to suppose that the shortening of the phosphoryl bond stems from the relatively high degree of π bonding obliged to concentrate in this link owing to the geometrical restrictions to P—O π bonding imposed by the oxygen "hinge effect" and the strain-lengthened P—O bond.

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Supplementary Material Available: A listing of structure factor amplitudes (5 pages). Ordering information is given on any current masthead page.

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Structure and Conformation of Cyclo(tri-L-prolyl) in the Crystalline State

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Abstract: Cyclo(tri-L-prolyl), $C_{15}H_{21}O_3N_3$, is made up of three prolyl residues joined by cis peptide bonds. A single-crystal x-ray diffraction study of the compound has been done. The crystals are orthorhombic, space group $P2_12_12_1$ with a = 15.942 (5), b = 19.097 (6), and c = 9.230 (3) Å and Z = 8. Intensity data were collected on a diffractometer using Mo x-radiation, and absorption corrections were made. The final R value for the 3167 data was 4.8%. In the peptides for the two molecules in the asymmetric unit, the N and its substituents show deviations from planarity, and the bond angles about N are different from those found in other prolyl residues. Two of the six prolyl rings have a C_α-envelope conformation, and the other four rings a C_α-C_β twist conformation. The conformations are closely related, and the barriers between them in solution must be small in order to explain the equivalence of the three C_α-protons in the proton magnetic resonance spectrum of cyclo(tri-L-prolyl). One of the H_α-C_α-C_β-H_β torsion angles is 90° in all six prolyl rings, but the other torsion angles show considerable variation. The packing involves several close oxygen to methylene carbon contacts.

The role of structural determinants such as amino acid sequence and chemical environment in overall peptide or protein conformation is a continuing interest of both theoreticians and experimentalists.[2,3] Proline residues in proteins and oligopeptides impart particular restraints on the conformational freedom of nearby peptide units, and, in turn, the degree of conformational flexibility of the proline residue is related to the overall polypeptide conformation. Cyclic peptides containing proline residues are of particular structural interest because the cyclization usually imposes additional conformational constraints.[4,5] There have been a number of crystallographic investigations of proline and proline-containing molecules,[6-19] and structural information from these studies and from NMR studies[5,20-25] has been related to prolyl ring conformation and used in conformational energy analyses.[4] Early model building studies of proline-containing peptides treated the pyrrolidine ring as either planar or puckered, but in all cases rigidity of the ring was assumed. Current experimental and theoretical evidence suggests that there is variability in the puckering of the prolyl ring, and that flexibility of the ring must be considered along with the effects of ring geometry on the backbone conformation.

Cyclo(tri-L-prolyl), which was first synthesized by Rothe et al.,[26] is unusual among peptides in its restricted conforma-

Table I. Crystal Data for Cyclo(tri-L-prolyl)

Crystal system: orthorhombic
Space group: $P2_12_12_1$
a = 15.942 ± 0.005 Å
b = 19.097 ± 0.006 Å
c = 9.230 ± 0.003 Å
Z = 8, 2 molecules/asymmetric unit
λ 0.71069 Å (Mo Kα)

tion. The molecule consists of three prolyl residues joined by cis peptide bonds. Proton magnetic resonance (^{1}H NMR) studies of cyclo(tri-L-prolyl)[20] show that the three α protons are equivalent, indicating either threefold symmetry or a rapid equilibrium among various conformers. In crystals, both cyclo(L-prolyl-L-prolyl-L-hydroxyprolyl) and cyclo(tri-L-prolyl) show considerable conformational variation,[12,14] suggesting less rigidity than anticipated for the prolyl rings. Crystals of cyclo(tri-L-prolyl) contain eight molecules in the orthorhombic unit cell, with two in the asymmetric unit. In order to examine six prolyl residues in the same crystal we undertook a detailed crystal and molecular structure analysis of this compound.

Experimental Section

Cyclo(tri-L-prolyl) was synthesized by Rothe et al.,[26] and crystals were prepared by slowly cooling a saturated aqueous solution. The crystal data are given in Table I. A crystal of dimensions 0.4 × 0.4 × 0.7 mm was used to collect x-ray diffraction data to $2\theta = 55°$ using monochromated molybdenum radiation and a Picker FACS-I diffractometer. The cell dimensions in Table I were derived by least-squares fit to the $\pm 2\theta$ values for 17 reflections. Two octants of data were collected using θ-2θ scans (2°/min) with 20-s background measurements at each end of the scan. Three reference peaks were monitored every 100 reflections, and these intensities remained constant (±4%). The data were corrected for absorption on the basis of a normalized plot of scan count vs. ϕ for two (00l) reflections (χ = 90°) and the equations of North et al.[27] The maximum correction was approximately 10%. After correcting for Lorentz and polarization effects, the symmetry related reflections were averaged; the average deviation from the mean of equivalent reflections was below 4%. The final observational data consisted of 3167 intensities above 2σ where σ was the standard deviation derived from counting statistics. Calculations were done on the IBM 370/168 system at the University of Chicago using programs cited in earlier publications,[28,29] and the pseudorotation program of Cremer and Pople[30] which Dr. R. F. Stewart kindly supplied. Scattering factors were taken from the International Tables.[31]

Table II. Positional and Thermal Parameters (×10^4) and Their Estimated Standard Deviations[a]

Atom	x/a	y/b	z/c	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
				A Molecule					
C(1)	5034 (2)	2925 (1)	7563 (3)	357 (12)	295 (11)	260 (11)	−26 (9)	28 (10)	0 (9)
O(1)	5167 (1)	3541 (1)	7284 (2)	478 (12)	319 (9)	557 (12)	−63 (8)	25 (10)	97 (9)
C(2)	5757 (2)	2429 (1)	7967 (3)	296 (12)	342 (11)	275 (11)	−40 (9)	−22 (10)	11 (10)
C(3)	6490 (2)	2830 (2)	8680 (3)	416 (14)	565 (17)	397 (15)	−139 (14)	−91 (13)	−39 (13)
C(4)	7020 (2)	3090 (2)	7421 (4)	404 (15)	598 (18)	679 (22)	−189 (14)	40 (16)	−78 (18)
C(5)	6857 (2)	2599 (2)	6166 (3)	375 (14)	581 (18)	469 (16)	−171 (12)	62 (13)	8 (14)
N(6)	6148 (1)	2149 (1)	6643 (2)	292 (10)	408 (11)	350 (11)	−48 (9)	65 (9)	−11 (10)
C(7)	6013 (2)	1535 (1)	5974 (3)	266 (10)	344 (11)	341 (12)	59 (9)	12 (10)	16 (10)
O(7)	6390 (1)	1382 (1)	4861 (2)	463 (10)	507 (11)	443 (11)	33 (9)	150 (10)	−85 (10)
C(8)	5359 (2)	1032 (1)	6604 (3)	320 (12)	318 (11)	335 (13)	55 (9)	36 (10)	−8 (10)
C(9)	5554 (2)	268 (2)	6201 (4)	559 (18)	345 (13)	592 (19)	82 (12)	164 (16)	−16 (13)
C(10)	5125 (3)	151 (2)	4746 (5)	997 (30)	490 (18)	660 (24)	104 (20)	41 (23)	−263 (18)
C(11)	4485 (2)	728 (2)	4556 (4)	563 (19)	632 (18)	466 (17)	−16 (15)	−15 (15)	−236 (16)
N(12)	4547 (1)	1151 (1)	5892 (2)	339 (10)	410 (11)	347 (11)	−16 (9)	−20 (9)	−86 (10)
C(13)	3901 (2)	1555 (1)	6321 (3)	331 (12)	339 (11)	375 (13)	9 (11)	−35 (11)	5 (11)
O(13)	3272 (1)	1615 (1)	5569 (3)	464 (12)	782 (17)	612 (14)	170 (11)	−227 (11)	−201 (13)
C(14)	3966 (1)	1937 (1)	7773 (3)	274 (10)	252 (9)	335 (12)	−18 (9)	35 (10)	29 (9)
C(15)	3102 (2)	2050 (1)	8472 (3)	302 (12)	358 (13)	486 (15)	1 (9)	70 (12)	29 (12)
C(16)	2775 (2)	2741 (2)	7836 (4)	331 (13)	451 (15)	634 (19)	109 (12)	110 (13)	92 (14)
C(17)	3526 (2)	3119 (1)	7214 (4)	371 (13)	326 (13)	622 (19)	72 (11)	18 (13)	102 (13)
N(18)	4251 (1)	2662 (1)	7513 (2)	288 (9)	255 (9)	416 (12)	4 (8)	13 (10)	52 (9)
				B Molecule					
C(1)	−101 (2)	4625 (1)	9607 (3)	329 (12)	277 (11)	248 (11)	−1 (9)	−18 (10)	13 (9)
O(1)	−358 (1)	4025 (1)	9509 (3)	455 (10)	262 (7)	688 (14)	−72 (8)	102 (10)	−56 (9)
C(2)	−697 (2)	5222 (1)	10044 (3)	292 (10)	238 (9)	286 (11)	−13 (9)	20 (10)	21 (9)
C(3)	−1480 (2)	4931 (1)	10806 (3)	350 (12)	386 (13)	459 (15)	−51 (11)	121 (12)	−3 (13)
C(4)	−2070 (2)	4729 (2)	9573 (5)	408 (15)	806 (24)	727 (23)	−228 (17)	−63 (17)	129 (21)
C(5)	−1858 (2)	5212 (2)	8340 (4)	309 (13)	606 (18)	553 (18)	−77 (12)	−83 (13)	−24 (16)
N(6)	−1051 (1)	5548 (1)	8736 (2)	277 (9)	344 (9)	344 (10)	13 (8)	−46 (9)	39 (9)
C(7)	−791 (2)	6116 (1)	8002 (3)	302 (12)	417 (13)	361 (13)	82 (11)	30 (10)	94 (11)
O(7)	−1157 (1)	6308 (1)	6914 (3)	497 (13)	870 (17)	545 (13)	−23 (12)	−139 (11)	358 (13)
C(8)	−35 (2)	6526 (1)	8583 (3)	369 (13)	269 (11)	323 (12)	54 (9)	57 (10)	43 (10)
C(9)	−91 (2)	7303 (1)	8211 (4)	675 (19)	249 (11)	543 (18)	82 (12)	115 (16)	83 (13)
C(10)	307 (3)	7365 (2)	6713 (4)	772 (24)	461 (17)	658 (22)	126 (17)	242 (19)	288 (17)
C(11)	907 (2)	6764 (2)	6568 (3)	629 (19)	442 (15)	396 (15)	17 (14)	132 (14)	146 (13)
N(2)	736 (1)	6312 (1)	7829 (2)	354 (10)	306 (9)	316 (10)	−5 (8)	84 (9)	52 (9)
C(13)	1320 (2)	5850 (1)	8266 (3)	339 (12)	304 (11)	342 (12)	−1 (9)	65 (10)	−2 (10)
O(13)	1969 (1)	5770 (1)	7576 (3)	524 (13)	703 (15)	614 (14)	220 (12)	299 (12)	205 (13)
C(14)	1166 (1)	5429 (1)	9656 (3)	265 (10)	289 (9)	288 (11)	−11 (9)	11 (10)	−18 (9)
C(15)	1995 (2)	5150 (1)	10276 (3)	290 (12)	446 (15)	411 (14)	32 (11)	−54 (11)	−16 (13)
C(16)	2161 (2)	4480 (2)	9428 (4)	339 (13)	493 (15)	536 (17)	138 (12)	−35 (13)	−26 (14)
C(17)	1299 (2)	4198 (1)	9023 (4)	386 (14)	311 (13)	581 (18)	94 (11)	39 (13)	−58 (13)
N(18)	709 (1)	4775 (1)	9335 (2)	302 (9)	233 (9)	348 (10)	27 (8)	14 (13)	−23 (8)

[a] Thermal parameters are of the form: $\exp(-2\pi^2 \Sigma_i \Sigma_j U_{ij} a_i^* a_j^*)$.

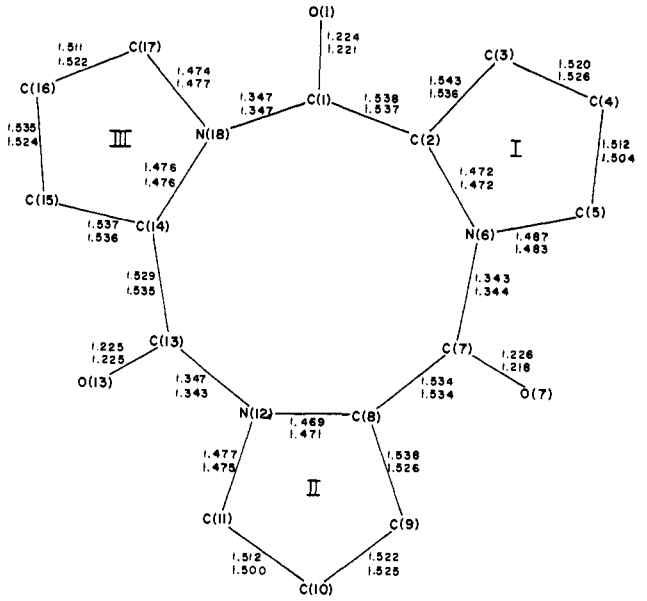

Figure 1. Bond distances (in Å) for cyclo(tri-L-prolyl). The top figures are for the A molecule and the bottom for the B molecule.

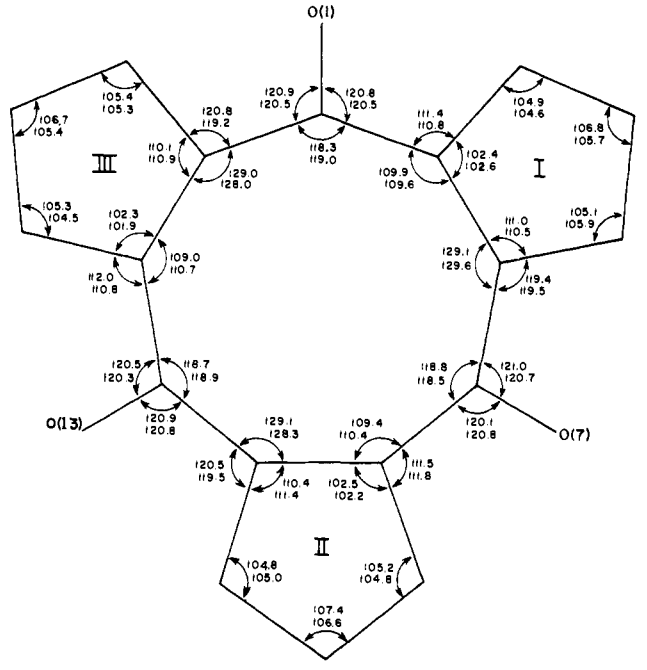

Figure 2. Bond angles (deg) for the A molecule (top) and B molecule of cyclo(tri-L-prolyl).

Solution and Refinement. The crystal structure of cyclo(tri-L-prolyl) was solved and partially refined by Kartha and Ambady using a set of data collected manually with Cu Kα radiation. The solution and refinement of the structure have been described.[12] The diffraction data were of poor quality, and the final agreement index was 13%. The significantly higher quality of the data we measured enabled us to refine the structure further, and to obtain a more accurate model for structural analysis. Refinement was by means of the least-squares procedure beginning with the positional parameters of Kartha et al.[12] The weighting system used was $\sigma_h = 3.8 + 0.038\ |F_o|$ where σ_h is $(1/w_h)^{1/2}$, and $h(= h, k, l)$ refers to an individual observation; the constants were derived from a plot of $\langle \Sigma(|F_o| - |F_c|)^2 \rangle^{1/2}$ vs. $\langle |F_o| \rangle$. Positional parameters for the 42 hydrogen atoms were assigned on the basis of small peaks (0.3-0.5 e/Å^3) in a difference Fourier synthesis, and these positional parameters were also refined. The final R value was 4.8%, where $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$, and the weighted R was 5.3%.

Table III. Positional Parameters (×10^3) for Hydrogen Atoms [a]

	x/a	y/b	z/c
A molecule			
H-C(2)	557	204	862
H1-C(3)	633	323	932
H2-C(3)	682	246	930
H1-C(4)	764	317	759
H2-C(4)	682	361	710
H1-C(5)	672	288	525
H2-C(5)	732	229	599
H-C(8)	530	111	770
H1-C(9)	525	−6	685
H2-C(9)	610	23	616
H1-C(10)	550	16	392
H2-C(10)	482	−32	475
H1-C(11)	391	56	446
H2-C(11)	460	104	368
H-C(14)	436	167	845
H1-C(15)	319	208	965
H2-C(15)	275	163	824
H1-C(16)	235	259	705
H2-C(16)	249	298	863
H1-C(17)	360	358	762
H2-C(17)	347	317	615
B molecule			
H-C(2)	−44	558	1067
H1-C(3)	−169	526	1147
H2-C(3)	−138	450	1142
H1-C(4)	−268	473	979
H2-C(4)	−193	417	919
H1-C(5)	−227	557	812
H2-C(5)	−182	491	746
H-C(8)	−1	645	955
H1-C(9)	27	758	883
H2-C(9)	−66	746	819
H1-C(10)	−12	733	593
H2-C(10)	60	785	654
H1-C(11)	151	691	666
H2-C(11)	85	648	559
H-C(14)	84	572	1039
H1-C(15)	192	505	1125
H2-C(15)	248	551	1006
H1-C(16	247	412	1002
H2-C(16)	252	459	850
H1-C(17)	114	378	972
H2-C(17)	125	405	798

[a] The hydrogens were given an isotropic B of 5 Å^2.

Results

The positional and thermal parameters for the 42 nonhydrogen atoms are given in Table II. The mean shift in atomic position from the initial coordinates is 0.03 Å, with a maximum shift of 0.07 Å. The estimated standard deviations of the C, N, or O positions are 0.004 Å, well above the maximum final least-squares shift (0.002 Å). The bond lengths not involving hydrogen should thus be accurate to ±0.01 Å or better, and the bond angles to ±0.3°. The bond distances and bond angles within the two independent molecules are given in Figures 1 and 2. The bond lengths are close to those expected;[32] the average C-C distance is 1.527 Å, the C′-N distance 1.345 Å, the C_α-N distance 1.476 Å, and the C′-O distance 1.223 Å. The bond lengths involving the γ-carbon atoms of the pyrrolidine rings appear short because of the significantly greater thermal motion of these atoms. Positional parameters for the hydrogen atoms are given in Table III. The mean C-H bond distance is 1.01 Å, with a range of 0.87-1.15 Å.

The C_α-C′-N angles average 118.7°, and the O-C′-N angles 120.6°, as they do in trans-prolyl residues.[33] Both of these angles differ significantly from the mean values of 116° and 123.5° observed for peptides other than proline.[32] The

Table IV. Dihedral Angles[a] (deg) for the Peptide Backbone

Angle	Ring	Molecule A	Molecule B
ϕ	I	−97.2	−94.8
	II	−94.9	−97.6
	III	−95.1	−106.0
ψ	I	94.7	87.3
	II	93.6	97.4
	III	96.8	88.7
ω	I	−1.2	12.5
	II	−2.7	5.7
	III	0.9	−2.5
θ''	I	91.4	92.2
	II	87.2	92.6
	III	87.0	90.0
$(\theta''-\phi)^b$	I	188.6	187.0
	II	182.1	190.2
	III	182.1	196.0

[a] ϕ, C′-N-C_α-C′; ψ, N-C_α-C′-N; ω, C_α-C′-N-C_α; θ'', C_δ-N-C_α-C′. Positive angles correspond to clockwise rotation of the far atom about the central bond. [b] $\theta''-\phi$ is 180° for a planar N environment.

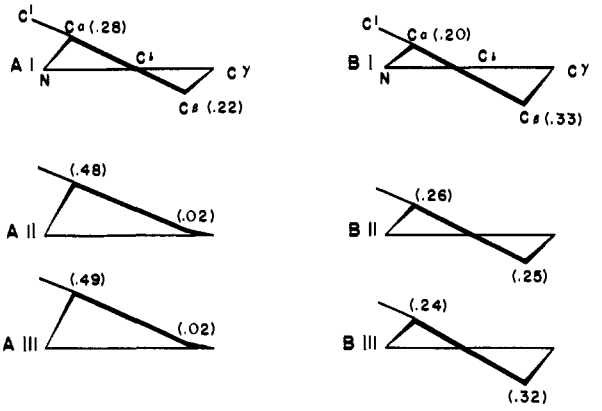

Figure 3. Projections of the prolyl rings along the C_γ-C_δ-N plane. Displacements from the plane (in Å) of C_α and C_β are given.

angles within the pyrrolidine rings are similar to those found by Kartha and Ambady in the cyclo(L-prolyl-L-prolyl-L-hydroxyprolyl) crystal structure,[13] and, except for the angle at the N, fall within the average range for trans-prolyl residues.[33] The C_α-N-C_δ angles average 110.9° in cyclo(tri-L-prolyl) and 110.3° in the hydroxyprolyl analogue, while the C′-N-C_δ angles average 119.8° in both structures. For trans-prolyl residues the mean values[33] of these angles are 113° and 126°. The cyclic peptide backbone in cyclo(tri-L-prolyl) is a nine-membered ring; the mean ring angles at the N are 128.8°, at C_α 109.8°, and at C′ 118.6°. The expected values for linear peptides[32] are 122°, 111°, and 116°, respectively; again the maximum angle deviation is at the N atom in the

Table V. Torsion Angles[a] (deg) for the Pyrrolidine Rings

Angle	Ring	Molecule A	Molecule B
χ_1	I	31.3	32.6
	II	29.0	31.3
	III	29.6	34.6
χ_2	I	−25.2	−28.5
	II	−17.6	−25.7
	III	−18.0	−29.5
χ_3	I	8.9	13.0
	II	−0.9	9.7
	III	−0.9	12.6
χ_4	I	11.9	8.2
	II	20.5	10.9
	III	20.9	10.0

[a] χ_1, N-C_α-C_β-C_γ; χ_2, C_α-C_β-C_γ-C_δ; χ_3, C_β-C_γ-C_δ-N; χ_4, C_γ-C_δ-N-C_α.

cyclic tripeptide. These angles are constrained both by the geometrical requirements for linking three nearly planar peptides and by the intramolecular H_α-H_α contact distances, which should be above 1.9 Å (ref 2). The H_α-H_α distances are 2.05 ± 0.05 Å, somewhat less than those observed in the hydroxyprolyl analogue[13] (2.21-2.24 Å) but well above the minimum. The torsion angles for the peptide groups are given in Table IV. The dihedral angles θ'' and φ involve N-atom substituents, and when the N and its substituents are coplanar, $\theta''-\phi$ will be 180°. None of the nitrogens in this structure is in a strictly planar environment, and the deviations from the planes of the three bound atoms are up to 0.11 Å. Distortions of the peptide units from planarity are indicated by the degree to which ω deviates from 0°, the value for a planar cis peptide. The maximum ω, 12.5°, is observed for residue BI; this corresponds to a mean deviation from the best plane of ±0.05 Å. The ϕ and ψ values in Table IV fall within the low energy region of a Ramachandran plot, and are close to a minimum region.[2] The torsion angles within the pyrrolidine rings are given in Table V. The χ_1 values average 31.4°, and show little variation in the six prolyl rings, but the other angles show considerable variation. Envelope and twist conformations involving C_β and C_γ of prolyl rings are often illustrated by projections along the C_α-N-C_δ plane.[33] The prolyl rings in cyclo(tri-L-prolyl) are in two conformations, an envelope conformation with C_α out of the plane for rings AII and AIII, and a C_α-C_β twist conformation for the other rings. These conformations are best illustrated by projections along the N-C_δ-C_γ plane, as shown in Figure 3 and by stereoscopic views such as that of the A molecule given in Figure 4. The conformations are closely related; if they are energetically discrete, the barriers between them in solution must be small in order to explain the equivalence of the three α protons in the ^{1}H NMR spectrum of cyclo(tri-L-prolyl).[20]

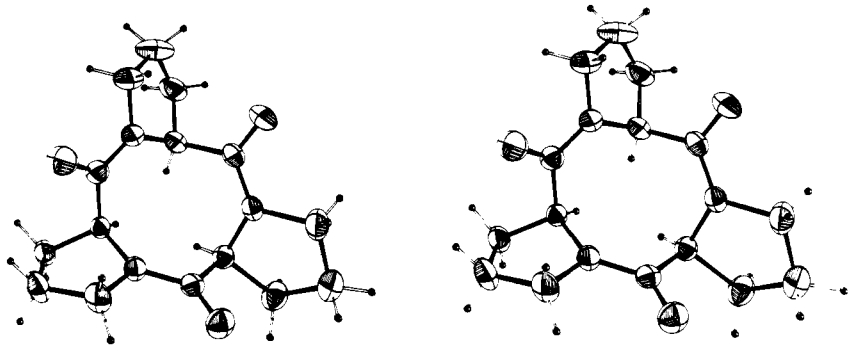

Figure 4. A stereoscopic view of the A molecule of cyclo(tri-L-prolyl) as found in the crystal.

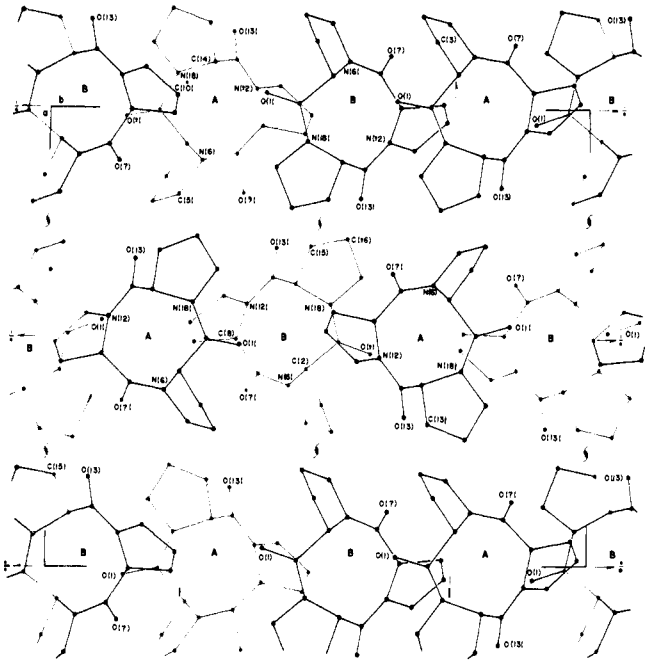

Figure 5. The crystal structure viewed down c. The independent A and B molecules are labeled.

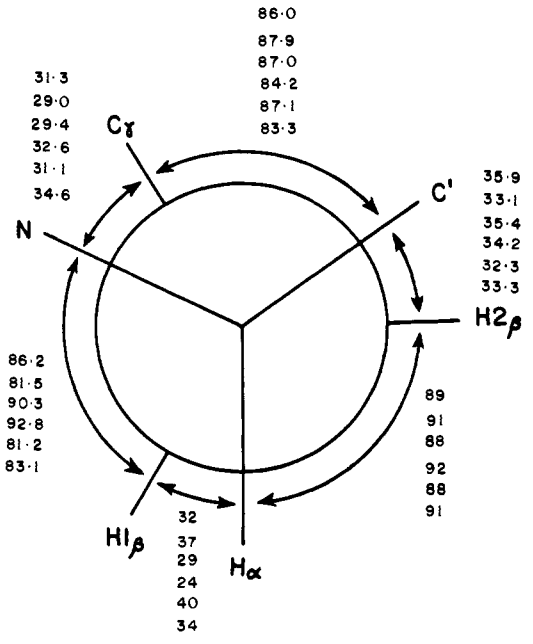

Figure 6. A Newman projection down the C_α-C_β bond. Estimated errors for dihedral angles involving hydrogens are ±4°.

There are no atoms capable of donating hydrogen bonds in the cyclo(tri-L-prolyl) molecule, and the question of what holds the crystal together is an important one, especially since the crystals are quite hard. A view of the crystal structure is given in Figure 5. The similarity in gross morphological shape of the two independent molecules is striking, especially since the AII and AIII rings have different conformations than the others. The maximum deviation on superimposing the two molecules is 0.3 Å. Presumably it is easier to pack similarly shaped molecules into a minimum energy structure. Intermolecular contact distances below 3.6 Å are given in Table VI. In all cases these are oxygen to methylene carbon contacts. The O-C distances are outside the range usually considered for O···H-C hydrogen bonds (3-3.1 Å),[34] but these are certainly polar contacts, and the collinearity of the O···H-C atoms shows an

Table VI. Intermolecular Contacts Below 3.6 Å[a]

O atom	C atom	Symmetry code[b]	d_{O-C}, Å	d_{O-H}, Å	∠O···H-C, deg
O(13)A	C(5)A	I	3.15	2.76	103
O(1)B	C(10)B	II	3.36	2.47	142
O(13)B	C(15)B	III	3.21	2.66	119
O(13)B	C(16)B	III	3.26	2.54	128
O(1)A	C(2)B	III	3.25	2.27	166
O(1)A	C(8)B	III	3.42	2.54	168
O(7)A	C(15)B	IV	3.49	2.46	168
O(1)B	C(14)A	V	3.29	2.35	153
O(7)B	C(3)A	III	3.45	2.56	147
O(7)B	C(15)A	VI	3.43	2.61	138

[a] All non-hydrogen atom contacts are between oxygen and carbon atoms; thus the oxygen to methylene hydrogen distance and the O···H-C angle are also given. [b] Symmetry code: I, $-\frac{1}{2}+x, \frac{1}{2}-y, 1-z$; II, $-x, -\frac{1}{2}+y, \frac{3}{2}-z$; III, $\frac{1}{2}-x, 1-y, -\frac{1}{2}+z$; IV, $1-x, -\frac{1}{2}+y, \frac{3}{2}-z$; V, $-\frac{1}{2}+x, \frac{1}{2}-y, 2-z$; VI, $-x, \frac{1}{2}+y, \frac{3}{2}-z$.

approximately linear correlation with the oxygen-hydrogen distance. Three of the O-H contacts, including the two shortest, involve H_α atoms. These hydrogens lie between the N and the carbonyl group, and are expected to be easily polarizable. Other contacts involve the hydrogens on C_β, C_γ, and C_δ, and are not as easy to rationalize. There are four symmetry-related contacts, one between A molecules and three between B molecules, and six contacts between independent A and B molecules.

Discussion

In predicting the structure of cyclo(tri-L-prolyl), Venkatachalam[35] found that three planar cis peptides could be cyclically linked but the resulting model structure contained close H_α-H_α contacts, and was not compatible with prolyl ring closure. Cyclo(tri-L-prolyl) is thus a molecule which must show geometrical distortions when compared to noncyclic peptides, and in terms of energy it is of interest to see where these distortions occur. Venkatachalam found that a satisfactory structure could be formed using an ω of about 25° to lengthen the H_α-H_α contact distances and permit closure of the prolyl rings;[35] the molecules in the crystalline state have acceptable contact distances with less distortion of the peptides from planarity. For the A molecule, the ω values (Table IV) range from −2.7° to 0.9°, and for the B molecule −2.5° to 12.5°. Deviations from planarity of the N and its substituents and changes in the bond angles about the N are also observed.

In the ^{1}H NMR spectrum of cyclo(tri-L-prolyl), the C_α proton resonances of all three prolyl residues overlapped to give a single doublet, while separate doublets were observed for each of the C_α protons in cyclo(L-prolyl-L-prolyl-L-hydroxyprolyl).[20] The occurrence of a single resonance peak for the three H_α protons in the case of cyclo(tri-L-prolyl) implies that these hydrogen atoms are equivalent and the molecule has threefold symmetry. The occurrence of a doublet suggests that one of the H_α-H_β coupling constants is near zero, and this was confirmed by spin-decoupling experiments.[20] A 90° H_α-C_α-C_β-H_β dihedral angle could account for a zero coupling constant. The χ_1 values in the pyrrolidine rings are all very similar (Table V), and the C_α-C_β projection (Figure 6) shows that the H_α-C_α-C_β-$H2_\beta$ angles are 90° (within error) for all six prolyl rings. Neither of the independent cyclo(tri-L-prolyl) molecules in the crystal has threefold symmetry; the NMR results can be explained if both the pyrrolidine rings and the peptide groups are sufficiently mobile in solution to give threefold symmetry on the NMR time scale. Proton magnetic resonance spectra at −60 °C show no detectable broadening of the H_α-doublet suggesting very low barriers to mobility.[36] Des-

Table VII. Puckering Parameters[a] for Prolyl Rings

Compound	Residue	q, Å	ϕ, deg	Ref
Cyclo(tri-L-prolyl)	AI	0.31 Å	52.3	—
	AII	0.30	34.7	—
	AIII	0.31	34.9	—
	BI	0.32	59.4	—
	BII	0.30	54.1	—
	BIII	0.34	57.5	—
Cyclo(L-Pro-L-Pro-L-3-Hyp)	I	0.33	65.3	(13)
	II	0.27	50.3	(13)
	III	0.32	347.0	(13)
Cyclo(Pro-Leu)	Pro	0.34	260.2	(11)
3-Hydroxyproline		0.33	292.2	(19)
Tosyl-L-Pro-L-3-Hyp	Pro[b]	0.33	122.2	(7)
	Hypro	0.34	299.6	(7)
L-Leu-L-Pro-Gly[c]	Pro[b]-1	0.30	87.7	(6)
	Pro[b]-2	0.21	314.8	(6)
p-BrZ-Gly-L-Pro-L-Leu-Gly	Pro[b]	0.19	269.6	(18)
L-Pro-L-Leu-Gly	Pro[b]	0.26	335.0	(8)
Na-antamanide	Pro-2	0.06	93.7	(9)
	Pro[b]-3	0.27	300.7	(9)
	Pro-7	0.07	276.6	(9)
	Pro[b]-8	0.32	295.6	(9)
Li-antamanide	Pro-2	0.15	96.0	(10)
	Pro-3[b]	0.20	59.4	(10)
	Pro-7	0.33	93.8	(10)
	Pro-8[b]	0.35	285.4	(10)
DL-Pro·HCl	Pro	0.34	41.5	(15)
S-Bz-L-Cys-L-Pro-L-Leu-Gly-NH_2	Pro-A	0.33	84.3	(17)
	Pro-B	0.34	95.0	(17)

[a] Calculated using Cremer's program, with q and φ from eq 1; IUPAC abbreviations are used. [b] A trans-prolyl residue. [c] The C_γ atom was disordered in this crystal structure; entries are for the two C_γ orientations observed. A similar disorder involving both C_β and C_γ was found in the crystal structure of *N*-acetyl-L-prolyl-L-lactylmethylamide.[16]

lauriers, Rothe, and Smith[37] determined the ^{13}C spin-lattice relaxation times (NT_1) of cyclo(tri-L-prolyl), and found that the relative atom mobilities were $C_\gamma > C_\beta \sim C_\delta > C_\alpha$. A test calculation by Somorjai and Deslauriers[38] has shown that these differences cannot be fully explained by a model involving only rigid body translation and rotation. Similar differences in spin-lattice relaxation times were observed for the prolyl atoms in a pentapeptide of glycine containing a central proline.[22] These relative mobilities correlate well with the mean-square displacements observed for the crystalline molecules (Table II), suggesting that the NT_1 values primarily reflect internal motion rather than overall rotation, and that the fixed conformations found in the crystal structure are representative of the range of possible conformations in solution. These observations, however, do not tell us about the mechanism for conformational change.

Five-membered rings are puckered in their lowest energy conformations, and the angle of maximum puckering can rotate around the ring, motion described as pseudorotation.[39] For cyclopentane the displacement of the jth carbon atom perpendicular to the unpuckered ring can be expressed as

$$z_j = (2/5)^{1/2} q \cos(\phi - 4\pi(j-1)/5) \quad (1)$$

where q is a puckering amplitude and ϕ a phase angle describing the various types of puckering. The lowest energy conformation for cyclopentane has a nonzero q, but the minimum is largely independent of ϕ.[39,40] Puckering parameters for a number of five-membered cis and trans prolyl rings are given in Table VII; these parameters were calculated using the method of Cremer and Pople,[30] which can be applied to heterocyclic ring systems without approximation. The pseudorotation angles are defined relative to a nitrogen envelope conformation, exo to C′, at 0°; the other envelope puckering conformations are at 36° intervals beginning with C_α-endo, the conformation shown in Figure 3 for rings AII and AIII of cyclo(tri-L-prolyl). Twist conformers occur midway between adjacent envelope conformations. Prolyl ring conformations tend to fall into two groups[4,33] with pseudorotation angles near 270 or 90°. The six pyrrolidine rings in cyclo(tri-L-prolyl) have similar conformations (Figure 3). The C_α-envelope conformations observed here and in DL-proline hydrochloride[15] have ϕ values about 20° below those for the C_α-C_β twist conformers, but the barrier to interconversion along a pseudorotation path seems likely to be small. The mechanism for interconversion between the major conformations is less clear. The occurrence of ϕ values of 335° for prolyl-leucyl-glycine and 347° for one of the pyrrolidine rings in the cyclo(prolyl-prolyl-hydroxyprolyl) crystal structure suggests that, if the major conformational change follows a pseudorotation path, it proceeds through 0° rather than 180°. Pseudorotation angles in different molecules can only be compared directly at constant q, however, and several of the entries in Table VII are well outside the usual puckering amplitude range. In particular the very low q values for some of the prolyl residues in Li- and Na-antamanide suggest that major conformational changes may proceed through a planar intermediate. Energetically, the restricted nature of the N and C_α environments might raise the barrier to a pseudorotation path above that for a planar intermediate.

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Supplementary Material Available: A listing of observed and calculated structure factors (33 pp). Ordering information is given on any current masthead page.

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Kinetics and Mechanism of Molybdate and Tungstate Complex Formation with Catechol Derivatives[1]

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Abstract: Rate constants for the complexation of molybdate and tungstate with catechol derivatives have been determined at 25 ± 1 °C and ionic strength 0.5 M (NH_4Cl) by the approach-to-equilibrium technique on a stopped-flow apparatus. Ligands studied were 1,2,4- and 1,2,3-trihydroxybenzene (pyrogallol), 3,4,5-trihydroxybenzoic acid (gallic acid), 3,4-dihydroxyphenylalanine (L-Dopa), and [3,4-dihydroxyphenyl]-2-methylaminoethanol (D-epinephrine). The formation of the mono (1:1) complex is more rapid for protonated than for unprotonated oxyanion. From the hydrogen ion dependence of the relaxation time it was determined that reactions of completely deprotonated ligand with completely deprotonated oxyanion, and completely protonated ligand with protonated oxyanion, do not contribute, within experimental error, to the observed rate of complexation. The relaxation times (standard deviations ±5%, except for pyrogallol and 1,2,4-trihydroxybenzene ±10%) consist of acid-independent and -dependent parts which contain kinetically indistinguishable terms for which upper limits could be deduced by setting all but one term equal to zero. Some of the upper limits exceed diffusion control allowing minimum limits to be set for the terms previously set equal to zero. For some pathways the upper and lower limits are approximately the same, leading to the actual value within experimental error and the uncertainties in the associated acid dissociation constants and estimated diffusion controlled rate constants. For molybdate and tungstate complexations with these and other ligands a trend in complex formation rate constant with basicity occurs. Namely, if the oxyanion is protonated the most basic ligand is most reactive. If the oxyanion is unprotonated, the least basic ligand is most reactive. The fastest rate of complex formation occurs when the protonated oxyanion reacts with the most basic ligand fully deprotonated at the binding sites. These trends are explained by assuming that the tetrahedral unprotonated oxyanion reacts by an addition mechanism, and the (postulated) octahedral protonated oxyanion reacts by a substitution mechanism. Ligand basicity then controls complex formation in substitution by assisting elimination of the OH^- groups to be replaced through a hydrogen-bond-transfer mechanism, but hinders addition through the same effect. For 1,2,4-trihydroxybenzene the kinetics of formation of mono and bis complexes has been determined at ionic strength 0.1 M. Unlike the formation of the mono complex, the formation rate of the bis complex decreases with decreasing pH. This effect is also explained by the fact that the reactive metal-containing species in the higher order complex formation step is already octahedrally coordinated. There is no conversion to octahedral form upon protonation, and the influence of ligand protonation dominates the process.

Metal-containing oxyanions readily form complexes with numerous different types of nucleophilic reagents.[2] In this regard, they resemble simple, aquated di- and trivalent metal ions. For the aquated metal ions, the mechanism of complexation is well understood, being based on one essential feature. Complex formation is a substitution process, controlled by the breaking of the metal-water bond in the formation of a reduced coordination-number activated complex.[3] Exceptions to this mechanism are few, and do not show wide variations in ligand substitution rate constants.[4] We now have several thorough kinetics studies of oxyanion-ligand systems: chromate,[5-7] vanadate,[8] molybdate,[9-12] and tungstate,[10,13] for example. It is thus appropriate to determine the context in which to view the mechanism of these reactions.

One ambiguity immediately presents itself, however, making it difficult to achieve a mechanistic synthesis similar to that for the simple aquated metal ions. The structures in solution of monomeric oxyanions are not clear. The unprotonated species (MoO_4^{2-}, WO_4^{2-}, etc.) are probably tetrahedral;[14] yet, addition of a proton to MoO_4^{2-} is not diffusion controlled,[15] implying that a structural change, perhaps tetrahedral to octahedral coordination, accompanies the reaction. Certainly, the known solid-state structures of molybdate and tungstate oxyanion complexes are octahedral,[16] with cis-dioxygen coordination in the absence of full occupation by the complexing ligand or ligands. This situation has led some investigators to regard oxyanion complexation and oxyanion polymerization (or condensation) as examples of addition rather than substitution.[9,17] This point, though interesting, is less fundamental than determining the trends, if any, in the complex formation rate constants with variations in ligand properties.

Ligand discrimination by oxyanions has been discussed for CrO_4^{2-},[7] where it may be present, but is obscured by accompanying catalytic behavior, and for MoO_4^{2-} and WO_4^{2-} with substituted 8-hydroxyquinolines.[10] With these ligands, it was established that the main pathways for complexation involve the protonated oxyanion. No conclusion was drawn with re-