Crystal Structure and Molecular Conformation of the Hydrated Cyclic Hexapeptide *cyclo*(L-Ala-L-Pro-D-Phe)$_2$

J. N. Brown* and R. G. Teller

Contribution from the Department of Chemistry, Illinois Institute of Technology, Chicago, Illinois 60616. *Received March 10, 1976*

Abstract: The crystal and molecular structure of the cyclic peptide *cyclo*(L-Ala-L-Pro-D-Phe)$_2$ hydrate has been determined by single-crystal x-ray diffraction analysis. The crystals are orthorhombic, space group $P2_12_12$ with a = 15.908 (1), b = 13.350 (1), and c = 9.643 (1) Å. Complete three-dimensional x-ray diffraction data were collected on a Picker diffractometer with copper radiation and refined to give residuals R = 0.13 and R_w = 0.08. The hexapeptide exhibits C_2 symmetry and has a conformation resembling that predicted by NMR data. Both proline residues are in the 2-positions of β turns, in which the expected strong 4–1 hydrogen bond is not found. The peptide is involved in an extensive network of intermolecular hydrogen bonding with water of crystallization and there are no peptide–peptide intermolecular hydrogen bonds.

With increasing frequency in recent years there have been reports in the literature of attempts to predict the conformational backbone angles of oligopeptides from proton magnetic resonance studies. Of particular interest has been the class of cyclic hexapeptides, since these molecules have a more limited number of possible configurations than do linear peptides or larger cyclic oligopeptides. There have been only a limited number of crystal studies on uncomplexed oligopeptides that have also been studied by nmr and minimum energy calculations. The cyclic hexaglycyl[1] was found to exist as four conformers, side-by-side, in the solid state and cyclic -Gly-Gly-D-Ala-D-Ala-Gly-Gly-[2] as a single conformer (analogous to one of the c-6-Gly conformers). In this latter case, the NMR study[3] indicated that in solution the molecule was freely interconverting between numerous conformers, none of which approximated the crystalline structure.

To try to build a more rigid molecule that might lend itself to a more definitive analysis, Kopple[4] and Blout[5] have synthesized cyclic hexapeptides with C_2 symmetry containing proline and have undertaken ^{1}H NMR studies to determine their conformations. Kopple has found that peptides of the type *cyclo*(L-X-L-Pro-D-Phe)$_2$, where X is Ala, Orn, or His, exist in two conformations with average C_2 symmetry and has proposed that the conformations are distinguished by *cis*- and *trans*-proline residues. In this paper we report the structure of the X-Ala molecule and generally confirm one of the conformations predicted by ^{1}H NMR.

Data Collection and Reduction

Numerous crystals of the title compound, grown from methanol–water solution, were examined for twinning and scattering efficiency. Nearly all crystals exhibited a larger falloff in intensity than is normal for reflections at high 2θ. Finally a crystal measuring 0.16 × 0.20 × 0.26 mm was found that had significant intensity at high angles. This crystal was mounted with the (001) axis coincident with the φ axis of a Picker diffractometer. Examination of the reciprocal lattice showed *mmm* symmetry with systematic extinctions $h = 2n + 1$ for (h00) and $k = 2n + 1$ for (0k0), uniquely characterizing the space group as $P2_12_12$. Lattice constants were determined by carefully measuring plus and minus 2θ values of the copper $K\alpha_1$–$K\alpha_2$ doublet for ten reflections with $2\theta > 60°$. The resultant parameters are a = 15.908 (1), b = 13.350 (1), and c = 9.643 (1) Å. Since the observed density is 1.26 ± 0.01 g/cm^3 (determined by flotation on several different crystals), the unit cell must contain a total weight of 1554 amu. Assuming two hexapeptides per unit cell (one-half per asymmetric unit), 631 amu each, and 16 waters of crystallization, the calculated density is 1.256 g/cm^3.

Two sets of intensity data ($\{A_1\}$ and $\{A_2\}$) were collected on a Picker FACS-I fully automated four-circle diffractometer using nickel filtered copper Kα radiation for the crystal described above and another complete data set ($\{A_3\}$) on a second crystal of inferior quality.[17] A θ–2θ scan rate of 2°/min with a variable scan width (2.0° + 0.3 tan θ) and 10-s background measurements at both extremities of the scan were used to measure 1861 independent reflections to a 2θ maximum of 125° (d = 0.87 Å) for each data set. Intensities of three standard reflections, monitored every 50 reflections, showed a systematic drop of approximately 5% during the collection of each data set. Absorption was corrected as a function of φ, crystal decay as a linear function of exposure time, and Lorentz polarization in the usual manner. The structure amplitudes and their estimated errors were calculated from the expressions $|F_o| = (QI_n)^{1/2}$ and $\sigma^2(F_o) = (Q/4I_n)[I_s + (t_s/t_b)^2 I_b + (0.02I_n)^2]$, where Q contains corrections for Lorentz polarization, absorption, decay, and attenuation, I_s and I_b are the scan and background intensities, t_s and t_b are the scan and background times, and I_n is the net integrated intensity. Reflections were considered observed if $|F_o| > 3\sigma(|F_o|)$. The agreement $(\Sigma w(|F_1| - K|F_2|)|/\Sigma w|F_1|)$ between the two sets of data collected on the same crystal ($\{A_1\}$ and $\{A_2\}$) is 8.4%, or 4.2% from the mean, and between the mean of these sets and that from the inferior crystal ($\{A_3\}$) is 12%. Because data set $\{A_1\}$ had a larger fraction of observed reflections (54%) than did data set $\{A_2\}$ (40%), only data set $\{A_1\}$, 1031 observed reflections, was used to solve and refine the structure.[18]

Since the crystals are air stable and do not lose water on standing, no attempt was made to seal a wet crystal in a capillary.

Structure Determination and Refinement

Phases for several of the normalized structure amplitudes, $|E|$'s, were initially determined by the symbolic addition procedure and later refined by the tangent formula, together with all reflections having $|E| > 1.5$. One of several E maps showed structural fragments which had reasonable bond distances and angles. Using the phase information from this partial structure in a recycling procedure, the remainder of the peptide nonhydrogen atoms were eventually located.

The entire peptide was refined (minimizing $\Sigma w(|F_o| - |F_c|)^2$) by five cycles of block-diagonal least squares[6] using $1/\sigma^2$ weights and isotropic temperature factors to R_w = 0.25 ($R_w = \Sigma w||F_o| - |F_c||/\Sigma w|F_o|$, $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$). A dif-

Table I. Fractional Atomic Coordinates, Temperature Factors, and Their Estimated Standard Deviations[a]

Atom	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
C(1)	−0.0819 (10)	0.3596 (12)	0.2840 (18)	39 (9)	21 (13)	140 (33)	−1 (19)	9 (36)	3 (38)
O(1)	−0.0698 (7)	0.4301 (9)	0.2008 (13)	26 (5)	74 (10	117 (19)	−5 (15)	14 (23)	40 (28)
N(1)	0.0693 (8)	0.3368 (10)	0.2787 (13)	31 (6)	37 (10)	60 (21)	−7 (17)	−20 (23)	2 (26)
$C(1)_\alpha$	−0.0095 (9)	0.3030 (13)	0.3562 (16)	5 (7)	84 (19)	80 (26)	5 (20)	−18 (27)	−12 (39)
$C(1)_\beta$	−0.0007 (12)	0.3390 (17)	0.5016 (18)	28 (8)	230 (30)	49 (24)	−10 (34)	6 (36)	33 (58)
C(2)	0.2239 (9)	0.5013 (14)	0.2944 (16)	31 (8)	74 (16)	52 (23)	−21 (23)	11 (29)	−30 (43)
O(2)	0.2257 (7)	0.4702 (9)	0.4175 (13)	63 (8)	58 (11)	156 (23)	−32 (17)	7 (27)	10 (28)
N(2)	0.1622 (7)	0.6653 (10)	0.3194 (14)	23 (6)	67 (12)	68 (21)	2 (17)	−5 (24)	33 (31)
$C(2)_\alpha$	0.2321 (10)	0.6091 (12)	0.2561 (17)	35 (9)	34 (14)	128 (34)	−7 (21)	7 (33)	−18 (36)
$C(2)_\beta$	0.3049 (9)	0.6628 (14)	0.3174 (20)	18 (8)	85 (18)	151 (34)	−8 (22)	−6 (32)	−14 (51)
$C(2)_\gamma$	0.2759 (10)	0.7698 (13)	0.3463 (20)	35 (9)	41 (14)	226 (42)	−6 (23)	−33 (38)	39 (47)
$C(2)_\delta$	0.1863 (10)	0.7541 (16)	0.3946 (17)	41 (10)	103 (18)	76 (29)	−29 (27)	−25 (29)	−21 (45)
C(3)	0.1356 (9)	0.2818 (12)	0.2789 (15)	41 (9)	47 (15)	36 (25)	4 (21)	−32 (28)	−20 (33)
O(3)	0.1379 (6)	0.1952 (8)	0.3198 (11)	28 (5)	87 (12)	65 (17)	3 (14)	−8 (20)	27 (25)
N(3)	0.2183 (7)	0.4298 (9)	0.1946 (14)	14 (6)	54 (11)	68 (19)	9 (15)	5 (24)	−1 (30)
$C(3)_\alpha$	0.2214 (9)	0.3225 (13)	0.2231 (16)	18 (7)	53 (14)	72 (26)	16 (20)	−5 (27)	0 (34)
$C(3)_\beta$	0.2499 (10)	0.2581 (15)	0.0973 (17)	26 (9)	79 (17)	99 (30)	2 (23)	0 (30)	52 (44)
$C(3)_\gamma$	0.3403 (10)	0.2870 (13)	0.0615 (17)	44 (11)	62 (17)	63 (28)	24 (23)	−29 (31)	−15 (35)
$C(3)_{\delta_1}$	0.4014 (10)	0.2329 (15)	0.1337 (18)	49 (11)	90 (19)	102 (32)	0 (28)	23 (33)	−17 (45)
$C(3)_{\delta_2}$	0.3637 (11)	0.3542 (13)	−0.0439 (18)	44 (11)	71 (19)	90 (31)	2 (25)	21 (33)	−11 (39)
$C(3)_{\epsilon_1}$	0.4866 (11)	0.2516 (17)	0.0991 (21)	25 (10)	109 (21)	316 (49)	19 (26)	−11 (41)	−142 (62)
$C(3)_{\epsilon_2}$	0.4454 (10)	0.3813 (15)	−0.0807 (23)	19 (10)	125 (24)	276 (49)	2 (27)	4 (38)	−19 (61)
$C(3)_z$	0.5030 (14)	0.3258 (16)	−0.0052 (23)	69 (13)	164 (28)	200 (40)	−23 (37)	45 (52)	−94 (62)
0X1[b]	0.0000 (0)	0.5000 (0)	0.9424 (20)	6.32 (57)					
0X2	0.1735 (7)	0.4668 (9)	0.9096 (1)	5.07 (35)					
0X3[c]	0.2786 (11)	0.0605 (13)	0.3312 (21)	3.29 (47)					
0X4[d]	0.2326 (17)	0.0182 (24)	0.3344 (32)	5.92 (88)					
0X5[e]	0.5000 (0)	0.5000 (0)	0.4310 (33)	17.81 (118)					

[a] Anisotropic thermal parameters × 10^4 and of the form $\exp[-(\beta_{11}h^2 + \beta_{22}k^2 + \beta_{33}l^2 + 2\beta_{12}hk + 2\beta_{13}hl + 2\beta_{23}kl)]$. [b] Occupancy is 0.5. [c] Occupancy is 0.58 ± 0.02. [d] Occupancy is 0.42 ± 0.02. [e] Occupancy is 0.50 ± 0.02.

ference electron density map indicated the positions of five unique water molecules. After several cycles of full matrix least-squares refinement, assuming anisotropic temperature factors for the peptide and isotropic temperature factors for the water molecules and in alternate cycles varying the weights and temperature factors of three of the water molecules, another electron density map was calculated to locate the hydrogen atoms. Since this map did not show positive electron density at all expected hydrogen positions, the coordinates of all hydrogen atoms except those bonded to the C_β methyl of alanine were calculated. Eight more cycles of refinement (peptide, anisotropic; water, isotropic and alternately varying weights and temperature factors of 0X3, 0X4, and 0X5) gave final values of $R_w = 0.08$ and $R = 0.13$. During this refinement, the sum of the occupancies for 0X3 and 0X4 were constrained to 1.0 and that for 0X5 to 0.5 or less. As can be seen in Table I, the temperature factor of 0X5 is ridiculously large. Numerous attempts were made to refine 0X5 with fixed weights or fixed temperature factors or both, in addition to various combinations of anisotropic and isotropic temperature factors. No one method proved any more successful than outlined above. The R_w value after five cycles of full matrix least squares excluding 0X5 was 0.132 and a difference electron density map had a peak of approximately 2 $e^-/Å^3$ at the 0X5 position. The Hamilton significance test clearly indicates that there is a very high probability that this atom should be included. A final difference map showed no density greater than 0.3 $e^-/Å^3$, indicating that any unaccounted mass in the unit cell must be disordered.

Results and Discussion

The fractional coordinates, anisotropic temperature factors, and estimated standard deviations for the nonhydrogen atoms are listed in Table I and the calculated hydrogen positions are given in Table II.

Table II. Calculated Hydrogen Coordinates

Atom	x	y	z
H(1)	0.068	0.403	0.227
$H(1)_\alpha$	−0.021	0.228	0.329
$H(2)_\alpha$	0.230	0.605	0.147
$H(2)_\beta$	0.357	0.663	0.255
$H(2)_{\beta'}$	0.322	0.630	0.408
$H(2)_\gamma$	0.279	0.814	0.260
$H(2)_{\gamma'}$	0.312	0.805	0.420
$H(2)_\delta$	0.183	0.748	0.499
$H(2)_{\delta'}$	0.150	0.816	0.369
H(3)	0.210	0.452	0.093
H(3)	0.262	0.320	0.304
$H(3)_\beta$	0.209	0.271	0.015
$H(3)_{\beta'}$	0.244	0.184	0.118
$H(3)_{\delta_1}$	0.384	0.183	0.211
$H(3)_{\delta_2}$	0.311	0.382	−0.104
$H(3)_{\epsilon_1}$	0.534	0.213	0.141
$H(3)_{\epsilon_2}$	0.462	0.437	−0.153
$H(3)_z$	0.565	0.337	−0.025

Figure 1 is a schematic drawing of the peptide with bond distances and bond angles indicated. The largest estimated standard deviations are less than 0.02 Å for distances, 1.5° for angles. As indicated, the proline residue has bond distances and angles generally consistent with several previous studies[7-9] except that the bond lengths are systematically shorter than the average observed value. The most notable differences are $C(2)_\alpha$–C(2) = 1.49 and $C(2)_\alpha$–$C(2)_\beta$ = 1.49 Å as compared to the expected 1.54 Å average for both. In both cases, these differences are only 0.01 Å greater than two estimated standard deviations and in all other cases the differences are no more than one estimated standard deviation from the expected value. As is commonly observed, $C(2)_\gamma$ is puckered out of the plane formed by $C(2)_\beta$–$C(2)_\alpha$–N(2)–$C(2)_\delta$, on the side op-

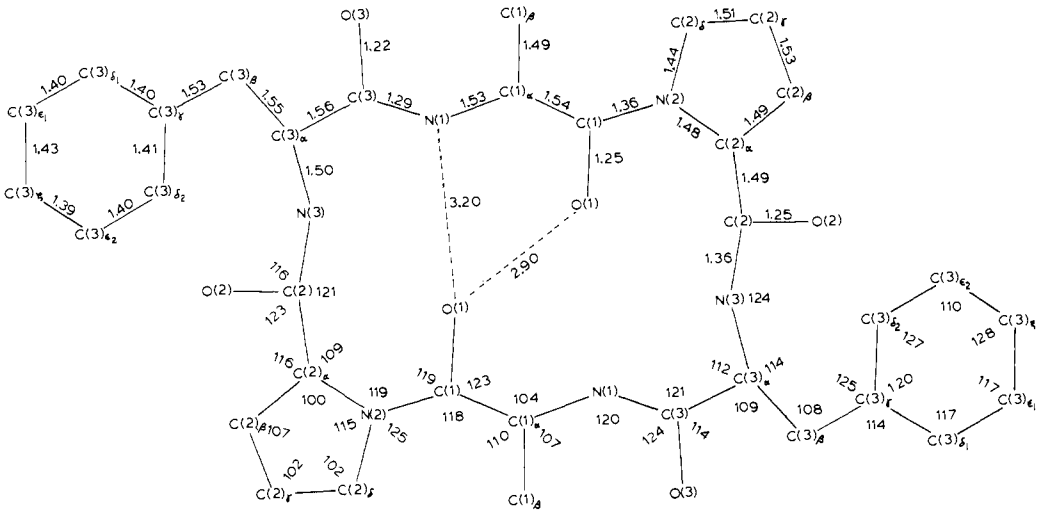

Figure 1. Bond distances and bond angles in the molecule *cyclo*(L-Ala-L-Pro-D-Phe)$_2$.

Table III. Backbone Conformational Angles[a] for *cyclo*(L-Ala-L-Pro-D-Phe)$_2$

	This crystal study			Kopple's NMR study[4]		
Residue	ϕ	ψ	ω	ϕ	ψ	ω
L-Ala	−157	172	178	−150	150	180
L-Pro	−60	122	171	−50	100	180
D-Phe	78	9	−169	150	−40	180

[a] Using conventions set forth by IUPAC-IUB Commission on Biochemical Nomenclature in *Biochemistry*, **9**, 3471 (1970).

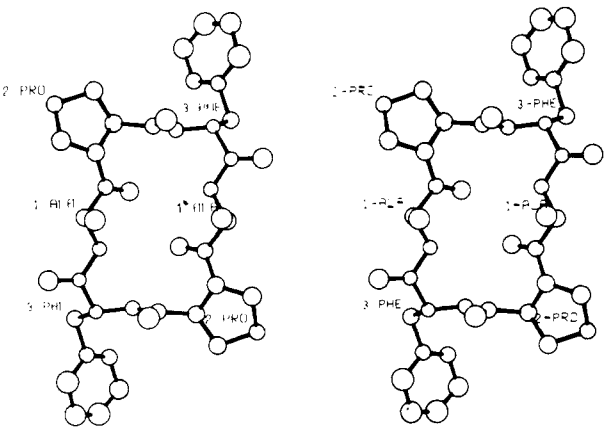

Figure 2. Stereoview of the molecule looking down the twofold axis.

posite the carbonyl oxygen O(2), by 0.53 Å. There appears to be no particular preference in previous studies of proline residues about which side of the plane C_γ lies. All bond angles within the proline ring are within two estimated standard deviations of accepted values.

The phenylalanine residue has internal aromatic bond distances and angles that are generally consistent with expected values. However, the temperature factors for these atoms are among the largest in the peptide and probably indicate a certain degree of twisting about the C_β–C_γ bond. Since the closest intermolecular contact for atoms within this ring is 3.4 Å, the ring is free to rotate several degrees about this bond. The ring is planar with a standard deviation of 0.02 Å. The C_β–C_γ bond is gauche with respect to the N–C_α bond with χ^1 torsional angle (about C_α–C_β) of 64°. The χ^2 angle, −86°, is close to ±90° as expected.

The backbone conformational angles are listed in Table III along with those predicted by Kopple[4] from ^{1}H NMR data. 1-L-Ala is in an extended conformation with $\phi_1 = -157$, $\psi_1 = 172$, and $\omega_1 = 178°$. These values are in agreement with Kopple's values of $\phi_1 = -150$, $\chi_1 = 150$, and $\omega_1 = 180°$. This ω_1 is the closest to 180° of all three residues and the amide plane $C(1)_\alpha$–C(1)–O(1)–N(2)–$C(2)_\alpha$ is the most planar of all three residues with a standard deviation of 0.01 Å. 2-L-Pro is the middle residue of a 4 → 1 LD type II β turn[10] and has conformational angles of $\phi_2 = -60$, $\psi_2 = 122$, and $\omega_2 = 171°$, again in agreement with the ^{1}H NMR values of $\phi_2 = -50$, $\psi_2 = 100$, and $\omega_2 = 180°$. As might be expected from the ω_2 value, the amide plane $C(2)_\alpha$–C(2)–O(2)–N(3)–$C(3)_\alpha$ shows significant distortion from planarity (±0.04 Å standard deviation). Similarly, the amide plane for 3-Phe $C(3)_\alpha$–C(3)–O(3)–N(1)–$C(1)_\alpha$ has a large standard deviation, ±0.06 Å, which is also a result of a large deviation of ω_3, −169°, from 180°.

The values of the conformation angles ϕ and ψ for L-Pro and D-Phe are consistent with previous structure determinations[11] containing this type of β turn. Turns with L,D or L,Gly substituents in positions 2 and 3, respectively, have ϕ_2 values that range from −57° for Ferrichrome A[12] to −67° for valinomycin[13] and ω_2 values from 128° for the linear peptide L-Pro-L-Leu-Gly-NH_2[14] to 140° for Ferrichrome A. ϕ_3 values have been observed from 72 to 96° and ψ_3 values from −1 to −8°.

The qualitative differences between the backbone conformation observed in this crystal stucture and that predicted for one of the two solution conformers by NMR are at 3-Phe. In the NMR report, ϕ_3 is given as near 150° rather than 78° as found in the crystal. Either angle corresponds to a H–N–C_α–H dihedral angle near 150°, which is indicated by the observed H–N–C_α–H spin–spin coupling constant. The choice of $\phi_3 = 150°$ was made in a model from the NMR data in order to avoid the short O(1)–O(1)′ separation between alanines, which was judged to be unfavorable but does, in fact, occur in the crystal. The NMR model was also constructed to avoid ψ_3 near 0°, which was at the time considered to be an unfavorable region of the dipeptide energy map (see recently suggested changes by G. N. Ramachandran[15]). However, the overall shape of the NMR model, including the position of the phenylalanine side chains, is similar to what we observe as shown in Figure 2.[16]

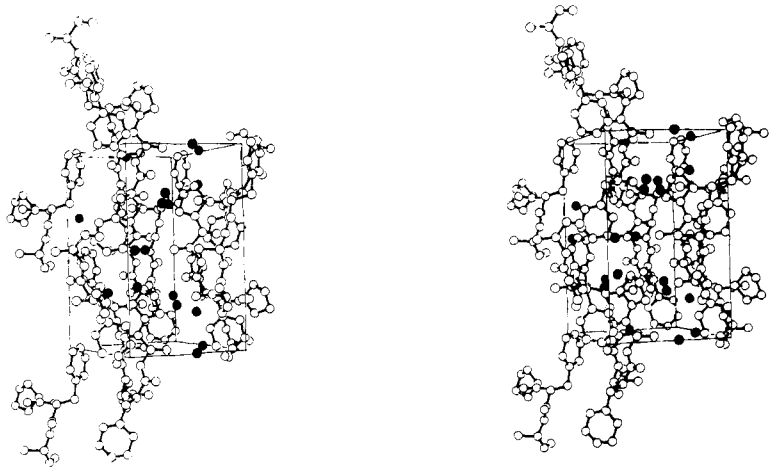

Figure 3. Unit cell contents stereoview. Water molecules have been darkened to emphasize their positions. The a axis is vertical (to the stereo frame of reference) and the twofold c axis is horizontal.

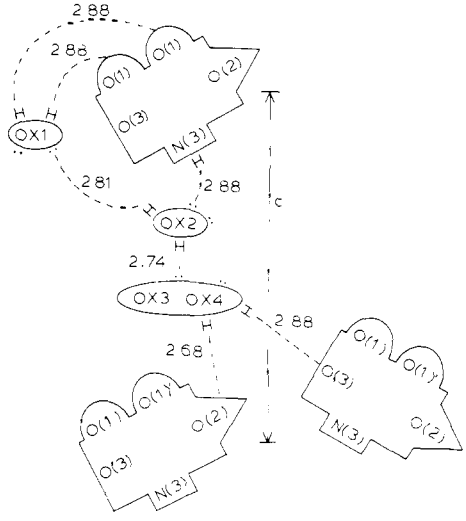

Figure 4. Schematic drawing of the intermolecular hydrogen bonding network. Distances listed about the 0X3:0X4 disordered pair are for the major hydration site, 0X3.

As mentioned in the experimental section, the unit cell must contain two hexapeptides and 16 solvent molecules to account for the observed density. We were able to locate 12 water molecules (per unit cell), based on occupancies applied to the scattering factors of the oxygens. Therefore, there are four water molecules unaccounted for per unit cell that must be disordered. Examination of the unit cell drawing, Figure 3, shows that the region about 0X5 is a rather large cavity of open space (the closest contact to 0X5 is $C(2)_\beta$ at a distance of 3.95 Å) and one can speculate that the remaining solvent should be found in this area. Since there are no hydrogen bonding atoms available, a disordered sea of solvent is reasonable.

Of all the a priori intramolecular peptide hydrogen bonds, there is only one candidate, O(1) · · · H(1)′-N(1)′, which need be considered seriously. The distance O(1)-N(1), 3.20 Å, and the angle, 132°, are at the limits of consideration and at best this interaction should be called an extremely weak hydrogen bond (since the hydrogen positions are calculated and not refined, angles involving hydrogen atoms may have large errors). This distance is 0.15–0.20 Å greater than observed in previous L,D or L,Gly type II β turns[11] and is somewhat at variance to the concept that a strong 4 → 1 hydrogen bond is a principal stabilization factor in a β turn. However, when one considers the overall cyclic nature of the molecule, the reason for a weaker bond is obvious. To make the distance O(1)-N(1)′ less than 3.0 Å and the angle O(1)-H(1)′-N(1)′ approximately 180° and maintain the backbone conformation as much as possible, one has to accept a O(1)-O(1)′ distance of 2.5 Å (assuming C_2 symmetry is preserved). Since this is ridiculously short, one has to conclude that a double L,D type II β turn does not allow a strong 4 → 1 hydrogen bond.

The peptide literally lies in a sea of solvent. There are no hydrogen bonds formed directly between peptide groups and, indeed, the shortest contact between peptides is 3.51 Å [O(2)-C(2) ($\frac{1}{2} - x, y - \frac{1}{2}, 1 - z$)]. However, all three unique carbonyl oxygens and the amide nitrogen of the phenylalanine residue are involved in an extensive intermolecular hydrogen bonding network with all the water molecules except 0X5. As illustrated in Figure 4, nitrogen N(3) is hydrogen bonded to 0X2 at a distance of 2.88 Å and an angle, N(3)-H · · · 0X2, of 165°. 0X1 lies on the twofold axis equidistant, 2.88 Å, from the symmetry related carbonyl oxygens, O(1) and O(1)′, of the alanine residues. The small angle O(1) · · · 0X1 · · · O(1)′, 60°, must result from a balance between the stabilization energy of the hydrogen bond and the repulsive forces of pushing the water further toward the peptide. To increase this angle to 105°, the resultant 0X1-O(1) distance would be 1.83 Å, an impossibility. 0X2 is also strongly hydrogen bonded to 0X1 at 2.81 Å and to the disordered pair 0X3:0X4 at 2.74 and 2.87 Å, respectively. The hydrogen bonding angles about 0X2 involving 0X1 (namely 99, 107, and 124° with N(3), 0X3, and 0X4, respectively) are within the expected range. The angles N(3)-0X2-0X3, 144°, and N(3)-0X2-0X4, 134°, are exceptionally large and lead one to speculate that the disorder observed for this pair and not for either 0X1 or 0X2 is the result of the weaker hydrogen bonding interactions. The 0X3:0X4 pair is in turn hydrogen bonded to two more peptide groups via carbonyl oxygens with distances 0X3-O(3) = 2.88, 0X3-O(2) = 2.68, 0X4-O(3) = 2.81, and 0X4-O(2) = 2.54 Å. The angles O(2)-0X3-O(3), 107°, and O(2)-0X4-O(3), 113°, are such that the water protons can be closely directed toward the carbonyl groups. It is difficult to imagine a hydrogen bond distance 0X4-H · · · O(2) of 1.54 Å. However, one must remember that 0X4 has an occupancy significantly less than that for 0X3 and is therefore the minor hydration site. The proton donating and accepting atoms in the hydrogen bonding scheme can be unambiguously assigned. Since water 0X1 must donate hydrogens to bonds with carbonyl oxygens O(1) and O(1)′, it must accept a hydrogen in its bond with 0X2. Likewise, the 0X3:0X4 pair must donate hydrogens to its bonds with O(2) and O(3) and therefore must also accept one from 0X2.

Acknowledgment. The authors wish to thank Professor K.

D. Kopple of our department for not only supplying the title compound, but also for providing useful comments about this work. We also appreciate the partial financial support of the Research Corporation, PHS-NIH-CA-17594, and the IIT Graduate School.

Supplementary Material Available: Listing of the structure amplitudes (10 pages). Ordering information is given on any current masthead page.

References and Notes

(1) I. L. Karle and J. Karle, *Acta Crystallogr.*, **16**, 969 (1963).
(2) I. L. Karle, J. W. Gibson, and J. Karle, *J. Am. Chem. Soc.*, **92**, 3755 (1970).
(3) A. E. Tonelli and A. I. Brewster, *J. Am. Chem. Soc.*, **94**, 2851 (1972).
(4) K. D. Kopple, T. J. Schamper, and A. Go, *J. Am. Chem. Soc.*, **96**, 2597 (1974).
(5) C. M. Deber, D. A. Torchia, S. C. K. Wong, and E. R. Blout, *Proc. Natl. Acad. Sci. U.S.A.*, **69**, 1825 (1972).
(6) Scattering factors for carbon, nitrogen, and oxygen are taken from the paper by D. Cromer and J. Waber, *Acta Crystallogr.*, **18**, 104 (1965), while that for hydrogen is from R. Stewart, E. Davidson, and W. Simpson, *J. Chem. Phys.*, **42**, 3175 (1965).
(7) G. Kartha and G. Ambady, *Acta Crystallogr., Sect. B*, **31**, 2035 (1975).
(8) A. D. Rudko and B. W. Low, *Acta Crystallogr., Sect. B*, **31**, 713 (1975).
(9) I. L. Karle, *J. Am. Chem. Soc.*, **96**, 4000 (1974).
(10) C. M. Venkatachalam, *Biopolymers*, **6**, 1425 (1968).
(11) R. Walter and J. Melenhofer, Eds., *Pept.: Chem., Struct., Biol., Proc. Am. Pept. Symp., 4th, 1975*, 68 (1975).
(12) A. Zalkin, J. D. Forrester and D. H. Templeton, *J. Am. Chem. Soc.*, **88**, 1810 (1966).
(13) I. L. Karle, *J. Am. Chem. Soc.*, **97**, 4379 (1975).
(14) T. Ueki, T. Ashida, M. Kakudo, Y. Sasade, and Y. Katsube, *Acta. Crystallogr., Sect. B*, **25**, 1840 (1969).
(15) G. N. Ramachandran in "Peptides, Polypeptides, and Proteins", E. R. Blout, F. A. Bovey, M. Goodman, and N. Latan, Ed., Wiley, New York, N.Y., 1974, pp 14–48.
(16) C. K. Johnson, ORTEP Report ORNL-3794, Oak Ridge National Laboratory, Oak Ridge, Tenn., 1965.
(17) The $\{A_3\}$ data set was actually collected first and discarded after six months because we were not able to solve the crystal structure. There was a one month time difference between data sets $\{A_1\}$ and $\{A_2\}$.
(18) This discussion about the various data sets is included to show the general degree of reliability of the intensity data.

The Novel Crystal and Molecular Structure of Bis[bis(2-pyridyl) disulfide]copper(I) Perchlorate

Mavis M. Kadooka, Larry G. Warner, and Karl Seff*

Contribution from the Chemistry Department, University of Hawaii, Honolulu, Hawaii 96822. *Received December 24, 1975*

Abstract: The structure of bis[bis(2-pyridyl) disulfide]copper(1) perchlorate, $[Cu(C_{10}H_8N_2S_2)_2]ClO_4$, has been determined by single-crystal x-ray diffraction techniques using counter methods and has been refined by full-matrix least-squares procedures to a final conventional R index of 0.051. The yellow crystals form as needles in the space group $P\bar{1}$ with a = 13.898, b = 21.867, c = 8.278 Å, α = 92.78, β = 100.79, γ = 84.37°, and Z = 4. The structure contains both isolated mononuclear complexed cations, CuL_2^+, and polynuclear cationic polymers, $Cu_nL_{2n}^{n+}$. In each case, Cu(1) is tetrahedrally coordinated by two ligands. One ligand coordinates through its two pyridyl nitrogen atoms to form a seven-membered chelate ring (SCNCuNCS) in the mononuclear species and a bridge between two Cu(1) ions in $Cu_nL_{2n}^{n+}$. The disulfide groups of this kind of ligand do not participate in coordination. The second ligand at each Cu(1) coordinates through one pyridyl nitrogen and the more distant sulfur atom, forming a five-membered chelate ring (CNCuSS); the remaining pyridine ring of this kind of ligand does not coordinate. Two independent times, then, the coordination geometry N_3S has occurred, which might be similar to that of Cu(1) at the ESR-inactive Cu site in ceruloplasmin and in the fungal laccases. Three of the S–S bonds in the four nonequivalent ligand molecules are approximately equal, averaging to 2.028 Å, somewhat longer than the 2.016 Å found in the structure of the uncoordinated ligand. The fourth S–S bond is significantly longer, 2.047 Å, and has occurred because the seven-membered chelate ring, of which it is a part, requires that the C–C–S–S and N–C–S–S torsion angles deviate widely from near 0 or 180°. The C–S–S–C torsion angles range within 9° of 90° and neither these angles nor coordination of sulfur to Cu(1) appears to affect the observed S–S bond lengths. Cu(1)–S distances average 2.42 Å in length, and Cu(1)–N averages to 2.024 Å. Coordination angles at Cu(1) range from 88.1 to 137.8°. Several times, whenever coordination requirements permit, a close intraligand C–H . . . S approach occurs (C . . . S = 3.2 Å), which could have been readily avoided, without changing the pattern of π interactions in the ligand, by a ring rotation of 180° about the C–S bond. This supports the existence of an energetically favorable C–H . . . S interaction. One sulfur atom of each uncoordinated disulfide group participates in a close charge-transfer interaction with the plane of a pyridine ring at distances of 3.31 and 3.37 Å, respectively. One or two water molecules are present per unit cell.

In order to elucidate the role of transition metal ions in the structure and function of some metalloenzymes and metalloproteins, attempts have been made to synthesize aliphatic disulfide complexes of transition metal ions and to determine their molecular structures. A more detailed introduction can be found in the reports of previous structures determined in this laboratory, particularly those of a Ni(II) complex,[1] chloro(bis{2-[(2-pyridylmethyl)amino]ethyl} disulfide)nickel(II) perchlorate, and of a Cu(I) complex,[2] *cyclo*-di-μ-{bis[2-(*N*,*N*-dimethylamino)ethyl] disulfide}dicopper(I) tetrafluoroborate, $[Cu(RSSR)]_2(BF_4)_2$. More recently, the crystal structure of another Cu(I) disulfide complex, {bis[2-(2-pyridyl)ethyl] disulfide}copper(I) perchlorate,[3] was determined. In both Cu(I) complexes, the Cu(I)–S coordination bonds are particularly short, 2.30 and 2.32 Å, respectively, and the corresponding S–S distances are long, approximately 2.08 Å in both structures; this is consistent with a π back-bonding scheme from Cu(I) to the disulfide group.[3]

A π interaction occurring between a metal ion and the disulfide group might be modified if the sulfur atoms participated in additional π interactions with the R groups in the R–S–S–R ligand itself. In the previous disulfide transition metal complexes which have been studied crystallographically, those of Ni(II),[1,4,5] Fe(III),[6] Cu(II) (the Cu–S distance is very long),[7] and Cu(I),[2,3,8] the carbon atoms bonded to sulfur are saturated in all cases except one. In that structure, that of chloro(bis{2-[(2-pyridylmethyl)imino]phenyl} disulfide)nickel(II) perchlorate,[4] the S–S bond was particularly long, 2.089 (8) Å, for