C(11′), 3.5 Å from the chlorine atom of a symmetry-related molecule and more than 3.6 Å from any other nonhydrogen atom. There were no other peaks in excess of 0.6 e/Å^3 except in the immediate vicinity of some chlorine atoms. It was also noted that atom Cl(12′) displayed unusually large thermal parameters. In a Fourier map that was phased with all atoms except those of the four chloroethyl side chains, the electron density at Cl(12′) was only about two-thirds that found at the positions of the other three chlorine atoms. These observations suggested that atom Cl(12′) might be disordered. Two positions, Cl(12′) and Cl(12A′), the latter corresponding to the residual peak, were assigned to this chlorine atom and population parameters for these two sites were allowed to vary when least-squares refinement was resumed. The population parameters, which were not constrained to a total of 1.0, converged to values of 0.556 (8) and 0.307 (9) for sites Cl(12′) and Cl(12A′), respectively. This result is consistent with the hypothesis that the chlorine atom occupies position Cl(12′) about two-thirds of the time and position Cl(12A′) about one-third of the time. Two feasible models for this disorder are (1) the C(10′)–C(11′)–Cl(12′) side chain was partially degraded during data collection, resulting in fragments that occupy the cavity around position Cl(12A′) or (2) the side chain assumes two or more conformations, perhaps under the effects of X-radiation, with the chlorine atom at site Cl(12′) or in the vicinity of site Cl(12A′).

The final R index, including all reflections, is 0.117 and the goodness-of-fit is 2.77. A final difference Fourier map shows no peaks or troughs exceeding 0.7 e/Å^3 in magnitude. The estimated errors in positional coordinates are about 0.002 Å for the two phosphorous atoms and 0.006 Å for other atoms of the two six-membered rings and their immediate substituents. In the chloroethyl side chains, the estimated errors in atomic positional coordinates are relatively large, ranging from 0.002 to 0.006 Å for chlorine atoms and from 0.01 to 0.03 Å for carbon atoms of the side chains, whereas the estimated error is 0.02 Å for position Cl(12A′).

Figure 1 depicts the chemical configuration, the conformation, and the heavy-atom thermal ellipsoids. As suggested by the data of Struck, *et al.*,[9] and of Takamizawa, *et al.*,[13] the compound is 4-peroxycyclophosphamide (4-hydroxycyclophosphamide anhydro dimer), with the two cyclophosphamide moieties joined by the peroxide linkage. The observed conformation is stabilized by two N–H--O intramolecular hydrogen bonds. It is likely that the low-field chemical shift of the NH protons found in the nmr experiment are a result of these bonds. Bond lengths and angles involving the central rings of the cyclophosphamide moieties and their immediate substituents are in agreement with the corresponding values reported for 4-ketocyclophosphamide.[14] The two C–O bonds of the peroxide linkage have lengths of 1.44 and 1.42 Å, respectively. As in hydrogen peroxide,[15] the O–O bond distance is 1.47 Å. The bond lengths within the chloroethyl side chain deviate greatly from their expected values. The C–Cl bond distances range from 1.47 to 1.94 Å with an average value of 1.77 Å, and the C–C and C–N bonds of the side chains range from 1.30 to 1.70 Å. These anomalous values are probably a consequence of the high degree of thermal motion and/or the extensive decomposition that occurred during data collection. Tables of atomic parameters and structure factors are included in the microfilm edition of this journal. See paragraph at end of paper regarding supplementary material.

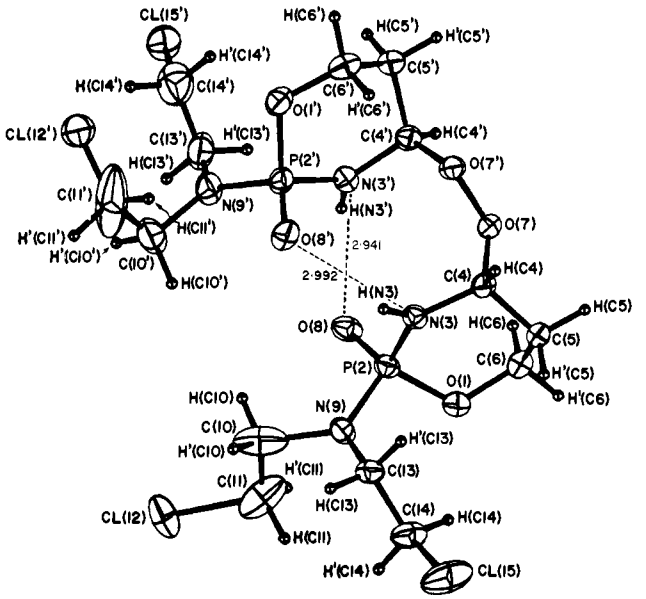

Figure 1. Conformation of 4-peroxycyclophosphamide. Nonhydrogen atoms are represented by thermal ellipsoids, which are defined by the principal axes of thermal vibration and are scaled to include 25% probability. The hydrogen atoms are represented by spheres of 0.1 Å radius. Only the major site of atom Cl(12′) is depicted. The donor–acceptor distances for the two intramolecular hydrogen bonds are shown.

Acknowledgment. We thank Miss Catherine Sims for assistance with the preparation of this manuscript. This research was supported by National Institutes of Health Grants CA-12159 and DE-02670.

Supplementary Material Available. A listing of structure factor amplitudes and atomic parameters will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D.C. 20036. Remit check or money order for $4.00 for photocopy or $2.00 for microfiche, referring to code number JACS-74-4014.

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Cyclic Peptides. VIII. ^{13}C and ^{1}H Nuclear Magnetic Resonance Evidence for Slow Cis′–Trans′ Rotation in a Cyclic Tetrapeptide[1]

Sir:

Cis–trans isomerism has been observed about X-imino acid peptide bonds (*e.g.*, Gly-Pro) where rota-

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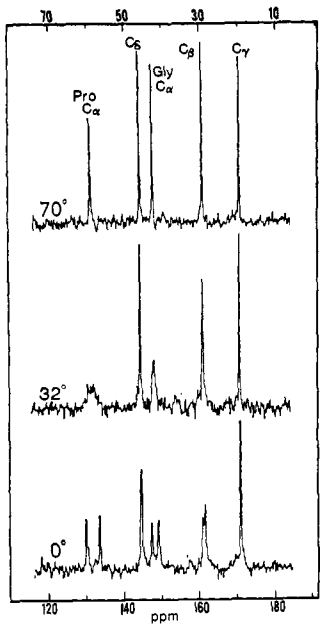

Figure 1. Upfield regions of Fourier transform ^{13}C nmr spectra (20 MHz) of *cyclo*(L-Pro-Gly)$_2$ in D_2O–CH_3CN (9:1 vol/vol) at 70, 32, and 0°, concentration 60 mg/ml. The following chemical shifts (given in ppm upfield from external CS_2 on the lower scale and in ppm downfield from TMS on the upper scale) were obtained at 70°: Pro C_α, 131.4; Pro C_δ, 144.4; Gly C_α, 147.9; Pro C_β, 161.2; and Pro C_γ, 171.2 (CH_3CN methyl carbon at 191.7 ppm). Carbonyl resonances showed similar coalescence behavior. Spectra are the result of 10–15,000 accumulations with a 0.5-sec recycle time. The 32° spectrum accumulated for 100,000 transients did not show any additional fine structure.

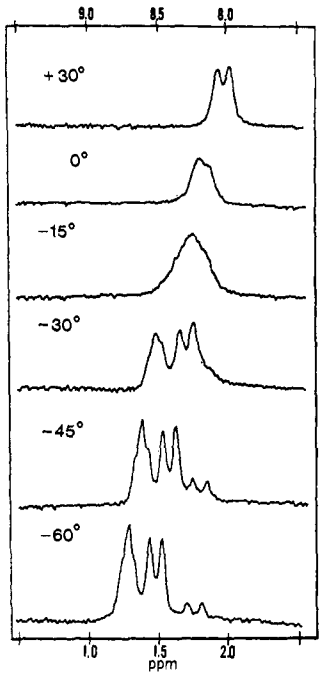

Figure 2. Peptide N–H regions of the proton nmr spectra (100 MHz) of *cyclo*(L-Pro-Gly)$_2$ in dimethyl-d_6 sulfoxide–methylene-d_2 chloride (2:5, vol/vol) at various temperatures, concentration 15 mg/ml. On the lower scale chemical shifts are given in ppm with TMS at 10.0 ppm (τ scale). On the upper scale chemical shifts are given in ppm with TMS at 0.0 ppm (δ scale).

tion between two isomers of similar energy is slow enough on the nmr time scale to permit observation of separate resonances for each form. This circumstance has been recorded in both synthetic[2-7] and biologically active[8-13] peptides. We now report ^{13}C and ^1H nmr evidence that relatively slow rotation about proline C_α–C=O single bonds (*i.e.*, cis′ ↔ trans′)[14] occurs in the cyclic tetrapeptide *cyclo*(L-Pro-Gly)$_2$.[15]

^{13}C nmr spectra of aqueous solutions of *cyclo*(L-Pro-Gly)$_2$ at 0, 32, and 70° are shown in Figure 1. Resonances were assigned through comparison with related peptides.[10,16-19] The resonances at 70° broaden as the temperature is lowered, with the Pro C_α and Gly C_α each separating into two sharp resonances of equal intensity at 0°, located 3.6 and 1.7 ppm apart, respectively. At 0°, the Pro C_β resonance has begun to split, while the Pro C_γ and Pro C_δ resonances remain fairly sharp.

Proton nmr spectra at 100 MHz of *cyclo*(L-Pro-Gly)$_2$, recorded in dimethyl-d_6 sulfoxide–methylene-d_2 chloride over the temperature range −60 to +30°, displayed similar coalescence behavior in their peptide N–H region (Figure 2). At −45°, the two Gly N–H protons have different chemical shifts and appear as two resonances of equal intensity: a barely resolved triplet (τ 1.4, estimated sum of $J_{N\alpha} \leq 7$ Hz) and a doublet (τ 1.6, $J_{N\alpha} = 9.5$ and ≤ 3 Hz). In addition, a second conformation in low population (small doublet τ 1.8, $J_{N\alpha} = 10$ Hz and ≤ 3 Hz) is distinguishable at −45°.

Estimates of the free energy of activation ($\Delta F^{\pm}$) for the conformational process giving rise to the observed magnetic nonequivalence, calculated[20] from coalescence

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(14) (a) A. E. Tonelli, *J. Amer. Chem. Soc.*, **95**, 5946 (1973). (b) The notations cis′ and trans′ correspond to regions of proline ψ angle values centered at −60 and 120°, respectively, in the recent convention used in rotational angle nomenclature (J. C. Kendrew, *et al.*, *Biochemistry*, **9**, 3471 (1970)). In the former convention (J. T. Edsall, *et al.*, *J. Biol. Chem.*, **241**, 1004 (1966)), ψ = *ca.* 120° for cis′ and *ca.* 300° for trans′.
(15) (a) cyclo(L-Pro-Gly-L-Pro-Gly) was synthesized *via* cyclization of the corresponding tetrapeptide *p*-nitrophenyl ester hydrochloride in pyridine at 90° in 25% yield, mass spectrum molecular ion peak *m/e* 308. For details of the synthesis and characterization, see ref 1. (b) An alternative synthesis of this peptide has been reported. M. Rothe, W. Schindler, R. Pudill, M. Kostrzewa, and R. Theysohn, *Angew. Chem.*, **83**, 927 (1971); M. Rothe, W. Schindler, R. Pudill, M. Kostrzewa, R. Theysohn, and R. Steinberger, "Peptides 1971," H. Nesvadba, Ed., North-Holland Publishing Co., Amsterdam, 1973, pp 388–399.
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temperatures and resonance separations at low temperature in ^{13}C and ^{1}H spectra, respectively, are 15 ± 1 kcal/mol in aqueous solution and 13 ± 1 kcal/mol in DMSO–CD_2Cl_2 solution.

cyclo(L-Pro-Gly)$_2$ is likely to adopt the cis–trans–cis–trans peptide bond backbone (with two cis Gly-Pro bonds),[2-13] which has been consistently found by Dale and Titlestad in sarcosine-containing cyclic tetrapeptides.[21-23] ^{13}C spectra have shown in several instances that the Pro C_γ resonance is the most sensitive and reliable indicator of a change in the conformational state of the X-Pro peptide bond,[10,16-19] but in *cyclo*(L-Pro-Gly)$_2$ spectra (Figure 1), Pro C_γ remains at the position attributed to cis peptide bonds (*ca.* 171 ppm). Furthermore, in contrast to the $\Delta F^{\ddagger}$ values calculated above, typical values for cis–trans isomerism of unhindered amides are in the range of 20 ± 1 kcal/mol.[24] Models indicate that interconversion between the cis′ and trans′ forms of the Pro-Gly units may be sterically difficult (due to transannular contacts) and might be expected to be "slow" on the nmr time scale. The foregoing data lead to the suggestion that the two Pro-Gly units differ by the orientation of the amide linkage joining the Pro and Gly residues (*i.e.*, by rotations about the Pro ψ and succeeding Gly ϕ angles). The nmr data are in accord with such an asymmetric conformation of *cyclo*(L-Pro-Gly)$_2$, containing the cis–trans–cis–trans peptide bond backbone,[21-23,25] but having one cis′ and one trans′ Pro C_α–C=O bond[26] (as shown schematically in Figure 3).

Preliminary nmr studies on two other cyclic tetrapeptides synthesized in this laboratory—*cyclo*(D-Pro-Gly-L-Pro-Gly) (see ref 1) and *cyclo*(L-Pro-Sar)$_2$—indicate no coalescence behavior in nmr spectra obtained under solvent and temperature conditions similar to those used for *cyclo*(L-Pro-Gly)$_2$. The ^{13}C chemical shift of the Pro C_γ resonance of each of the two latter peptides is in the region ascribed to cis X-Pro peptide bonds, providing evidence that the cis–trans–cis–trans peptide backbone again prevails in these molecules.

The results obtained herein for *cyclo*(L-Pro-Gly)$_2$ suggest that, in cases where comparable steric hindrance is absent (*e.g.*, in larger cyclic peptides and in linear peptides), cis′–trans′ interconversion is likely to be considerably faster.

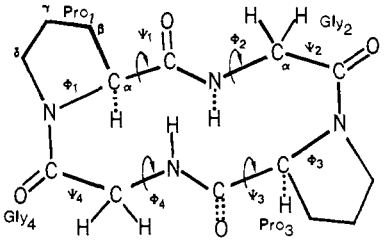

Figure 3. A diagrammatic representation of the asymmetric conformation proposed for *cyclo*(L-Pro-Gly)$_2$ in solution. The dotted bonds extend below the plane of the paper. Gly_4-Pro_1 and Gly_2-Pro_3 peptide bonds are cis; Pro_1-Gly_2 and Pro_3-Gly_4 peptide bonds are trans. The Pro_1 ψ_1 angle is trans′, and the Pro_3 ψ_3 angle is cis′. Interconversion with the identical conformer but with ψ_1 cis′ and ψ_3 trans′ occurs by rotation of the amide unit joining Pro_1 and Gly_2 (about the bonds denoted by arrows), with the N–H proton passing through the interior of the cyclic peptide ring and a similar rotation of the Pro_3—Gly_4 amide unit.

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Acknowledgments. This work has been supported, in part, by U.S. Public Health Service Grants AM07300 and AM10794. We thank the National Science Foundation (under Grant GB-41535) for providing major support for a ^{13}C spectrometer, the Muscular Dystrophy Association of America for a fellowship (to E. T. F.), and the Department of Chemistry for the use of the Varian XL-100-15 nmr instrument (provided by the National Science Foundation under Grant GP-32317). We are grateful to Dr. Vincent Madison for several stimulating discussions.

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Synthesis, Structure, and Bonding of a Cubane-Like Cobalt–Nitrosyl Complex, $Co_4(NO)_4(\mu_3\text{-}NC(CH_3)_3)_4$. Stereochemical Nonconformity of the Metal Cluster Geometry to That Predicted by a First-Order Jahn–Teller Effect

Sir:

A concentrated synthetic and stereochemical investigation of cubane-like metal clusters for the purpose of correlating the detailed changes in geometry with different MO-electronic configurations[1] has resulted in the preparation and characterization of $Co_4(NO)_4(\mu_3\text{-}NR)_4$ (where R = $C(CH_3)_3$). This metal nitrosyl tetramer is of prime interest not only in being the first characterized cubane-like molecule in which the metal atoms are each coordinated to a nitrosyl group as a single terminal ligand but also in representing the first such case for a metal cluster system in which its presumed idealized geometry does not conform to that expected from considerations of the first-order Jahn–Teller effect.

The successful synthesis of a metal cluster $M_4(NO)_4(\mu_3\text{-}X)_4$ system was accomplished by the reaction of $Co(NO)(CO)_2P(C_6H_5)_3$ with excess $\{(CH_3)_3CN\}_2S$ in refluxing toluene which produced a very soluble brown crystalline complex isolated in small yields (5–10%) from other products. Elemental analysis together with a parent ion peak at *m/e* 640 in its mass spectrum provided the initial evidence for the tetrameric nature of $Co_4(NO)_4(\mu_3\text{-}NC(CH_3)_3)_4$. A solid-state infrared spectrum (KBr pellet) exhibits a single strong nitrosyl band at 1722 cm^{-1} in accord with one terminal nitrosyl

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