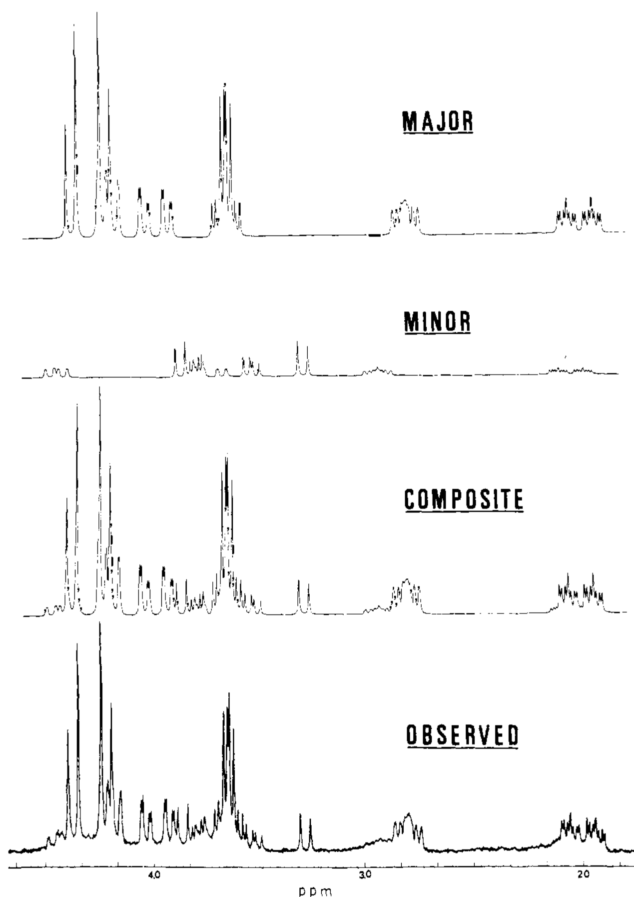
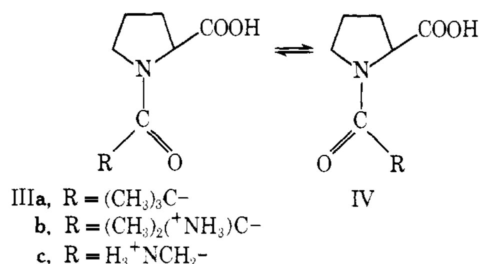


Figure 2. The upfield ^{1}H NMR spectrum of II at 360 MHz. The calculated spectra, generated using the chemical shifts and coupling constants in Table II, show how the spectra of the major and minor forms combine to make up the observed spectrum. The placing of the calibration to represent resonance positions relative to Me_4Si is approximate; see text.

Discussion

In designing a peptide for this work, we initially sought to minimize the presence of the second rotational isomer. Nishihara et al. have reported that pivaloylproline (IIIa) exists as



only one rotamer,[19] confirming an observation we had also made.[20] This result suggested that 2-methylalanine would be a logical choice for the N-terminal amino acid of our peptide (IIIb), since the 2-methylalanyl group would be expected to be isosteric with the pivaloyl group. Unfortunately, we were not able to synthesize 2-methylalanyl-L-prolyl-L-tryptophan or its fluorinated analogues because of an unusual decomposition reaction[21] and the *N*-glycyl peptide was settled on as the next best choice. The observed ratio of conformers (83:17) for II is very similar to the results obtained in other related systems[11,17,18,22] and, as in these systems, is due to the slow rate of the process analogous to the III $\rightleftharpoons$ IV exchange in II.

The spectral resolution at both 100 and 360 MHz was about 2 Hz and precluded the determination of a number of long range coupling constants. The free energy of activation for rotation in acetylproline is 20 ± 0.5 kcal/mol in aqueous so-

Table I. NMR Spectral Parameters of Glycyl-*trans*-4-fluoro-L-prolyl-L-tryptophan[a]

Major	Minor	Major	Minor
(a) 4-Fluoroproline			
$\delta_1 = 4.8639^b$	$\delta_1 = 4.9389^b$	$J_{2,4} = 1.1$	$J_{2,4} = 0.9$
$\delta_2 = 2.7986$	$\delta_2 = 2.9267$	$J_{2,5} = 1.9$	$J_{2,5} = 2.4$
$\delta_3 = 2.0000$*	$\delta_3 = 2.0406$	$J_{2,6} = 0.2$	$J_{2,6} = 0.2$
$\delta_4 = 5.5861$	$\delta_4 = 5.4708$	$J_{2,F} = 19.2$	$J_{2,F} = 20.4$
$\delta_5 = 4.1164$	$\delta_5 = 4.4008$	$J_{3,4} = 4.0$	$J_{3,4} = 4.8$
$\delta_6 = 3.9506$	$\delta_6 = 3.6936$	$J_{3,5} = 0.3$	$J_{3,5} = 0.2$
$\delta_F = 0.1063$	$\delta_F = 0$	$J_{3,6} = 0.7$	$J_{3,6} = 0.8$
$J_{1,2} = 8.0$	$J_{1,2} = 8.5$	$J_{3,F} = 41.5$	$J_{3,F} = 40.8$
$J_{1,3} = 10.0$	$J_{1,3} = 8.8$	$J_{4,5} = 0.8$	$J_{4,5} = 0.8$
$J_{1,4} = 0.6^c$	$J_{1,4} = 0.7^c$	$J_{4,6} = 2.9$	$J_{4,6} = 2.9$
$J_{1,5} = -0.3$	$J_{1,5} = -0.1$	$J_{4,F} = 51.8$	$J_{4,F} = 51.8$
$J_{1,6} = 0.4$	$J_{1,6} = 0.6$	$J_{5,6} = -13.4$	$J_{5,6} = -14.5$
$J_{1,F} = -0.4^c$	$J_{1,F} = -0.4^c$	$J_{5,F} = 20.9$	$J_{5,F} = 21.4$
$J_{2,3} = -15.0$	$J_{2,3} = -15.0$	$J_{6,F} = 38.0$	$J_{6,F} = 37.3$
(b) Glycine			
$\delta_7 = 4.3339$	$\delta_7 = 3.8322$	$J_{7,8} = -16.6$	$J_{7,8} = -16.3$
$\delta_8 = 4.1886$	$\delta_8 = 3.2700$		
(c) Tryptophan			
$\delta_9 = 4.8972^b$	$\delta_9 = 4.8083^b$	$J_{9,10} = 7.7$	$J_{9,10} = 10.8$
$\delta_{10} = 3.5961$	$\delta_{10} = 3.5067$	$J_{9,11} = 6.2$	$J_{9,11} = 5.2$
$\delta_{11} = 3.6511$	$\delta_{11} = 3.7592$	$J_{10,11} = -14.8$	$J_{10,11} = -14.8$
$\delta_{12} = 7.665^d$	$\delta_{12} = 7.660^d$	$J_{13,14} = 8.23$	$J_{13,14} = 8.23^e$
$\delta_{13} = 8.0869$	$\delta_{13} = 8.1075$	$J_{13,15} = 1.14$	$J_{13,15} = 1.14^e$
$\delta_{14} = 7.5200$*	$\delta_{14} = 7.5414$	$J_{13,16} = 0.68$	$J_{13,16} = 0.68^e$
$\delta_{15} = 7.6136$	$\delta_{15} = 7.6264$	$J_{14,15} = 7.25$	$J_{14,15} = 7.25^e$
$\delta_{16} = 7.9025$	$\delta_{16} = 7.9225$	$J_{14,16} = 0.95$	$J_{14,16} = 0.95^e$
		$J_{15,16} = 8.32$	$J_{15,16} = 8.32^e$

[a] Sample was 0.09 M in D_2O at apparent pH 2.7. Chemical shifts are given relative to tetramethylsilane, assuming δ_{HOD} = 5.05 ppm. These shifts are approximate relative to the standard. For the spectral analyses, the starred chemical shifts were taken as zero; relative chemical shifts within each group are accurate to ~±0.001 ppm. Fluorine shifts are downfield relative to the shift of the minor isomer. [b] These shifts are obscured by the residual water peak. The values given are those assumed for the computer simulation. [c] These coupling constants could not be determined and are taken from the data obtained for the free imino acid. [d] Measured directly from the spectra. [e] Assumed to be the same as the corresponding value for the major isomer.

lution at pH 2,[23] a value in general agreement with the barriers found for various acylprolines in nonaqueous solvents.[19,24] If the rotational barrier in II is approximately of this size, the exchange broadening effect on the spectra should be less than 0.1 Hz. However, if an activation barrier as low as 16 kcal/mol obtains in II, then the exchange broadening contribution to the resolution would be in excess of 2 Hz. Unfortunately, no control experiments on instrumental resolution were done but, since both instruments used are capable of better resolution than this and the spectra were obtained by experienced operators, we tend to believe that a substantial portion, if not all of the line-widths observed at 100 and 360 MHz is a result of chemical exchange broadening. Other than instrumental factors, a contribution to the degraded resolution from paramagnetic impurities, either from the hydrogenation catalyst or the deuterium oxide solvent,[25] or from solute self-association[26] cannot be excluded.

In attempting to ascertain the conformation of the 4-fluoroproline ring in this tripeptide we utilized the same approach that was taken in the earlier study of the corresponding imino acid.[12] In brief, the qualitative validity of the Karplus equation is assumed, so that each proton on the five-membered ring can be identified by its vicinal coupling constants to other protons or the fluorine nucleus. It is then assumed that the magnitude of these coupling constants may be quantitatively related to

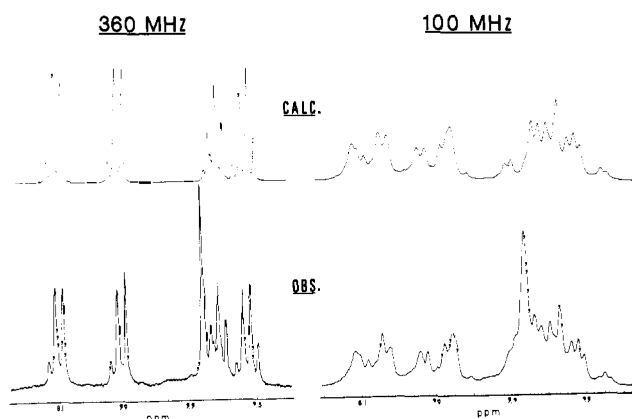


Figure 3. The aromatic portion of the ^{1}H NMR spectrum of II at 100 and 360 MHz. Calculated curves were obtained using the data in Table II.

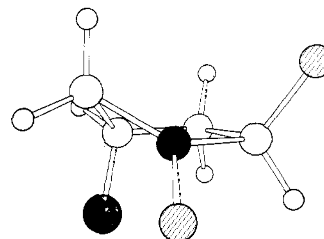


Figure 4. The dominant ring conformation of the major and minor forms of glycyl-4-fluoro-L-prolyl-L-tryptophan suggested by the vicinal coupling constant data. This ring structure was located by the fitting procedure described in the text and is very similar for both rotamers. The black atom is the imino nitrogen, while the lightly shaded atoms are carboxyl carbons. The more heavily shaded atom is the fluorine.

the dihedral angle (φ) between the C–H (or C–F) bonds which hold the spin-coupled nuclei to the ring skeleton according to the equation

$$J_{vic} = A \cos^2 \varphi + b \cos \varphi + C \qquad (1)$$

A computer program is used to generate a series of conformations for a somewhat idealized five-membered ring[27] and, for each conformation, a least-squares fit of eq 1 to the available coupling constant data is made. (Separate calculations are done for the proton–proton and proton–fluorine coupling constants.) That conformation which gives the best fit to the data is regarded as the dominant form of the proline ring.

This procedure converged very sharply with the free amino acid,[12] that is, one conformation gave a distinctly excellent fit of eq 1 to the coupling constant data. However, with both rotational isomers of II the approach to the "best fit" conformation was less precipitous, and a number of similar conformations were found which gave nearly equivalent accommodation to eq 1 in each case. Figure 4 shows the "best-fit" structures, defined by the bond angles in Table II, for the dominant and minor forms of the tripeptide. The striking characteristic of these conformations is the appearance of C_δ above the mean plane through the remainder of the ring atoms. All conformations which gave adequate fits of eq 1 to the coupling constants had the same general form as these structures. In both the major and minor isomer, the fluorine substituent takes up a quasi-axial position as it does in the free amino acid.[12]

For the major isomer, the parameters of eq 1 for H–H coupling constants at the best fit conformation were A = 14.1 Hz, B = −8.2 Hz, and C = 2.0 Hz. The corresponding quantities for the minor form were A = 13.6 Hz, B = −6.6 Hz, and C = 1.5 Hz. Proton–fluorine coupling constants in the major form were described by eq 1 with A = 44 Hz, B = 0, and C = −1.1 Hz, and in the minor conformer A = 37 Hz, B = 0, and

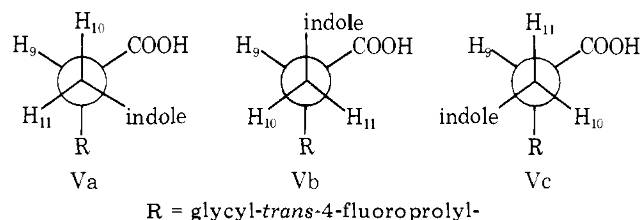
Table II. Parameters Resulting from Conformational Analysis of Both Rotational Isomers of Glycyl-*trans*-4-fluoro-L-prolyl-L-tryptophan

	Major	Minor
	(a) Bond Angles, deg	
$NC_\alpha C_\beta$	96	97
$C_\alpha C_\beta C_\gamma$	128	118
$C_\beta C_\gamma C_\delta$	66	81
$C_\gamma C_\delta N$	134	117
$C_\delta N C_\alpha$	89	100
	(b) Dihedral Angles,[a] deg	
φ_N	1	2
φC_α	−40	−37
φC_β	48	50
φC_γ	−53	−47
φC_δ	41	33
	(c) H-H Vicinal Coupling Constants,[b] Hz	
J_{12}	8.0 (8.0)	8.5 (8.5)
J_{13}	10.0 (10.0)	8.8 (8.8)
J_{24}	1.0 (1.1)	0.9 (0.9)
J_{34}	4.1 (4.0)	4.8 (4.8)
J_{45}	0.9 (0.8)	0.8 (0.8)
J_{46}	2.9 (2.9)	2.9 (2.9)
	(d) Vicinal Coupling Constants,[b] Hz	
J_{2F}	24.7 (19.2)	27.0 (20.4)
J_{3F}	37.8 (41.5)	34.8 (40.8)
J_{5F}	18.6 (20.9)	18.7 (21.4)
J_{6F}	41.0 (38.0)	39.3 (37.3)

[a] The dihedral angles are defined according to Hendrickson's notation.[28] [b] The experimental values for the coupling constants are given in parentheses.

C = 3.5 Hz. Given the accuracy of the various spin coupling constants, these coefficients for eq 1 probably should be regarded as identical for both isomers; they are similar to those found to describe the spin coupling in *trans*-4-fluoroproline, but are not identical. However, it is not clear that eq 1 should be quantitatively transferable from structure to structure.

Conformational isomerism is also possible about the C_α–C_β bond of the tryptophan residue, resulting in the three possible staggered rotamers shown below.[29] Since rotation at this bond is surely rapid, the observed vicinal coupling constants $J_{9,10}$ and $J_{9,11}$ will be weighted averages of their values in each of these rotamers. Structure Vc is analogous to the most stable form of the tryptophan anion[29] and the dipeptide glycyl-L-tryptophan.[30]



R = glycyl-*trans*-4-fluoroprolyl-

The magnitudes of $J_{9,10}$ and $J_{9,11}$ for the minor isomer (Table I) suggest that at the tryptophan residue this form of II is predominantly present as either rotamer Vb or Vc. However, the corresponding coupling constants do not support this conclusion as strongly in the case of the dominant structure of II and, instead, suggest that two or more of the rotamers Va–c are significantly populated.[31]

Consideration of a molecular model of II and the chemical shifts for the glycyl residue of the tripeptide provide a persuasive confirmation of the conclusion that Vc is the preferred rotamer at the C_α–C_β bond of the tryptophan residue in the minor isomer of II. The amide rotational isomer analogous to IVc is surely the minor form of II. When the tryptophan residue is present as rotamer Vc, the indole ring of this amino acid comes very close to the methylene group of the glycine. Both of these protons in the minor structure are shifted substantially upfield relative to their resonance position in the major form. Moreover, the chemical shift difference between the two methylene protons is dramatically increased. The model of this structure suggests that the methylene protons can be within 2–3 Å of the plane of the indole ring, more than sufficient for an upfield ring current effect.[32] Because of the asymmetric nature of the indole ring, the two methylene protons should be differently affected by the ring current; the model suggests that one of these atoms lies above the six-membered ring of the indole skeleton, while the other is above the five-membered component.

It is curious that the relative amounts of the rotamers Va–c in the major isomer (analogous to IIIc) appear to be so sensitive to the position of the glycine residue. In the major form, the carbonyl group of the glycine–fluoroproline amide bond is oriented toward the indole ring in rotamer Vc. Although sterically less demanding than the $-CH_2NH_3^+$ group, this carbonyl seems to repel the indole ring, resulting in a destabilization of this structure.

Conclusions

Through considerations of the vicinal H-H and H-F coupling constants in the *trans*-4-fluoroproline ring, we have shown that this residue in the tripeptide, glycyl-*trans*-4-fluoro-L-prolyl-L-tryptophan, is nonplanar and that, contrary to precedent, C_δ is the atom buckled out of the plane defined by the remaining atoms of the ring. This conformation is not greatly affected by the position of the glycine residue, in agreement with the results of a study of *N*-acetyl-4-hydroxy-L-proline.[10c] However, it appears that conformational equilibration at the C_α–C_β bond of the tryptophan residue is sensitive to reorientation of the glycine residue.

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References and Notes

(1) This work is the basis for a portion of the Ph.D. thesis of R.S.M. at the University of California, Santa Barbara.
(2) B. Love and J. T. Edsall, "Currents in Biochemical Research", Interscience, New York, N.Y., 1956, p 378.
(3) R. E. Dickerson and I. Geis, "The Structure and Action of Proteins", Harper and Row, New York, N.Y., 1969, p 49.
(4) Y. C. Leung and R. E. Marsh, *Acta Crystallogr.*, **11**, 17 (1958).
(5) T. Ueki, T. Ashida, M. Kakudo, Y. Sasada, and Y. Katsube, *Acta Crystallogr., Sect. B*, **25**, 1840 (1969).
(6) A. D. Rudko, F. M. Lovell, and B. W. Low, *Nature (London), New Biol.*, **232**, 18 (1971).
(7) T. Matsuzaki and Y. Iitaka, *Acta Crystallogr., Sect. B*, **27**, 507 (1971).
(8) I. L. Karle, *J. Am. Chem. Soc.*, **94**, 81 (1972).
(9) G. Kartha, G. Ambady, and P. V. Shankar, *Nature (London)*, **247**, 204 (1974).
(10) Recent examples include, (a) L. G. Pease, C. M. Deber, and E. R. Blout, *J. Am. Chem. Soc.*, **95**, 258 (1973); (b) M. Ellenberger and L. Pogliani, *Biochem. Biophys. Res. Commun.*, **58**, 613 (1974); (c) T. Prange, C. Garbay-Jaureguiberry, B. Roques, and M. Anteunis, *ibid.*, **61**, 104 (1974).
(11) R. Deslauriers, I. C. P. Smith, and R. Walter, *J. Biol. Chem.*, **249**, 7006 (1974).
(12) J. T. Gerig and R. S. McLeod, *J. Am. Chem. Soc.*, **95**, 5725 (1973).
(13) L. M. Jackman and S. Sternhell, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry", 2d ed, Pergamon Press, New York, N.Y., 1959, Chapter 4.
(14) "Organic Syntheses", Coll. Vol. III, Wiley, New York, N.Y., 1955, p 168.
(15) R. A. Boissonnas, S. Guttmann, R. L. Huguenin, P. A. Jaquenoud, and E. Sandrin, *Helv. Chim. Acta*, **41**, 1867 (1958).
(16) (a) P. Diehl, H. Kellerhals, and E. Lustig, "NMR. Basic Principles and

Progress", Vol. 6, Springer-Verlag, New York, N.Y., 1972; (b) A. A. Bothner-By and S. M. Castellano, "Quantum Chemistry Program Exchange", No. 111, Chemistry Department, Indiana University, Bloomington, Ind.
(17) D. E. Dorman and F. A. Bovey, *J. Org. Chem.*, **38**, 2379 (1973).
(18) V. Madison and J. A. Schellman, *Biopolymers*, **9**, 511 (1970).
(19) H. Nishihara, K. Nishihara, T. Uefuji, and N. Sakota, *Bull. Chem. Soc. Jpn.*, **48**, 553 (1975).
(20) R. S. McLeod, Ph.D. Thesis, University of California, Santa Barbara, 1975.
(21) J. T. Gerig and R. S. McLeod, *J. Org. Chem.*, **41**, 1653 (1976).
(22) W. A. Thomas and M. K. Williams, *J. Chem. Soc., Chem. Commun.*, 994 (1972).
(23) J. T. Gerig, unpublished results.
(24) H. L. Maia, K. G. Orrell, and H. N. Rydon, *Chem. Commun.*, 1209 (1971).
(25) H. Pearson, D. Gust, I. M. Armitage, H. Huber, J. D. Roberts, R. E. Stark, R. R. Vold, and R. L. Vold, *Proc. Natl. Acad. Sci. U.S.A.*, **72**, 1599 (1975).
(26) R. Deslauriers, R. Walter, and I. C. P. Smith, *FEBS Lett.*, **37**, 27 (1973). These authors observed self-association of a proline-containing tripeptide in dimethyl sulfoxide solution, but not in deuterium oxide at solute concentrations of ~0.2 M.
(27) We assumed all bond lengths of the proline ring to be 1.52 Å, the average of bond lengths found in a number of crystal structures. Four angles are needed to specify a particular conformation.[28] As before, three contiguous bond angles and a dihedral angle were used for this purpose.[12]
(28) J. B. Hendrickson, *J. Am. Chem. Soc.*, **83**, 4537 (1961).
(29) J. R. Cavanaugh, *J. Am. Chem. Soc.*, **92**, 1488 (1970).
(30) R. A. Pasternack, *Acta Crystallogr.*, **9**, 341 (1956).
(31) Accepting Cavanaugh's values J_{gauche} = 2.6 Hz and J_{trans} = 13.6 Hz,[28] we estimate that the major form of II consists of 21% Va, 33% Vb, and 46% Vc. The minor structure is calculated to be a mixture of 2% Va, 24% Vb, and 74% Vc. Cavanaugh's constants may not be transferable to this system, but probably give population estimates that are qualitatively correct.
(32) (a) C. W. Haigh and R. B. Mallion, *Org. Magn. Reson.*, **4**, 203 (1972); (b) C. Giessner-Prettre and B. Pullman, *C. R. Acad. Sci., Ser. D*, **268**, 1115 (1969); (c) *J. Theor. Biol.*, **27**, 87, 341 (1970).

Photoisomerization Kinetics of 11-*cis*-Retinal, Its Schiff Base, and Its Protonated Schiff Base

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Abstract: Kinetics of the photoisomerization of retinal (RET), a retinylidene Schiff base (SB), and a protonated Schiff base (PSB) have been studied using nanosecond laser photolysis techniques. Two paths of isomerization were observed, one occurring over 10's of nanoseconds and one occurring instantaneously in the time frame of the experiment. Data are presented identifying the former process as being mediated through the triplet state. Isomerization is the net result of a delicate balance between competing processes, and the relative amounts of isomerization via the two paths vary with the compound and with the solvent. A model is presented which allows an adequate and consistent interpretation of the experimental results. The significance of our results to visual pigments is discussed. The protonated Schiff base, which is the closest chemical analogue to rhodopsin we investigated, isomerizes through a singlet mechanism. The extremely rapid rate we observe for this isomerization is consistent with prelumirhodopsin being photogenerated from rhodopsin in picoseconds.

When rhodopsin is exposed to light it undergoes a series of spectral changes corresponding to the presence of various transient photoproducts. The first observable spectral change at 77 K or above involves absorption of a photon by rhodopsin to yield prelumirhodopsin. This photoproduct has been isolated and studied by cooling the rhodopsin solution to liquid nitrogen temperature.[1-3] Subsequent warming of the solution results in the formation of successive transient species. Only the first reaction in the series requires light for its initiation. The subsequent reactions, the so-called "dark reactions", proceed spontaneously at physiological temperatures in the absence of light.

The chromophore in rhodopsin is the 11-cis isomer of retinal[4] bound to the protein, opsin, through a protonated Schiff base linkage.[5-7] The final products of photolysis of bovine rhodopsin are opsin and retinal. This solution appears yellow compared to the red initial solution so it has become customary to refer to the photolysis process as "bleaching". When bleached with light the chromophore is released from the protein in the form of *all-trans*-retinal.[4] When "bleached" with heat, however, the opsin is denatured and releases the chromophore as 11-*cis*-retinal.[8] Thus, one deduces that the interaction of light with rhodopsin causes the cis → trans isomerization of the chromophore.[9] Since only the transition from rhodopsin to prelumirhodopsin requires light, one might suspect that this step involves isomerization of the retinylidene chromophore about the 11–12 double bond.

Recently several workers have investigated the kinetics of the photolysis of rhodopsin to produce prelumirhodopsin at physiological temperatures. Using a pulsed nitrogen laser to photolyze rhodopsin, Rosenfeld et al. found that the buildup of prelumirhodopsin occurred on a time scale faster than 10 ns.[10] Using a mode locked Nd^{3+} glass laser, Busch et al. established that the upper limit for the buildup of prelumirhodopsin is less than 6 ps.[11] Thus, there is strong evidence documenting the rapid conversion of rhodopsin to prelumirhodopsin and linking this reaction to a cis → trans isomerization.

Much work has been done in trying to determine the mechanism for isomerization of visual pigments. Most of this work has involved studies of the isomerization of retinal as a model system for the chromophoric moiety. Unfortunately, much of this work, both theoretical and experimental, is inconclusive or contradictory.

Some theoretical studies indicate that there is a high barrier to rotation for the isomerization of 11-*cis*- to *all-trans*-retinal out of the first $\pi\pi^*$ singlet state but a low barrier from the first $\pi\pi^*$ triplet state.[12,13] However, Becker et al. find low barriers to rotation in both the singlet and triplet states[14] and Pullman et al. find high barriers to rotation in both states.[15]

Experimental results tend to support the hypothesis that 11-*cis*-retinal isomerizes efficiently out of the first triplet state but the degree and nature of the triplet involvement are not clear. Raubach and Guzzo report that the isomerization quantum yield, ϕ_{isom}, is about 0.75 out of the triplet state, as determined by triplet sensitization studies.[16] Taking the intersystem crossing quantum yield to be $\phi_T = 0.11$[17] and the isomerization yield to be $\phi_{isom} = 0.2$[18] following direct irradiation, they determined that about half the isomerization of