conformation, generally denoted as ap,ap (where ap stands for anti periplanar),[2b] corresponds to a local maximum, which lies well above (by ~7 kcal/mol) the calculated minimum ($\omega = \omega' = 75°$ or, by symmetry, $\omega = \omega' = 285°$). This minimum, as expected (see above), occurs near the gauche-gauche conformation and is denoted as −sc,−sc or +sc,+sc (where sc stands for syn clinal).[2b,14] Of the 18 phosphodiesters whose experimental ω,ω' values are listed in ref 2b–d, including many polynucleotides, and three dinucleoside phosphates, 11 are of the −sc,−sc or +sc,+sc type, with ω,ω' values corresponding to energies <1 kcal/mol above our calculated minimum energy. The remaining seven phosphodiesters are of the sc,ap type and have energies (based on the present calculations) within 3 kcal/mol of the minimum. It is reasonable that these sc,ap conformations should be accessible, if one recognizes that the intrinsic energy cost (~3 kcal/mol) could be moderated by longer range interactions involving the sugar and bases, effects beyond the scope of the present work. It is considerably less likely that the energetic demands of the ap,ap conformation would be satisfied, since the intrinsic barrier separating the sc,sc and ap,ap conformations is calculated to be ~7 kcal/mol. Hence, the absence of any experimentally observed ap,ap cases[15] is readily understood.[16] Our calculated torsional potential energy surface thus appears to be in good general accord with experimental ω,ω' data, in spite of the limitations inherent in using the symmetrical dimethyl phosphate as a model for much more complicated and less symmetrical phosphodiesters. We wish to emphasize the sharp difference between the present ω,ω' torsional potential, characterized by a large barrier and the absence of threefold periodicity, and the traditionally used threefold potential, with smaller barriers. Furthermore, it is suggested that the accuracy of future empirical or semiempirical calculations on phosphate esters would be improved by employing intrinsic ω,ω' torsional potentials for PO bonds, which are consistent with the results of the present model calculations.

As a final point, we consider the possible effect of phosphorus 3d orbitals on the calculated ω,ω' potential energy surface. Although charge densities might be sensitive to these functions, previous work[13b] indicates that addition of 3d orbitals does not strongly affect calculated rotational barriers. This point is currently being tested with further *ab initio* calculations. In the meantime, we have carried out semiempirical CNDO/2 calculations[17] on dimethyl phosphate, both with and without 3d functions on phosphorus. The 3d functions have a negligible effect on the rotational barriers and both calculated surfaces are in good agreement with the *ab initio* minimal basis results, with a sc,sc minimum at $\omega = \omega' = 66°$ and an ap,ap maximum 5 kcal/mol above the minimum.

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(14) Note that +sc,−sc conformation (*e.g.*, $\omega = +60$, $\omega' = -60$) is very unfavorable, due to combined dipolar and steric interactions. For regions where one of the angles is between ~120 and 240°, the coupling between the two rotors is rather small.

(15) Crystal structures for some triphosphate polyanions are available,[2b] which in some cases do indeed exhibit ap,ap conformations. There is, however, some indication that the preferred conformations may be dependent on the degree of negative charge in these polyanions.[2b] Simple electrostatic calculations for $P_3O_{10}^{5-}$, based on plausible point charges, suggest some preference for the ap,ap conformation over the sc,ap conformation. At any rate, conformation changes in polyphosphate anions include effects (*viz.* changes of interatomic distance for atom pairs in which *both* atoms have large formal charges) absent in the present model compound, dimethyl phosphate monoanion. Hence, one might approach the problem of polyphosphate conformations either by supplementing the intrinsic torsional contributions (Figure 2) with appropriate electrostatic terms or by carrying out molecular orbital calculations on larger model systems (*e.g.*, $P_3O_{10}^{5-}$). Such studies are presently being initiated.

(16) In some previous calculations,[4b] the ap,ap conformation was found to be less favorable than the sc,sc and sc,ap cases, for certain parameter choices; however, the energy differences were slight (−1.0 kcal/mol), and the ap,ap case always represented at least a local minimum.

(17) J. A. Pople and G. A. Segal, *J. Chem. Phys.*, **44**, 3289 (1966); D. P. Santry and G. A. Segal, *ibid.*, **47**, 158 (1967).

Marshall D. Newton
Department of Chemistry
Brookhaven National Laboratory
Upton, New York 11973
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Cyclic Peptides. V. ^1H and ^{13}C Nuclear Magnetic Resonance Determination of the Preferred β Conformation for Proline-Containing Cyclic Hexapeptides[1]

Sir:

Two C_2 symmetric intramolecularly hydrogen-bonded conformations are possible for the cyclic hexapeptide *cyclo*(Gly-L-Pro-Gly)$_2$. In a previous study,[2] 100-MHz ^1H nmr was used to analyze the solution structure of this peptide, but ambiguity in the nmr spectrum introduced by two pairs of magnetically nonequivalent glycine residues in the sequence did not allow a clear choice between structures A and B. With the aid of specifically deuterated and ^{13}C-enriched samples, the conformation of *cyclo*(Gly-Pro-Gly)$_2$ in DMSO and in aqueous solution has now been determined through the use of 250-MHz ^1H and 25.16-MHz ^{13}C nmr spectroscopy.

Previous nmr investigations of the solution conformations of *cyclo*(Pro-Ser-Gly)$_2$ and *cyclo*(Ser-Pro-Gly)$_2$[3,4] demonstrated that the favored conformation for each of these in aqueous solution contains type II β turns[5] (as in structure A) with the residue preceding the proline intramolecularly H bonded, and the proline in the trans′ ($\psi \approx 300°$)[6] conformation. In contrast, the proline-containing cyclic decapeptide gramicidin S assumes[7] type II′ β turns[5] (analogous to structure B), where the residue (Val) following the Pro is intramolecularly H bonded, and the Pro is cis′ ($\psi \approx 125°$).

(1) For the preceding paper, see C. M. Deber, D. A. Torchia, S. C. K. Wong, and E. R. Blout, *Proc. Nat. Acad. Sci. U. S.*, **69**, 1825 (1972).
(2) R. Schwyzer and U. Ludescher, *Helv. Chim. Acta*, **52**, 2033 (1969).
(3) D. A. Torchia, A. diCorato, S. C. K. Wong, C. M. Deber, and E. R. Blout, *J. Amer. Chem. Soc.*, **94**, 609 (1972).
(4) D. A. Torchia, S. C. K. Wong, C. M. Deber, and E. R. Blout, *ibid.*, **94**, 616 (1972).
(5) C. M. Venkatachalam, *Biopolymers*, **6**, 1425 (1968).
(6) For an explanation of conventions used in dihedral angle nomenclature, see J. T. Edsall, P. J. Flory, J. C. Kendrew, A. M. Liquori, G. Nemethy, G. Ramachandran, and H. A. Scheraga, *J. Biol. Chem.*, **241**, 1004 (1966).
(7) Yu. A. Ovchinnikov, V. T. Ivanov, V. F. Bystrov, A. I. Miroshnikov, E. N. Shepel, N. D. Abdullaev, E. S. Efremov, and L. B. Senyavina, *Biochem. Biophys. Res. Commun.*, **39**, 217 (1970).

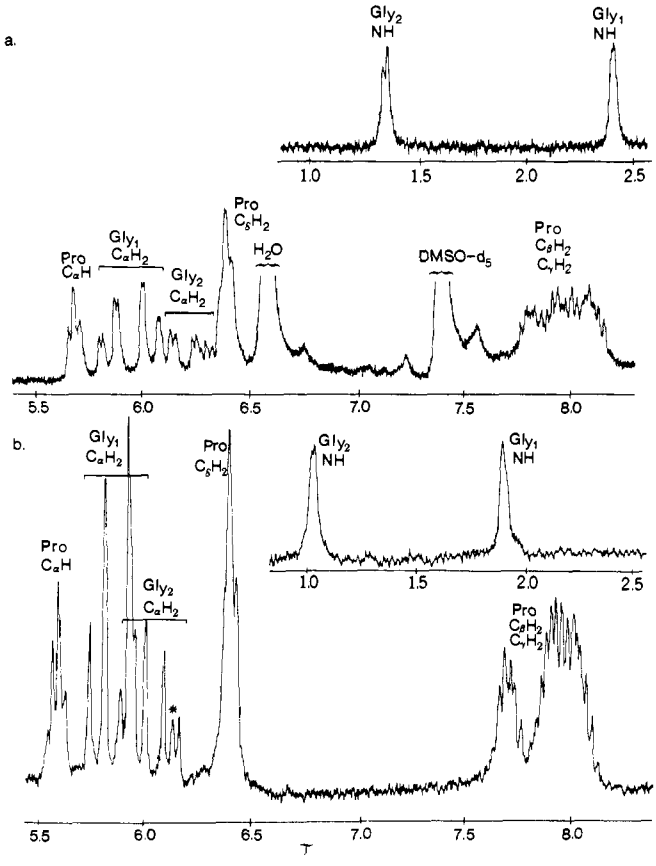
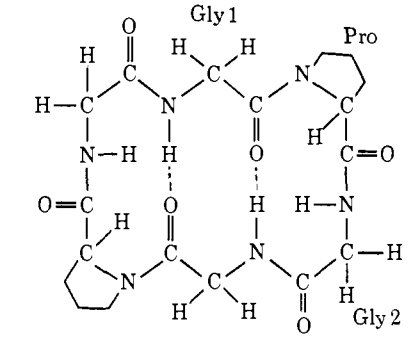
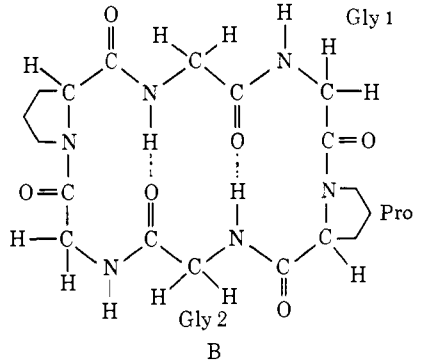


Figure 1. ^1H spectra (250 MHz) of *cyclo*(Gly-L-Pro-Gly(d_2))$_2$ at *ca.* 25°: (a) in DMSO-d_6; *c* 20 mg/ml; (b) in D_2O and (NH region) in H_2O–CH_3COOH, 98:2 by volume; *c* 15 mg/ml. The Gly_2 residue was 80% deuterated at the α position. Chemical shifts given in ppm, with TMS at 10.0 ppm (τ scale). The indicated resonance (*) at τ 6.2 in (b) is due to Gly_2 $HC_\alpha D$ species.



A
type II β turn



B
type II′ β turn

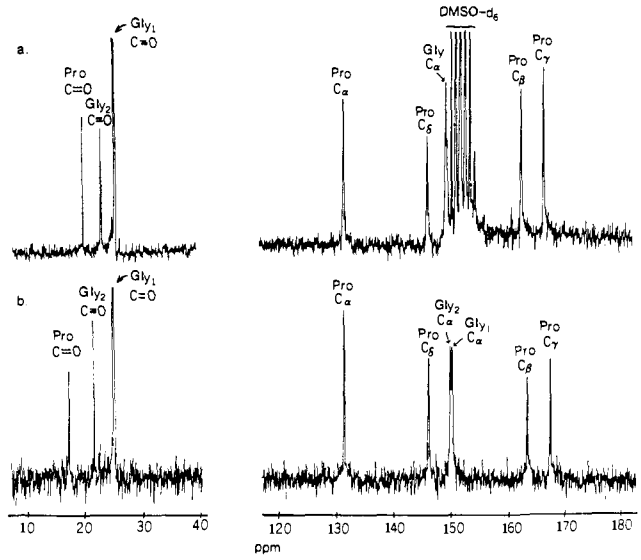


Figure 2. Fourier transform ^{13}C spectra (25.16 MHz) of *cyclo*-(Gly-L-Pro-Gly)$_2$ at 31°: (a) in DMSO-d_6; (b) in D_2O; *c* 20 mg/ml. Chemical shifts are given in ppm upfield from external $^{13}CS_2$. The resonance of the Gly_1 carbonyl carbon (enriched ~6% with ^{13}C) is off-scale in both (a) and (b). The upfield region was assigned by comparison with ^{13}C spectra of related peptides (F. A. Bovey, *Proc. 3rd Amer. Peptide Symp.*, in press). In both spectra, the pulse sequence was comprised of 0.8-sec acquisition time and 3.0-sec pulse delay.

In Figure 1 the 250-MHz ^1H spectra of *cyclo*(Gly-Pro-Gly(d_2))$_2$[8] in D_2O and in DMSO-d_6 are shown. The extent of deuteration (*ca.* 80%) of the Gly_2 residue has left sufficient Gly_2 $C_\alpha H_2$ species such that these protons appear as an AB quartet of diminished intensity. Thus, no coupling information is lost, and the fine splitting of the AB quartets for both glycines can be measured in DMSO-d_6, yielding the following $J_{N\alpha}(HNC_\alpha H)$ coupling constants:[9] for Gly_1, 4.0 and 3.0, and for Gly_2 (80% d_2), 5.7 and 5.5. Spin decoupling experiments involving the two NH resonances revealed that the *upfield* resonance is coupled to the *undeuterated* glycine (Gly_1), and the *downfield* resonance is coupled to the *deuterated* glycine (Gly_2). Furthermore, when the temperature dependence over the range 30–115° of the NH region of the spectrum in DMSO-d_6 was determined, a shift to higher field of 0.0013 ppm/deg was observed for the upfield resonance and of 0.0053 ppm/deg for the downfield resonance, values typical for internal (buried or H-bonded) protons and external (exposed to solvent) N–H protons, respectively.[3,4,10,11]

These results demonstrate that the glycine preceding the proline is intramolecularly hydrogen bonded, as in conformation A. From the experimentally obtained $J_{N\alpha}$ coupling constants for the glycines, and from molecular models, it is possible to describe a C_2 sym-

(8) The cyclic hexapeptide was synthesized *via* cyclodimerization of the tripeptide active ester H-Gly-Pro-Gly-*p*-nitrophenyl ester hydrochloride, either in triethylamine–dimethylformamide (see J. A. Reader and P. W. G. Smith, *J. Chem. Soc.*, 3479 (1965)) or, preferably, in pyridine.

(9) The experimentally measured $J_{N\alpha}$ were modified to yield the best fit of a computer-simulated spectrum, using a line width at half-height of 3.5 Hz.

(10) M. Ohnishi and D. W. Urry, *Biochem. Biophys. Res. Commun.*, **36**, 194 (1969).

(11) K. D. Kopple, A. Go, R. H. Logan, Jr., and J. Savrda, *J. Amer. Chem. Soc.*, **94**, 973 (1972).

metric backbone conformation[12] for *cyclo*(Gly-Pro-Gly)$_2$ in DMSO-d_6 solution

	Gly_1	Pro	Gly_2
ϕ	~0°	120°	270°
ψ	~0°	300°	180°

Nmr spectra in aqueous solution suggest that the same skeletal conformation exists in this solvent.

The ^{13}C spectra of the enriched sample (Figure 2) permitted assignment of the highest field carbonyl resonance to the Gly_1 carbonyl carbon—the one involved in H bonding. This result is somewhat surprising, since shifts to lower field of 5–10 ppm have been reported for intermolecularly H-bonded carbonyl carbons,[13] although shielding due to magnetic anisotropy of the end peptide group of the β turn provides a possible explanation in this case.[14] Further, in spectra of the deuterated sample, both in DMSO-d_6 and in D_2O, it was noted that the central carbonyl resonance had markedly reduced intensity relative to the other carbonyls, while in the enriched sample (undeuterated) this was not the case.[15] Since the dominant ^{13}C spin–lattice relaxation mechanism is through neighboring protons, deuteration of the C_α of Gly_2 should lead to a less efficient relaxation (longer T_1) and, hence, to the observed reduced peak size. Thus, the Gly_2 C=O may be assigned to the central resonance, and the Pro C=O to the remaining downfield peak (Figure 2a and b).

Although the observed coupling constants are interpreted in terms of a single conformation for *cyclo*(Gly-Pro-Gly)$_2$, described by the ϕ,ψ angles indicated, the $J_{N\alpha}$ values are equally consistent with a weighted average of similar conformational states related by rotations (of perhaps ±30°) around the C_α bonds of the four glycyl residues. In contradistinction to the serine-containing cyclic hexapeptides,[3,4] *cyclo*(Gly-Pro-Gly)$_2$ showed no asymmetric conformations or structures containing *cis*-X-Pro peptide bonds in the solvents examined. The results with *cyclo*(Gly-Pro-Gly)$_2$[16] establish a general conformational preference for this class of proline-containing cyclic hexapeptides, which favors a trans′ orientation of the proline residue, and intramolecular H bonding of the residue *preceding* the proline.

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(12) The estimated uncertainty in all (ϕ,ψ) angles obtained from $J_{N\alpha}$ data is ±10°.

(13) F. A. Bovey in "Nuclear Magnetic Resonance Spectroscopy," Academic Press, New York, N. Y., 1969, p 233, and references therein.

(14) D. W. Urry and M. Ohnishi in "Approaches to Biomolecular Conformation," D. W. Urry, Ed., American Medical Association, Chicago, Ill., 1970, pp 263–299, and references therein.

(15) ^{13}C spectra of the deuterated sample taken with a longer pulse interval (acquisition time plus pulse delay = 8.5 sec) show recovery of the amplitude of the central resonance relative to the other carbonyl resonances. This result confirms that the observed effects are due to a change in T_1.

(16) Note Added in Proof. Professor Robert Schwyzer of the Eidg. Technische Hochschule, Zürich-Hönggerberg, has informed us that related investigations on this compound have recently been carried out in his laboratory (*Helv. Chim. Acta*, in press).

Lila G. Pease, Charles M. Deber, Elkan R. Blout*
Department of Biological Chemistry
Harvard Medical School
Boston, Massachusetts 02115
Received October 18, 1972

Ferromagnetic Behavior of Phenanthrolineiron(II) Dichloride and Related Compounds

Sir:

Examples of inorganic ferromagnetic order among transition metal organometallic compounds are rare. The only such compound known to us is the five coordinate, spin $^3/_2$ bis(diethyl dithiocarbamato)iron-(III) chloride studied by Wickman[1] and coworkers. This complex is not polymeric and thus the magnetic exchange is probably due to a weak (T_c = 2.5 K) interaction[2] between discrete molecules. In this communication we present Mössbauer and magnetic data for Fe(phen)Cl_2, in which a cooperative ferromagnetic behavior is observed, and for some similar systems.

Powder susceptibility measurements for analytically pure[3] samples of Fe(phen)Cl_2 over the temperature range 4.2–300 K and at various fields (Figure 1) have been made. Fits with a Curie–Weiss law yield a paramagnetic Curie temperature $\theta = +12 \pm 4$ K. The Curie–Weiss constant $C = 1.24 \times 10^{-2}$ emu/g determined at high temperatures ($T > 10\theta$) corresponds to an effective magnetic moment of 5.4 μB, consistent with quintet iron(II). This is also suggested by the iron-57 Mössbauer isomer shift [δ(300 K) = +1.04 mm/sec, ΔE = +1.45 mm/sec] relative to iron metal. The temperature dependence of dc magnetization at low fields (Figure 2) and ac susceptibility indicates a Curie temperature, $T_c = 8 \pm 2$ K. In addition, the field dependence of magnetization for high fields and $T < 4.2$ K clearly shows that Fe(phen)Cl_2 is not completely saturated for fields up to 50 kG (Figure 3), and hysteresis is detected at low fields as expected for a ferromagnetic material. The low-field data in Figure 3 show a rapid rise of magnetic moment, σ, *vs.* applied field largely reflecting the demagnetizing field of the particular sample shape. The dashed initial portion indicates the region where hysteresis is observed and the region above ~10 kG shows a gradual almost linear increase of σ *vs.* field. The moment σ = 55.6 emu/g at 48 kG corresponds to 3.07 μB/Fe atom in Fe(phen)Cl_2 and magnetic saturation is clearly not achieved. Similar data down to 1.4 K show the same general features with slightly (<5%) higher values of σ at high field. In order to saturate the Fe moment, these preliminary experiments must be extended to much higher applied fields. We also have made similar magnetic measurements for the complexes Fe(2,2′-bipy)Cl_2 and Fe(5,5′-diMe-2,2′-bipy)Cl_2.

Mössbauer spectroscopy confirms that Fe(phen)Cl_2 is ordered at 4.2 K. The well-resolved Zeeman spec-

(1) H. H. Wickman, A. M. Trozzolo, H. J. Williams, G. W. Hull, and F. R. Merritt, *Phys. Rev.*, **155**, 563 (1967).

(2) H. H. Wickman, *J. Chem. Phys.*, **56**, 976 (1972).

(3) Calculated % C = 46.95, H = 2.63, N = 9.12, Fe = 18.60; measured % C = 46.77, H = 2.66, N = 9.34, Fe = 18.20.