Crystal Structures and Conformations of the Cyclic Dipeptides *cyclo*-(Glycyl-L-tyrosyl) and *cyclo*-(L-Seryl-L-tyrosyl) Monohydrate[1a]

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Received March 9, 1973

Abstract: The crystal and molecular structures of *cyclo*-(glycyl-L-tyrosyl) (*cyclo*-(Gly-L-Tyr)) and *cyclo*-(L-seryl-L-tyrosyl) (*cyclo*-(L-Ser-L-Tyr)) monohydrate were determined by single crystal X-ray diffraction techniques. Both peptides are in the folded conformation ($\chi^1 = 55°$), with the aromatic ring in the tyrosine side chain facing the diketopiperazine ring. This finding is in agreement with proton magnetic resonance and molecular orbital studies of a number of cyclic dipeptides containing arylmethyl side chains. In *cyclo*-(Gly-L-Tyr), the diketopiperazine ring assumes a boat conformation with the C^α–C^β bond in a quasi-axial "flagpole" position, but in *cyclo*-(L-Ser-L-Tyr), this ring is nearly planar with the α carbons deviating about 0.2 Å from the least-squares plane through the two peptide bonds. Analysis of presently available crystallographic and nmr cyclic dipeptide data indicates that, in general, the diketopiperazine ring favors a conformation which maximizes the diketopiperazine–aromatic ring attractive interaction without allowing steric interference between side chains. Crystals of *cyclo*-(Gly-L-Tyr) are orthorhombic, space group $P2_12_12_1$ with $a = 7.775 \pm 0.003$, $b = 21.475 \pm 0.001$, $c = 6.170 \pm 0.001$ Å, and $Z = 4$. The crystals of *cyclo*-(L-Ser-L-Tyr) are monoclinic, space group $P2_1$, with $a = 11.445 \pm 0.002$, $b = 6.191 \pm 0.001$, $c = 8.828 \pm 0.001$ Å, $\beta = 99.10 \pm 0.01°$, and $Z = 2$. The X-ray intensity data were collected with a four circle diffractometer and refined by least-squares procedures to an R factor of 9.9% for the former compound and 5.3% for the latter.

The current interest in conformations of cyclic dipeptides (2,5-piperazinediones) began with the discovery, through proton magnetic resonance studies, that an arylmethyl side chain prefers the folded conformation with the aromatic ring facing the diketopiperazine (DKP) ring.[2-4] Since the attractive force between these two rings which stabilizes this conformation may play a role in molecules of biological interest, we have sought X-ray crystallographic evidence for the folded conformation in the solid state.

We have also tried to see if there is any systematic relationship between the amino acid composition of a cyclic dipeptide and the conformation of its piperazinedione backbone, the DKP ring. Within the restriction of amide planarity, the DKP ring can be planar or assume two possible boat conformations, "flagpole" with cis α-carbon substituents quasi-axial and "bowsprit" with these substituents quasi-equatorial.[2] These three ring conformations are shown with the folded form of an arylmethyl side chain in Figure 1. The folded form or F rotamer[4] corresponds to $\chi^1 = 60°$ and is represented in Newman's projection in Figure 1D. The simplest cyclic dipeptide, diketopiperazine, is planar in the crystalline state,[5,6] but replacement of one or both glycines may change the conformation greatly. Studies in solution of a number of diketopiperazines have shown that nmr[2-4] and optical rotatory data[7-9] must be interpreted in terms of a major contribution from some kind of nonplanar boat form of the DKP ring. Recently this finding was confirmed by X-ray crystallographic analyses of *cyclo*-(L-alanyl-L-alanyl)[10,11] and *cyclo*-(L-prolyl-L-leucyl).[12] The two structures reported here introduce aromatic side chains. A preliminary report of the *cyclo*-(glycyl-L-tyrosyl) crystal structure showed that the molecule assumes a folded form with a flagpole DKP ring as shown in Figure 1B.[13] The prediction was made that replacement of the glycine residue by a larger group may alter the conformation of the DKP ring. Inclusion of a serine residue in the present comparative study allows an evaluation of this effect.

Experimental Section

The cyclic dipeptides *cyclo*-(glycyl-L-tyrosyl) (*cyclo*-(Gly-L-Tyr)) and *cyclo*-(L-seryl-L-tyrosyl) (*cyclo*-(L-Ser-L-Tyr)) were prepared as previously described[14] and donated by Professor K. D. Kopple. The *cyclo*-(Gly-L-Tyr) compound was crystallized from hot water as thin colorless needles which were used directly after drying over phosphorus pentoxide in an evacuated desiccator. The *cyclo*-(L-Ser-L-Tyr) material was dissolved in an ethanol–water solution and crystallized as colorless needles by slow evaporation.

Preliminary precession pictures showed the *cyclo*-(Gly-L-Tyr)

(1) (a) Presented in part at the Summer Meeting of the American Crystallographic Association, Ames, Iowa, August 1971, No. G10, G11. (b) Abstracted in part from the thesis of C.-F. Lin submitted in partial fulfillment of the Doctor of Philosophy degree at Illinois Institute of Technology. (c) Illinois Institute of Technology. (d) Washington University School of Medicine.

(2) G. Gawne, G. W. Kenner, N. H. Rogerts, R. C. Sheppard, and K. Titelstad in "Peptides 1968," E. Bricas, Ed., Wiley, New York, N. Y., 1968, p 28.

(3) K. D. Kopple and D. H. Marr, *J. Amer. Chem. Soc.*, **89**, 6193 (1967).

(4) K. D. Kopple and M. Ohnishi, *ibid.*, **91**, 962 (1969).

(5) R. B. Corey, *ibid.*, **60**, 1598 (1938).

(6) R. Degeilh and R. E. Marsh, *Acta Crystallogr.*, **12**, 1007 (1959).

(7) K. Bláha and I. Frič in ref 2, p 40.

(8) J. A. Schellman and B. E. Nielsen in "Conformation of Biopolymers," Vol. 1, G. N. Ramachandran, Ed., Academic Press, New York, N. Y., 1967, p 109.

(9) D. Balasubramanian and D. B. Wetlaufer in ref 8, p 147.

(10) E. Bendettl, P. Corradini, and C. Pedone, *Biopolymers*, 7, 751 (1969).

(11) E. Sletten, *J. Amer. Chem. Soc.*, **92**, 172 (1970).

(12) I. Karle, *ibid.*, **94**, 81 (1972).

(13) C.-F. Lin and L. E. Webb, *ibid.*, **93**, 3818 (1971).

(14) K. D. Kopple and H. G. Ghazarian, *J. Org. Chem.*, **33**, 862 (1968).

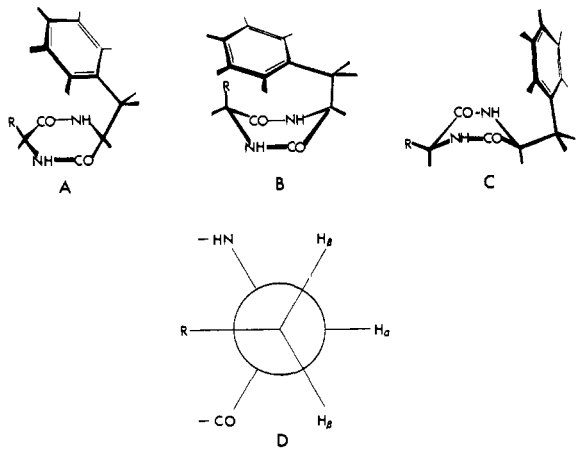

Figure 1. Planar (A), flagpole boat (B), and bowsprit boat (C) conformations of the diketopiperazine ring in a cyclic dipeptide with an aromatic amino acid residue. The folded form is illustrated as a conformation about the α–β bond in (D).

crystals to be orthorhombic with the needle axis parallel to the crystallographic c axis. Systematic absences established the space group uniquely as $P2_12_12_1$. The *cyclo*-L-(Ser-L-Tyr) crystals are monoclinic, space group $P2_1$, with the needle axis parallel to the crystallographic b axis.

All lattice constants and intensity measurements were made with a Picker four circle diffractometer and a scintillation counter. The quoted cell constants were calculated from a least-squares fit to 20 or more 2θ values measured manually by the Bond method.[15] The uncertainties given are estimated standard deviations derived from the least-squares matrix. Radiation from the copper target X-ray tube was nickel filtered to give the Cu Kα wavelength which was assumed to be 1.54178 Å for all calculations.

For intensity measurements, appropriate thicknesses of nickel foil with experimentally determined filter factors were used to maintain linearity in the scintillation counter response. A pulse height analyzer was used in the usual way to accept about 80–90% of the desired radiation. The take-off angle was 4°.

cyclo-(Glycyl-L-tyrosyl). The cell constants are $a = 7.775 \pm 0.003$, $b = 21.475 \pm 0.011$, $c = 6.170 \pm 0.001$ Å. The density of the crystals as measured by flotation implied that no water of crystallization was present. With the formula $C_{11}H_{12}O_3N_2$, $d_{calcd} = 1.418$ g/cm^3 for $Z = 4$ and $d_{obsd} = 1.415$ g/cm^3.

For intensity measurements, a 0.05 × 0.08 × 0.5 mm crystal was mounted with the c axis parallel to the spindle axis. Integrated intensities were measured by θ–2θ scans at 1° min. The scan ranges were calculated according to the equation $2\theta = A + B \tan\theta$.[16] The constant A, based on the required scan range for strong low-order reflections, was 2.0° for $2\theta < 90°$, but was increased to 3.0° for $2\theta > 90°$. The Cu Kα dispersion factor was $(360/\pi)(\Delta\lambda/\lambda) = 0.285$. Background radiation was counted for 10 sec on either side of the scan. Two standard reflections, measured every 2–3 hr, showed no systematic deviations; experimental instabilities accounted for maximum fluctuations from the mean of 1.5%.

The 1045 measured intensities were taken from one octant of the reciprocal lattice out to $2\theta = 130°$. A relative standard deviation σ_{rel} based on counting statistics was calculated for each measured intensity. For all calculations but the final least-squares cycles, the 1023 measurements with $\sigma_{rel} < 1.0$ were classified as observed. No absorption corrections were applied; the linear absorption coefficient is 8.84 cm^{-1}.

cyclo-(L-Seryl-L-tyrosyl). The cell constants are $a = 11.445 \pm 0.002$, $b = 6.191 \pm 0.001$, $c = 8.828 \pm 0.001$ Å, and $\beta = 99.10 \pm 0.01°$. The calculated density assuming one water per molecule, formula $C_{12}H_{14}O_4N_2 \cdot H_2O$, is 1.44 g/cm^{-3}; the density measured by flotation is 1.43 g/cm^{-3}.

For intensity measurements, it was possible to select a more equidimensional crystal than was possible with the extremely thin *cyclo*-(Gly-L-Tyr) needles. A 0.1 × 0.1 × 0.3 mm crystal was mounted with the b axis parallel to the spindle axis. Intensity data were taken manually by the stationary crystal–stationary counter method to a maximum of $2\theta = 135.8°$ or $\sin\theta/\lambda = 0.60$. Each peak was counted for 10 sec and background counts were taken for 10 sec on either side. Standard reflections measured twice per day showed no systematic deviation. The net peak counts were converted to integrated intensities with an I_i/I_p calibration curve[17] which included 49 intensities measured by θ–2θ scans. Data were obtained for 1241 independent reflections of which 31 were too weak to measure. An estimated relative standard deviation based on counting statistics, σ_{rel}, was assigned to each of the remaining 1210 intensities.

Structure Determinations

After application of Lorentz polarization corrections, normalized structure factors, $|E|$, were derived using a K curve and the anisotropic fit suggested by Maslen.[18,19] The magnitudes of these normalized structure factors differed by as much as 25% from those calculated with a single overall temperature factor.

The structures were solved by application of the tangent formula[20] to a starting set of phases determined by the symbolic addition procedure.[21] Programs developed by S. Hall[19] were used to assist in selecting the starting set and for the tangent refinement. Both structures were refined by full matrix least squares with unit weights, until anisotropic refinement and difference maps allowed location of the hydrogens. The coordinates and anisotropic temperature factors for the C, N, and O atoms and the coordinates of all hydrogen atoms were varied in the final least-squares cycles. Isotropic temperature factors for the hydrogens were set equal to those of the atom of attachment. The atomic scattering factors were those from the "International Tables for X-Ray Crystallography."[22]

For *cyclo*-(Gly-L-Tyr), selection of the origin and enantiomorph allowed the phases π, $-\pi/2$, $\pi/2$, and $\pi/2$ to be arbitrarily assigned to the reflections 045, 1,13,0, 160, and 037, respectively. These reflections were chosen to determine useful phases in all parity groups. Symbolic addition,[21] with output from the MAGIA program[23] as a helpful reference, gave multiple indications that the phases for the 125, 2,12,0, 240, and 285 reflections were at or near $\pi/2$, π, 0, and π, respectively, and these phases were added to the starting set for the tangent formula.

The highest 16 peaks of the 303 term E map corresponded to the 16 nonhydrogen atoms of the peptide in the D form. After refinement of the L coordinates and anisotropic temperature factors, all but the two amide hydrogens were located unambiguously in a difference map. The amide hydrogen coordinates were derived from known bond geometry and included in the refinement.

The final least-squares cycles minimized the function $\Sigma w(|F_o| - |F_c|)^2$ for a total of 1027 reflections. For observed reflections, $w = 1$ for $|F_o| < 25$ and $w = 25/|F_o|$ for $|F_o| > 25$. All 51 reflections with $\sigma_{rel} > 0.4$ were classified as unobserved. Those unobserved reflec-

(15) W. L. Bond, *Acta Crystallogr.*, **13**, 814 (1960).
(16) L. E. Alexander and G. S. Smith, *ibid.*, **17**, 1195 (1964).
(17) L. E. Alexander and G. S. Smith, *ibid.*, **15**, 983 (1962).
(18) E. N. Maslen, *ibid.*, **22**, 945 (1967).
(19) J. M. Stewart, F. A. Kundell, and J. C. Baldwin, Ed., "The X-Ray System of Crystallographic Programs," University of Maryland Computer Science Center, Baltimore, Md., 1970.
(20) J. Karle and H. Hauptman, *Acta Crystallogr.*, **9**, 635 (1956).
(21) J. Karle and I. L. Karle, *ibid.*, **21**, 849 (1966).
(22) "International Tables for X-Ray Crystallography," Vol. III, The Kynoch Press, Birmingham, England, 1962.
(23) R. B. K. Dewar, "Multiphase Automatic Generation in Acentric Crystals," Information Science Center, Illinois Institute of Technology, Chicago, Ill., 1969.

Table I. Fractional Coordinates and Thermal Parameters[a]

Atom	x	y	z	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
				cyclo-(Glyceryl-L-tyrosyl)					
N_1	0.3278 (10)	0.0688 (4)	1.0538 (12)	2.93 (33)	4.28 (37)	1.69 (27)	0.38 (30)	0.36 (26)	−0.28 (28)
C_1^{α}	0.2252 (13)	0.0920 (5)	0.8722 (16)	3.37 (43)	4.68 (51)	2.25 (36)	1.14 (42)	−0.17 (37)	−0.49 (37)
C_1	0.2979 (11)	0.0743 (4)	0.6523 (15)	2.62 (36)	2.40 (33)	2.78 (36)	0.76 (30)	0.16 (34)	0.44 (31)
O_1	0.2059 (9)	0.0828 (3)	0.4924 (10)	4.20 (32)	4.31 (31)	2.05 (24)	0.86 (29)	0.20 (28)	0.36 (24)
N_2	0.4534 (11)	0.0525 (4)	0.6447 (12)	2.53 (32)	4.10 (35)	2.06 (30)	0.13 (28)	0.53 (28)	−0.09 (30)
C_2^{α}	0.5759 (12)	0.0461 (4)	0.8292 (15)	3.29 (41)	3.40 (38)	2.10 (35)	0.83 (34)	−0.54 (35)	−0.22 (33)
C_2	0.4883 (11)	0.0508 (4)	1.0447 (13)	2.60 (36)	2.89 (35)	1.78 (32)	0.48 (31)	−0.19 (31)	0.73 (29)
O_2	0.5705 (9)	0.0334 (3)	1.2044 (10)	3.97 (32)	4.24 (30)	2.61 (26)	1.00 (27)	−0.04 (27)	0.34 (25)
C^{β}	0.7213 (13)	0.0951 (4)	0.8106 (20)	3.19 (41)	3.60 (41)	3.88 (48)	0.78 (35)	0.29 (43)	0.35 (39)
C^{γ}	0.6565 (12)	0.1615 (4)	0.7931 (17)	3.02 (38)	2.86 (36)	4.27 (45)	−0.35 (34)	−1.38 (40)	−0.35 (36)
$C^{\delta 1}$	0.5810 (13)	0.1921 (4)	0.9721 (17)	4.12 (45)	2.24 (35)	3.85 (45)	−0.32 (34)	0.90 (45)	−0.87 (35)
$C^{\delta 2}$	0.6774 (13)	0.1953 (5)	0.6075 (16)	3.55 (44)	3.71 (44)	3.00 (40)	−0.01 (39)	0.07 (40)	0.19 (35)
$C^{\epsilon 1}$	0.5251 (13)	0.2531 (5)	0.9542 (16)	3.54 (43)	4.10 (45)	3.01 (45)	−0.66 (38)	0.81 (39)	−0.25 (38)
$C^{\epsilon 2}$	0.6253 (16)	0.2565 (5)	0.5907 (16)	4.77 (50)	4.36 (50)	2.63 (37)	0.49 (45)	0.48 (40)	0.15 (37)
C^{ζ}	0.5498 (12)	0.2859 (4)	0.7649 (17)	3.04 (38)	2.10 (33)	4.60 (49)	−0.74 (31)	−1.03 (39)	−0.22 (35)
O^{η}	0.4959 (10)	0.3471 (3)	0.7588 (12)	4.10 (32)	2.85 (27)	4.11 (35)	−0.52 (24)	−0.17 (29)	−0.06 (25)
				cyclo-(L-Seryl-L-tyrosyl)					
N_1	0.5496 (5)	0.6573 (11)	0.1972 (7)	3.13 (23)	1.35 (21)	3.80 (25)	−0.33 (19)	0.76 (19)	0.15 (20)
C_1	0.5207 (6)	1.0523 (13)	0.1870 (7)	2.89 (27)	2.24 (28)	2.58 (25)	−0.17 (25)	0.18 (20)	−0.03 (23)
C_1^{α}	0.4658 (6)	0.8324 (15)	0.1999 (8)	2.57 (28)	3.31 (36)	3.87 (32)	−0.05 (26)	0.49 (25)	−0.13 (28)
O_1	0.4600 (4)	1.2149 (11)	0.2005 (6)	3.80 (23)	1.66 (19)	5.23 (27)	0.42 (19)	0.69 (20)	0.21 (19)
N_2	0.6318 (5)	1.0627 (11)	0.1609 (6)	3.34 (23)	0.87 (19)	2.85 (22)	−0.08 (18)	0.22 (18)	−0.14 (18)
C_2	0.6571 (6)	0.6658 (12)	0.1614 (7)	3.30 (26)	1.19 (24)	2.00 (23)	−0.14 (22)	0.18 (19)	−0.14 (20)
O_2	0.7179 (4)	0.5018 (—)[b]	0.1494 (5)	3.47 (20)	1.61 (19)	3.78 (21)	0.29 (17)	0.95 (17)	−0.18 (17)
C_2^{α}	0.7080 (6)	0.8833 (11)	0.1319 (7)	3.04 (27)	1.06 (24)	2.22 (23)	−0.06 (21)	0.38 (20)	0.00 (19)
C_1^{β}	0.4097 (8)	0.8222 (16)	0.3461 (11)	4.55 (38)	2.76 (37)	6.90 (47)	−0.04 (33)	3.23 (36)	0.51 (36)
O_1^{γ}	0.4942 (8)	0.8826 (15)	0.4741 (7)	11.20 (45)	5.23 (37)	4.00 (30)	0.12 (40)	2.94 (32)	0.05 (28)
C_2^{β}	0.8335 (5)	0.9187 (14)	0.2216 (6)	2.91 (25)	2.45 (28)	1.95 (22)	−0.06 (23)	0.50 (19)	−0.38 (22)
C_2^{γ}	0.8397 (5)	0.9553 (14)	0.3931 (6)	2.29 (23)	2.56 (28)	1.96 (22)	−0.16 (21)	0.26 (18)	−0.07 (21)
$C^{\delta 1}$	0.8193 (7)	0.7915 (14)	0.4914 (8)	4.69 (34)	1.47 (24)	2.86 (27)	0.04 (25)	0.41 (24)	−0.03 (23)
$C^{\delta 2}$	0.8708 (6)	1.1551 (14)	0.4565 (7)	3.32 (27)	2.56 (30)	2.11 (24)	−0.19 (26)	0.37 (21)	0.07 (24)
$C^{\epsilon 1}$	0.8301 (7)	0.8212 (14)	0.6485 (8)	5.07 (37)	1.98 (30)	2.45 (26)	−0.41 (27)	0.57 (24)	0.30 (23)
$C^{\epsilon 2}$	0.8815 (6)	1.1919 (14)	0.6140 (7)	3.22 (26)	2.11 (27)	2.36 (24)	−0.26 (24)	0.17 (20)	−0.37 (23)
C^{ζ}	0.8609 (5)	1.0229 (14)	0.7086 (7)	2.94 (25)	2.71 (28)	2.01 (23)	0.15 (23)	0.44 (18)	−0.01 (23)
O^{η}	0.8724 (4)	1.0492 (12)	0.8661 (5)	5.54 (24)	3.79 (24)	1.69 (16)	−0.38 (24)	0.62 (16)	−0.11 (19)
O_3[c]	0.8962 (5)	0.4421 (12)	0.9743 (6)	4.88 (25)	4.34 (28)	3.80 (22)	−0.26 (23)	0.94 (19)	−1.36 (22)

[a] Unit cell translations are included to put all atoms in one molecule. The thermal parameters are of the form

$$-1/4\sum_{i=1}^{3}\sum_{j=1}^{3} B_{ij}h_ih_ja_i^*a_j^*$$

with the B_{ij} in Å^2 units. The estimated standard deviations are given in parentheses and refer to the last decimal places. [b] Fixed during refinement. [c] Water oxygen atom.

tions with $|F_c| > |F_o|$ were included in the refinement with $w = 0.5$. The final conventional R factor for the 994 observed reflections was 0.099.[24] The largest shift/standard deviation ratio in the last refinement cycle was less than one for all parameters except for four hydrogen coordinates for which this ratio varied from 1.13 to 1.28.

The less straightforward solution of the *cyclo*-(L-Ser-L-Tyr) structure was begun by arbitrarily assigning phases of 0 to the 205, 30$\bar{1}$, and 510 reflections to fix the origin and a phase of $\pi/2$ to the 537 reflection to fix the enantiomer. Selection of the 537 reflection and its use in symbolic addition with the 510 reflection led to phases which allowed an unambiguous determination of the origin along the b axis even though all reflections with $k = 1$ were weak. Calculations with the Σ_2 formula, with acceptance of phases by criteria suggested by Karle,[25] allowed the unambiguous assignment of ten additional phases. This procedure verified a phase of 0 predicted by the Σ_1 formula[20] for the 006 reflection. Multiple indications that the phase of the 208 reflection was π were taken as evidence that the Σ_1 prediction of 0 for this phase was incorrect. The total of 14 phases derived in this way were refined and expanded by the tangent formula.

Of the highest 30 peaks in the 383 term E map, 19 corresponded to the atomic positions and revealed the structure of the molecule. After all hydrogen coordinates except those in the water molecule and the seryl hydroxyl group were determined from a difference map, the final least-squares cycles minimized the function $\Sigma w(|F_o| - |F_c|)^2$ for a total of 1241 reflections. For observed reflections, $w = 1$ for $|F_o| < 15$ and $w = 15/|F_o|$ for $|F_o| > 15$. The 66 reflections with $|F_o| < 1.0$ were classified as unobserved since σ_{rel} for these was 0.5 or greater. Those unobserved reflections with $|F_c| > |F_o|$ were included in the refinement with $w = 0.5$. The final conventional R factor for the 1175 observed reflections was 0.053.[24] The largest shift/standard deviation ratio in the last refinement cycle was less than one for all parameters except for the water oxygen z parameter (1.04) and the z parameters of H_1^{α} and $H_1^{\beta 2}$ (1.40 and 1.05).

The final atomic parameters and their standard

(24) See paragraph at end of paper regarding supplementary material.

(25) I. L. Karle in "Crystallographic Computing," F. R. Ahmed, Ed., Munskgaard, Copenhagen, 1969, pp 19–25.

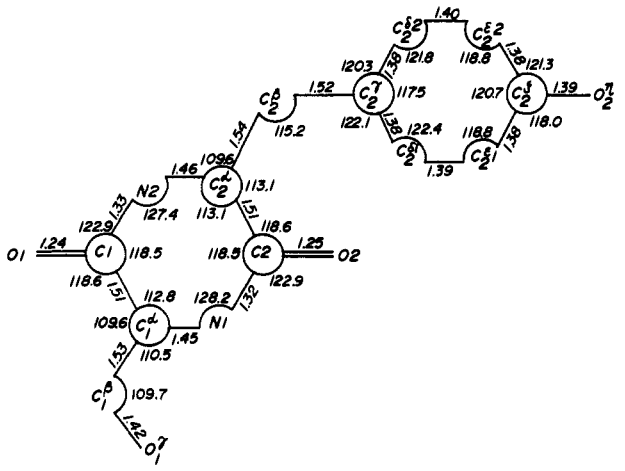

Figure 2. Bond distances and bond angles in *cyclo*-(L-seryl-L-tyrosyl).

deviations, derived in the usual way from the variance–covariance matrix, are given in Tables I and II. The

Table II. Fractional Coordinates and Isotropic Temperature Factors for Hydrogen Atoms[a]

Atom	*x*	*y*	*z*	*B*
		cyclo-(Gly-L-Tyr)		
H_1	0.275 (15)	0.064 (5)	1.202 (19)	2.88
H_2	0.494 (18)	0.052 (6)	0.556 (20)	2.77
$H_1^{\alpha 1}$	0.093 (15)	0.081 (5)	0.869 (20)	3.35
$H_1^{\alpha 2}$	0.224 (15)	0.139 (5)	0.876 (20)	3.35
H_2^{α}	0.652 (14)	0.007 (5)	0.848 (20)	3.08
$H^{\beta 1}$	0.791 (17)	0.075 (6)	0.699 (20)	3.62
$H^{\beta 2}$	0.808 (17)	0.075 (6)	0.916 (21)	3.62
$H^{\delta 1}$	0.553 (15)	0.167 (5)	1.100 (20)	3.24
$H^{\delta 2}$	0.732 (16)	0.172 (5)	0.498 (20)	3.44
$H^{\epsilon 1}$	0.475 (16)	0.271 (5)	1.071 (20)	3.46
$H^{\epsilon 2}$	0.639 (17)	0.277 (6)	0.478 (22)	3.88
H^{η}	0.569 (16)	0.363 (6)	0.666 (22)	3.70
		cyclo-(L-Ser-L-Tyr)		
H_1	0.5170 (74)	0.533 (17)	0.216 (10)	2.60
H_2	0.6571 (73)	1.210 (18)	0.160 (10)	2.30
H_1^{α}	0.3910 (75)	0.755 (17)	0.077 (10)	3.52
H_2^{α}	0.1742 (65)	0.878 (16)	0.010 (9)	2.19
$H_1^{\beta 1}$	0.3399 (85)	0.940 (19)	0.339 (11)	4.14
$H_1^{\beta 2}$	0.3708 (82)	0.677 (20)	0.352 (11)	4.14
$H_2^{\beta 1}$	0.8818 (73)	0.792 (17)	0.206 (9)	2.20
$H_2^{\beta 2}$	0.8695 (70)	1.053 (16)	0.179 (9)	2.20
$H_2^{\delta 1}$	0.8106 (76)	0.642 (17)	0.473 (10)	2.76
$H_2^{\delta 2}$	0.8956 (73)	1.274 (17)	0.406 (10)	2.50
$H_2^{\epsilon 1}$	0.8095 (76)	0.720 (18)	0.704 (10)	3.09
$H_2^{\epsilon 2}$	0.9063 (69)	1.373 (17)	0.648 (9)	2.37

[a] Standard deviations estimated from least-squares refinement are given in parentheses. Isotropic temperature factors were not refined but were set equal to those of the atom of attachment.

structure refinements and standard deviations suggest that the *cyclo*-(Gly-L-Tyr) structure is slightly less accurate than the *cyclo*-(L-Ser-L-Tyr) structure, presumably because of the difficult data collection from the extremely thin crystals of the *cyclo*-(Gly-L-Tyr) compound. This assessment is consistent with comparative internal consistencies of the bond parameters discussed below. The nuclear coordinates listed in Table III were calculated from the C, N, and O coordinates assuming standard sp^2 or sp^3 geometry and bond distances of 1.09 and 1.00 Å for C–H and N–H, respectively.

Table III. Nuclear Fractional Coordinates for Hydrogen Atoms[a]

Atom	*x*	*y*	*z*
	cyclo-(Gly-L-Tyr)		
H_1	0.266	0.072	1.196
H_2	0.506	0.041	0.501
$H_1^{\alpha 1}$	0.095	0.072	0.885
$H_1^{\alpha 2}$	0.216	0.142	0.882
H_2^{α}	0.644	0.002	0.817
$H^{\beta 1}$	0.799	0.085	0.667
$H^{\beta 2}$	0.803	0.092	0.955
$H^{\delta 1}$	0.568	0.169	1.114
$H^{\delta 2}$	0.732	0.175	0.480
$H^{\epsilon 1}$	0.464	0.273	1.077
$H^{\epsilon 2}$	0.645	0.280	0.451
	cyclo-(L-Ser-L-Tyr)		
H_1	0.513	0.514	0.220
H_2	0.672	1.205	0.152
$H_1^{\beta 1}$	0.332	0.933	0.336
$H_2^{\beta 2}$	0.375	0.658	0.364
$H_2^{\beta 1}$	0.888	0.775	0.204
$H_2^{\beta 2}$	0.873	1.058	0.172
$H_2^{\delta 1}$	0.796	0.642	0.446
$H_2^{\delta 2}$	0.884	1.279	0.388
$H_2^{\epsilon 1}$	0.815	0.697	0.718
$H_2^{\epsilon 2}$	0.907	1.337	0.659
H_1^{α}	0.392	0.810	0.107
H_2^{α}	0.724	0.889	0.013

[a] These positions are calculated assuming standard sp^2 or sp^3 geometry around the C, N, and O positions (C–H = 1.09 Å, N–H = 1.00 Å).

Results

Bond parameters and standard peptide notation are shown for *cyclo*-(L-Ser-L-Tyr) in Figure 2. The bond distances and bond angles of the *cyclo*-(Gly-L-Tyr) and *cyclo*-(L-Ser-L-Tyr) peptide backbones reflect the small differences expected between a cyclic dipeptide and open cis and trans peptides.[11,26-28] The comparison in Table IV with the unsubstituted cyclic

Table IV. Bond Lengths[a] (Å)

Bonds	*cyclo*-(Gly-L-Tyr)	*cyclo*-(L-Ser-L-Tyr)	DKP[b]
N_1–C_1^{α}	1.463 (13)	1.450 (10)	1.452
C_1^{α}–C_1	1.518 (13)	1.511 (12)	1.509
C_1–O_1	1.232 (11)	1.240 (10)	1.243
C_1–N_2	1.297 (12)	1.330 (9)	1.334
N_2–C_2^{α}	1.491 (12)	1.459 (9)	1.452
C_2^{α}–C_2	1.497 (12)	1.506 (10)	1.509
C_2–O_2	1.232 (11)	1.245 (10)	1.243
C_2–N_1	1.307 (12)	1.319 (9)	1.334
C_2^{α}–C_2^{β}	1.548 (14)	1.542 (8)	
C_2^{β}–C_2^{γ}	1.517 (13)	1.522 (8)	
C_2^{γ}–$C^{\delta 1}$	1.413 (14)	1.378 (11)	
$C^{\delta 1}$–$C^{\epsilon 1}$	1.383 (14)	1.386 (10)	
$C^{\epsilon 1}$–C^{ζ}	1.378 (14)	1.380 (12)	
C^{ζ}–O^{η}	1.381 (10)	1.386 (7)	
C^{ζ}–$C^{\epsilon 2}$	1.377 (14)	1.382 (11)	
$C^{\epsilon 2}$–$C^{\delta 2}$	1.380 (15)	1.395 (9)	
$C^{\delta 2}$–C^{γ}	1.365 (14)	1.381 (11)	
C_1^{α}–C_1^{β}		1.531 (13)	
C_1^{β}–O_1^{γ}		1.416 (11)	

[a] Standard deviations are given in parentheses and refer to the last decimal places. [b] The measured bond lengths all have a standard deviation of 0.007 Å and are corrected for rigid body thermal motion. See ref 6.

(26) R. B. Corey and L. Pauling, *Proc. Roy. Soc., Ser. B*, 141 (1953).
(27) R. E. Marsh and J. Donohue, *Advan. Protein Chem.*, 22, 235 (1967).
(28) G. N. Ramachandran and V. Sasisekharan, *ibid.*, 23, 283 (1968).

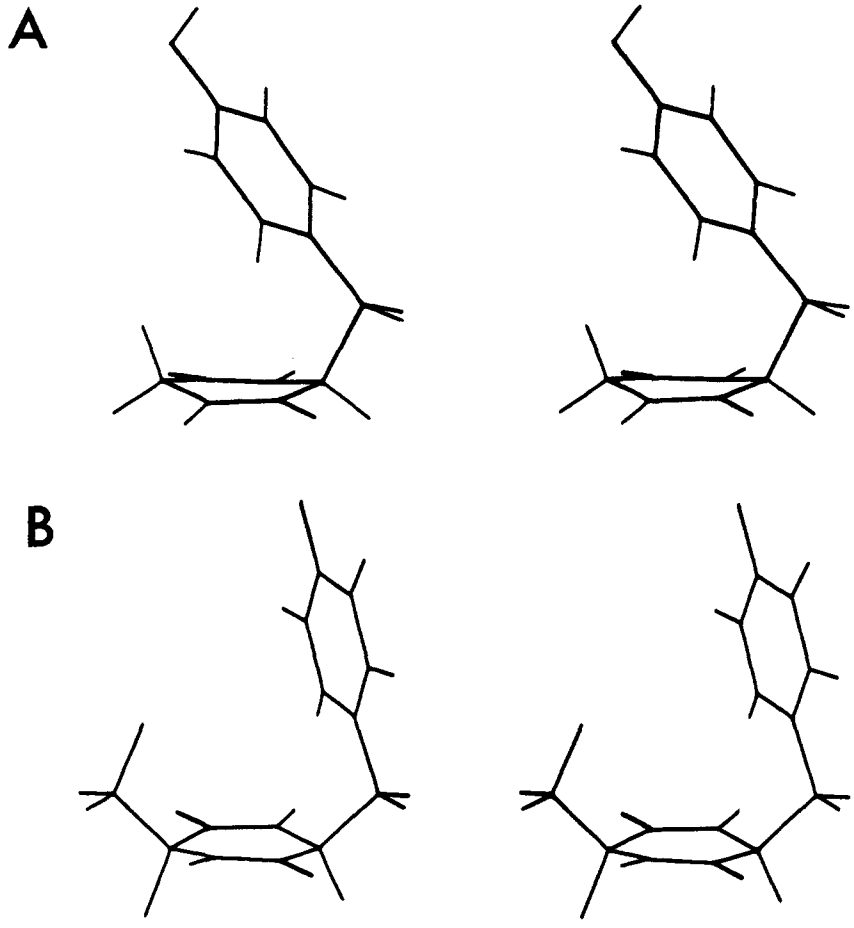

Figure 3. Stereopicture of the *cyclo*-(Gly-L-Tyr) molecule (A) and the *cyclo*-(L-Ser-L-Tyr) molecule (B) in the orientation shown in Figure 1. The atoms can be identified by referring to Figure 1, and the figures should be viewed with a stereoviewer. All stereopictures were obtained directly from interactive cathode ray tube displays generated from the crystallographic coordinates by a system of computer programs designed for molecular models.[29]

dipeptide, diketopiperazine (DKP), reveals no significant differences in bond lengths. The apparent increased N_2–C_2^{α} distance in *cyclo*-(Gly-L-Tyr) is not significant.

Although the bond angles at the carbonyl carbon and nitrogen atoms are very close to those found in DKP, there is more flexibility at the α carbons (Table V). In the monosubstituted *cyclo*-(Gly-L-Tyr) the internal angles at C^{α} follow an expected trend and are larger than the corresponding angles in the disubstituted diketopiperazine, *cyclo*-(L-Ala-L-Ala) (112.0 and 110.5°),[11] but smaller than those in the unsubstituted DKP molecule, *cyclo*-Gly-Gly (115.1°).[6] The corresponding internal angles in *cyclo*-(L-Ser-L-Tyr) (112.8 and 113.1°) are smaller than those in *cyclo*-(Gly-Gly) even though the DKP ring in *cyclo*-(L-Ser-L-Tyr) is planar as it is in *cyclo*-(Gly-Gly). The expected effect of substitution on C^{α} is consistent with this observation. The *cyclo*-(L-Ser-L-Tyr) C^{α} internal angles remain larger than those in the nonplanar *cyclo*-(L-Ala-L-Ala) molecule.

The bond parameters in the side chains in both *cyclo*-(Gly-L-Tyr) and *cyclo*-L-Ser-L-Tyr are not significantly different from expected values, although deviations from the average benzene ring values are slightly larger in *cyclo*-(Gly-L-Tyr). The increase in the $C^{\alpha}C^{\beta}C^{\gamma}$ angles over the tetrahedral value is often found in amino acids.[12]

Table V. Bond Angles[a] (deg)

Angles	*cyclo*-(Gly-L-Tyr)	*cyclo*-(L-Ser-L-Tyr)	DKP[b]
C_2–N_1–C_1^{α}	126.0 (8)	128.3 (7)	126.0 (3)
N_1–C_1^{α}–C_1	113.4 (8)	112.8 (6)	115.1 (3)
C_1^{α}–C_1–N_2	118.0 (8)	118.5 (7)	118.9 (3)
C_1^{α}–C_1–O_1	117.5 (8)	118.6 (6)	118.5 (3)
O_1–C_1–N_2	124.5 (8)	122.9 (7)	122.6 (3)
C_1–N_2–C_2^{α}	126.9 (8)	127.4 (7)	126.0 (3)
N_2–C_2^{α}–C_2	112.4 (8)	113.1 (6)	115.1 (3)
N_2–C_2^{α}–C_2^{β}	110.3 (8)	109.6 (6)	
C_2^{β}–C_2^{α}–C_2	110.6 (8)	113.1 (6)	
C_2^{α}–C_2–N_1	119.5 (8)	118.5 (7)	
C_2^{α}–C_2–O_2	117.0 (8)	118.6 (6)	
O_2–C_2–N_1	123.4 (8)	122.9 (7)	
C_2^{α}–C_2^{β}–C_2^{γ}	113.7 (8)	115.2 (5)	
C_2^{β}–C_2^{γ}–$C^{\delta 1}$	121.3 (9)	122.1 (7)	
C_2^{β}–C_2^{γ}–$C_2^{\delta 2}$	121.3 (9)	120.3 (7)	
$C^{\delta 2}$–C^{γ}–$C^{\delta 1}$	117.3 (8)	117.5 (6)	
C^{γ}–$C^{\delta 1}$–$C^{\epsilon 1}$	120.6 (9)	122.4 (8)	
$C^{\delta 1}$–$C^{\epsilon 1}$–C^{ζ}	120.5 (9)	118.8 (7)	
$C^{\epsilon 1}$–C^{ζ}–$C^{\epsilon 2}$	119.1 (8)	120.7 (6)	
$C^{\epsilon 1}$–C^{ζ}–O^{η}	117.9 (9)	118.0 (7)	
O^{η}–C^{ζ}–$C^{\epsilon 2}$	123.0 (9)	121.3 (7)	
C^{ζ}–$C^{\epsilon 2}$–$C^{\delta 2}$	120.2 (9)	118.8 (7)	
$C^{\epsilon 2}$–$C^{\delta 2}$–C^{γ}	122.3 (9)	121.8 (7)	
N_1–C_1^{α}–C_1^{β}		110.5 (7)	
C_1–C_1^{α}–C_1^{β}		109.6 (7)	
C_1^{α}–C_1^{β}–O_1^{γ}		109.7 (7)	

[a] Standard deviations are given in parentheses. [b] See ref 6.

Conformation. The tyrosine rings in both peptides of this study are clearly in the folded or F conformation found previously in solution by nmr methods.[2-4] Computer displays[29] of the structures are shown in Figure 3. The torsional angles (IUPAC–IUB conventions[30]) are listed in Table VI. In *cyclo*-(L-Ser-L-Tyr), the oxygen of the seryl hydroxyl group is also folded over the DKP ring ($\chi_1^1 = 72°$).

Table VI. Torsional Angles (deg) in Cyclic Dipeptides[a]

Angle	DPK[b]	*cyclo*-(L-Ala-L-Ala)[c]	*cyclo*-(Gly-L-Tyr)	*cyclo*-(L-Ser-L-Tyr)
$\phi_1(N_1, C_1^\alpha)$	−1	−32	19	−12
$\psi_1(C_1^\alpha, C_1)$	1	21	−13	6
$\omega_1(C_1, N_2)$	1	8	−4	5
$\phi_2(N_2, C_2^\alpha)$		−25	16	−10
$\psi_2(C_2^\alpha, C_2)$		27	−10	5
$\omega_2(C_2, N_1)$		1	−7	6
$\chi_1^1(C_1^\alpha, C_1^\beta)$				72
$\chi_2^1(C_2^\alpha, C_2^\beta)$			55	55
$\chi_2^{2,1}(C_2^\beta, C_2^\gamma)$			71	72

[a] The conventions of the IUPAC–IUB Commission are followed.[30] [b] See ref 6. [c] See ref 11.

In the folded conformation ($\chi_1^1 = 60°$), χ^2 must be within about 30° of 90° or −90° in order to prevent interference between the tyrosine ring and the DKP ring. The intermediate χ_1^2 values of 71° in *cyclo*-(Gly-L-Tyr) and 72° in *cyclo*-(L-Ser-L-Tyr) also reflect the crystal packing and hydrogen bonding forces discussed below. The aromatic ring plane is tilted 18 or 19° away from a position perpendicular to the $C^\alpha C^\beta C^\gamma$ plane (Figure 3).

The DKP rings in *cyclo*-(Gly-L-Tyr) and *cyclo*-(L-Ser-L-Tyr) assume conformations which allow the maximum interaction with the arylmethyl side chain without violating van der Waals contacts (Figure 3). The torsional angles in the DKP rings of several cyclic dipeptides are compared in Table VI with those in diketopiperazine itself, *cyclo*-(Gly-Gly), which is planar.

The ϕ, ψ, and ω angles show that the DKP ring in *cyclo*-(Gly-L-Tyr) is, with very little deviation from amide planarity, buckled to the extent of 10–20° toward the flagpole boat conformation (Figure 1B). This conformation contrasts with the bowsprit conformation of *cyclo*-(L-Ala-L-Ala),[10,11] which is buckled in the opposite direction with the methyl groups quasi-equatorial (Figure 1C).

In *cyclo*-(L-Ser-L-Tyr), however, the DKP ring appears to be nearly planar (Figure 3). The ϕ and ψ angles are much smaller than in *cyclo*-(L-Ala-L-Ala), and the α carbons deviate only 0.2 Å from the least-squares plane formed by the four atoms, N_1, N_2, C_1, and C_2. As the signs of the ϕ and ψ angles show, this deviation is in the same direction as in *cyclo*-(L-Ala-L-Ala) (compare Figure 1C).

Detailed examination of Figure 3 and Table VI reveals slight deviations from amide planarity in both *cyclo*-(Gly-L-Tyr) and *cyclo*-(L-Ser-L-Tyr). Estimation of errors in the ω angles indicates that these deviations, though small, are significant and impart a small "twist" to the DKP rings. In *cyclo*-(L-Ala-L-Ala), characterized as a "twist boat," one of the ω values (8°) is significantly larger than those found for *cyclo*-(Gly-L-Tyr) and *cyclo*-(L-Ser-L-Tyr).[11]

The overall molecular conformations produce no unusually close contacts between the DKP and tyrosine rings. The shortest distance between atoms in these rings is the C^α–C^γ distance which is 2.57 Å in *cyclo*-(Gly-L-Tyr) and 2.59 Å in *cyclo*-(L-Ser-L-Tyr). As Figure 3 shows, the other distances between these rings are much longer. The perpendicular distance between the seryl oxygen and the plane of the tyrosine ring is 4.0 Å. Any appreciable buckling of the DKP ring toward the flagpole conformation would clearly make this distance too small. The implications of this observation for other cyclic peptide structures will be discussed below. Other intramolecular contacts fall within normal values. Some typical distances are listed in Table VII.

Table VII. Principal Contact Distances (Å) and Hydrogen Bonds[a] (Å)

cyclo-(Gly-L-Tyr)[b]		*cyclo*-(L-Ser-L-Tyr)[c]	
A. Intramolecular Contacts			
N_1–$C^{\delta 1}$	3.34	C_2^γ–C_2^α	2.59
N_1–$H^{\delta 1}$	2.8	C_1^α–O_1^γ	2.41
$H_1^{\alpha 2}$–$C^{\delta 1}$	3.1	C_2–$C_2^{\delta 1}$	3.28
N_2–C^γ	2.97	C_2–$H_2^{\delta 1}$	3.0
C_2^α–C^γ	2.57	O_2–$H_2^{\delta 1}$	3.0
C_2–$C^{\delta 1}$	3.15	C_1^α–$H_1^{\beta 2}$	2.1
C_2–C^γ	3.13	C_2^γ–$H_2^{\beta 2}$	2.1
C_2–$H^{\delta 1}$	2.6		
O_2–$H^{\delta 1}$	2.9		
B. Intramolecular Contacts			
O_1–C_2 (i)	3.26	O_1^γ–O_1 (i)	3.02[d]
C_1–N_1 (i)	3.27	O_1^γ–O_1^γ (i)	3.13[d]
O_1–N_1 (i)	3.28	C_2–O_1 (ii)	3.28
		N_1–C_1 (ii)	3.42
C. Hydrogen Bonds			
N_2–H···O_2 (ii)	2.89	O_1···H–N_1 (iii)	2.93
N_1–H···O_1 (iii)	2.88	N_2–H···O_2 (iii)	2.90
O^7–H···O_1 (iv)	2.70	O_2^7–H···O_3 (iv)	2.61
		O_3–H···O_2 (v)	2.77
		O_3–H···O_2^7 (vi)	2.87

[a] The first atom is in the molecule selected in Table I and shown with hydrogens in Figures 4 and 5. [b] (i) $1/2 - x, -y, -1/2 + z$; (ii) $x, y, -1 + z$; (iii) $x, y, 1 + z$; (iv) $1/2 + x, 1/2 - y, 1 - z$. [c] (i) $1 - x, -1/2 + y, 1 - z$; (ii) $1 - x, -1/2 + y, -z$; (iii) $x, 1 + y, z$; (iv) x, y, z; (v) $x, 1 + y, 1 + z$; (vi) $2 - x, 1/2 + y, 2 - z$. [d] Possible weak hydrogen bonds.

Crystal Packing and Hydrogen Bonding. Hydrogen bonds and intermolecular contacts of interest are listed in Table VII. Hydrogen bonds of the N–H···O type bind the DKP rings in rows parallel to the *c* axis in *cyclo*-(Gly-L-Tyr) and *b* axis in *cyclo*-(L-Ser-L-Tyr). The two N–H···O distances for each DKP ring (Table VII) agree with those found in other examples of associating DKP rings and in amino acids in general.[31,32] The short crystallographic repeat distances dictated by this hydrogen bonding (6.17 Å in *cyclo*-(Gly-L-Tyr) and 6.19 Å in *cyclo*-(L-Ser-L-Tyr)) favor the tilted arrangement of the tyrosine rings mentioned

(29) E. Hill, D. Tsernoglou, L. Webb, L. Banaszak, T. Jacobi, R. Ellis, and J. Fritsch, *J. Mol. Biol.*, 72, 577 (1972).
(30) IUPAC–IUB Commission on Biological Nomenclature, *Biochemistry*, 9, 3471 (1970).
(31) B. K. Vainshtein, *Advan. Struct. Res. Diffr. Methods*, 1, 24 (1964).
(32) G. V. Gurskaya, "The Molecular Structure of Amino Acids," Plenum Publishing Co., New York, N. Y., 1968, p 113.

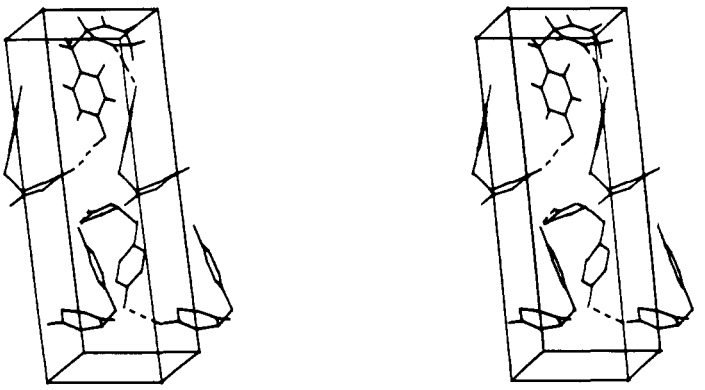

Figure 4. Stereopicture of the molecular packing and hydrogen bonding in *cyclo*-(Gly-L-Tyr) crystals. The view is approximately normal to the *ab* plane. The molecule chosen for the asymmetric unit in Tables I and VII is shown with hydrogens. Hydrogen bonds are indicated by dashed lines. Other hydrogen bonds bind the diketopiperazine rings in rows parallel to the *c* axis.

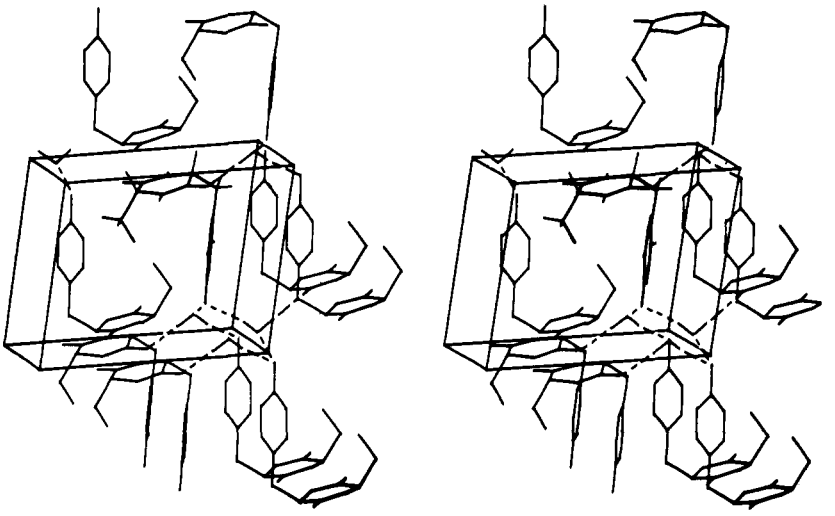

Figure 5. Stereopicture of the molecular packing and hydrogen bonding in *cyclo*-(L-Ser-L-Tyr) viewed approximately normal to the *ac* plane. The molecule chosen for the asymmetric unit in Tables I and VII is shown with hydrogens. The presumed hydrogen bonding scheme involving the water molecules is shown by dashed lines. Hydrogens on the water molecules were not positioned crystallographically but are placed in accordance with the hydrogen bonding scheme. The N–H···O hydrogen bonds between the diketopiperazine rings are not shown.

above. The aromatic ring must tilt away from a position parallel to these crystallographic axes in order to avoid an overlap of hydrogen atoms.

The molecular packing in the crystal and the remaining hydrogen bonds are shown in Figures 4 and 5. The DKP and aromatic rings interact at the normal stacking distances of about 3.3 Å (Table VII). The seryl side chain makes it impossible for *cyclo*-(L-Ser-L-Tyr) molecules to pack as tightly as the *cyclo*-(Gly-L-Tyr) molecules in the crystal. The relatively loose binding of the seryl hydroxyl groups and water molecules in *cyclo*-(L-Ser-L-Tyr) is reflected by the relatively high temperature factors of the seryl and water oxygens (Table I).

All the molecules in *cyclo*-(L-Ser-L-Tyr) crystals are bound by a three-dimensional network of hydrogen bonds, but the only interaction between adjacent layers in *cyclo*-(Gly-L-Tyr) crystals is the van der Waals attraction between overlapping DKP rings. There are three hydrogen bonds for each *cyclo*-(Gly-L-Tyr) molecule; two of these bonds connect the DKP rings. The other hydrogen bond is the connecting link for the ribbons of molecules which are perpendicular to *b* as shown in Figure 4. However, for each peptide molecule in *cyclo*-(L-Ser-L-Tyr) crystals there are at least five hydrogen bonds; two of them connect the DKP rings along the *b* axis, and three of them involve a water molecule. The phenolic oxygen of the molecule in the asymmetric unit is hydrogen bonded through a water molecule to the carbonyl oxygen and the phenolic oxygen of the molecules shown in Figure 5. Peptide and water molecules are alternately hydrogen bonded in a spiral along each *b* twofold screw axis. The intermolecular distances in Table VII suggest two possible weak hydrogen bonds from the seryl oxygen to molecules related by the twofold screw axis at the center of the unit cell. Our inability to locate the seryl hydroxy hydrogen in the difference map precludes further characterization of this interaction. A distribution of the hydrogen atom between two or more positions is likely.

Discussion

The present crystallographic results substantiate the previous conclusion[3] that the preference for the folded form in solution results from a direct interaction be-

tween the DKP and aromatic rings rather than from a release of ordered solvent molecules. In solution, nmr studies showed the folded form to be favored by an enthalpy change of about 3 kcal/mol, although it has a more negative entropy by about 4 cal/(mol deg).[3,4] Molecular ortital calculations on the *cyclo*-(Gly-L-Phe) molecule give a global energy minimum at $\chi^1 = \chi^2 = 60°$, and favor the folded conformation over the two unfolded conformations by 1.6 kcal/mol.[33] Empirical potential energy calculations find the lowest energy at $\chi^1 = 60°, \chi^2 = 90°$.[34]

The nmr spectrum of *cyclo*-(Gly-L-Tyr) in dimethyl sulfoxide solution is consistent with a flagpole boat conformation of the DKP ring with a twist no larger than that found crystallographically. When Kopple and Ohnishi[4] compared observed NH–C$^\alpha$H coupling constants with those expected for a twist boat and a bowsprit boat conformation, they concluded that the conformation of several *cyclo*-(Gly-X) dipeptides must be a twist boat. But when the flagpole boat conformation is considered, it is clear that this model also fits the observed coupling constants.[35]

Structure Correlations. An examination of X-ray and nmr data now permits correlations to be made between the amino acid sequence and the preferred conformation of the cyclic peptide backbone. Aromatic ring shielding effects together with peptide H$^\alpha$–H$^\beta$ and NH–C$^\alpha$H coupling constants allow one to identify which of the three DKP ring conformations shown in Figure 1 is the major contributor in solution. A recently developed expression relating observed NH–C$^\alpha$H coupling constants to the dihedral angle ϕ has been shown to be valid for several cyclic oligopeptides[36] including the *cyclo*-(Gly-L-Tyr) structure.[35]

The flagpole conformation (Figure 1B) was first proposed to explain the observed aromatic ring shielding effects in the nmr spectra of *cyclo*-(Gly-L-Phe) and *cyclo*-(D-Ala-L-Phe).[2] Coupling constants similar to those of *cyclo*-(Gly-L-Tyr), which are observed for a number of other nonsubstituted diketopiperazines, including *cyclo*-(Gly-L-Phe), *cyclo*-(Gly-L-Trp), and *cyclo*-(Gly-L-Val), indicate that these also prefer a boat conformation with the substituent in a flagpole type of orientation.[4,35]

Although the preference for the folded form appears to be less pronounced for *cyclo*-(Gly-L-Val) than for the aromatic residue case,[4,33,35] an interaction with the DKP ring, probably of a dipole–induced dipole type, appears to exert some influence in all cases. One can easily see from Figure 1 that, in the folded form, this attractive interaction is greatest for the DKP ring in the flagpole form with the substituent quasi-axial.

When the second amino acid is bulkier than glycine, additional steric considerations become important. Space-filling models clearly show that if both amino acids have the same configuration, as in Figure 1, side-chain interference may exclude the flagpole conformation, particularly when one substituent is aromatic. However, a near planar conformation of the DKP ring (Figure 1A) can, as in *cyclo*-(L-Ser-L-Tyr), remove this interference and still maintain as much interaction as possible between a substituent and the DKP ring. A bowsprit boat conformation, on the other hand, would sacrifice most of this interaction. This analysis has been used to explain the dominance of the planar conformation in *cyclo*-(L-Ala-L-Phe) as determined by nmr.[2] Additional confirmation of this hypothesis is given by the similarity between coupling constants of *cyclo*-(L-Val-L-Try) and *cyclo*-(Gly-Gly). Application of the above mentioned coupling constant relationship shows that the DKP rings in both are predominantly planar.[35]

Since cyclic dipeptides with no aromatic side chains are apparently less affected by attractive interactions between the substituent and DKP ring, one might expect the DKP ring to minimize ring strain by assuming one (or both) boat conformations. Interactions between side chains might favor one boat form over the other. However, the lessened importance of intramolecular interactions with the DKP ring apparently makes the various DKP ring conformations very close in energy and makes other effects such as crystal packing forces more important. Thus, in the crystalline state, DKP itself and the DKP ring in the trans compound, *cyclo*-(D-Ala-L-Ala), are essentially planar.[5,6,37] On the other hand, *cyclo*-(L-Ala-L-Ala) assumes a boat conformation as expected;[10,11] the bowsprit form is observed, although side-chain interference would not exclude the flagpole form. Analyses of forces in the crystals will have to await detailed calculations, but one can see how in each case the observed molecular shape accommodates close packing, hydrogen bonding, and the intermolecular stacking interactions.

In certain cases, the nature of the side chain may strongly favor one DKP conformation. For example, in *cyclo*-(L-Pro-L-Pro) the DKP ring would favor the bowsprit form.[8] For *cyclo*-(L-Pro-L-Leu), the flagpole form is excluded, and in the crystalline state, the DKP ring is in the bowsprit boat form with a large deviation from planarity.[12] In this peptide, the presence of the proline ring may enhance the stability of this boat form relative to that of the planar form. The opposite kind of boat, the flagpole form, has been found recently in a cyclic dipeptide whose DKP ring conformation is highly restricted by a tetrasulfide bridge between the α carbons.[38] These observations, together with the *cyclo*-(Gly-L-Tyr) and *cyclo*-(L-Ala-L-Ala) structures, stress the importance of specifying which type of boat, flagpole or bowsprit, characterizes a particular nonplanar cyclic dipeptide structure.

The relationship between amino acid sequence and the DKP ring conformation is thus reasonably clear for cyclic peptides with aromatic residues, although ambiguities may exist when these are not present. The maximization of DKP aromatic ring interaction and the avoidance of side-chain interference appear to have the greatest influence on DKP ring conformation. One would therefore expect *cyclo*-(Gly-X), where X is aromatic, to be a flagpole boat. In *cyclo*-(L-Y-L-X), the introduction of the Y residue prevents the flagpole form; unless Y is too bulky, a nearly planar DKP ring

(33) J. Caillet, B. Pullman, and B. Maigret, *Biopolymers*, **10**, 221 (1971).
(34) G. N. Ramachandran, University of Chicago, personal communication, 1971.
(35) K. D. Kopple, Illinois Institute of Technology, personal communication, 1971.
(36) G. N. Ramachandran, R. Chandrasekaran, and K. D. Kopple, *Biopolymers*, **10**, 2113 (1971).
(37) E. Benedetti, P. Corradini, and C. Pedone, *J. Phys. Chem.*, **73**, 2891 (1969).
(38) B. R. Davis and I. Bernal, *Proc. Nat. Acad. Sci. U. S.*, **70**, 279 (1973).

allows interaction between X and the DKP ring without interference between X and Y. For the *cyclo*-(Y-Z) case where Y and Z are both L or D and are not aromatic, one can at lease predict some kind of boat subject to Y-Z steric effects.

Acknowledgments. This work was begun with Research Corporation assistance and completed under National Science Foundation Grant No. GB-7376 and National Science Foundation Instrumentation Grant No. GP-10343. We are also grateful to Professors K. Kopple and G. Ramachandran and to Drs. A. Lakshminarayanan and R. Chandrasekaran for helpful discussions and computations. We are indebted to Mr. B. Stevens for computational assistance. The molecular displays[29] were made possible by the Computer Systems Laboratory of Washington University Medical School and the kind assistance of Dr. J. M. Fritsch.

Supplementary Material Available. Observed and calculated structure factors for *cyclo*-(Gly-L-Tyr) and *cyclo*-(L-Ser-L-Tyr) 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, 20× 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 $7.00 for photocopy or $2.00 for microfiche, referring to code number JACS-73-6803.

Structure of Copper(II) *n*-Propylporphine. Effect of a Metallo Substitution on the Free Base Macrocycle

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Received March 14, 1973

Abstract: The crystal and molecular structure of copper(II) α,β,γ,δ-tetra-*n*-propylporphine has been determined by X-ray crystallographic methods. The compound crystallizes in the space group $P2_1/c$ with two molecules per unit cell of dimensions $a = 5.010$ (7), $b = 11.55$ (4), $c = 22.50$ (9) Å, $\beta = 99.1$ (2)°. The metallo structure is isomorphous with the free base and it was solved from a sharpened, three-dimensional Patterson function. The structure was refined by full-matrix, least-squares procedures with the final R factor being 0.059 ($R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$). The molecule is centrosymmetric with half of a molecule per asymmetric unit. The two independent pyrrole rings have very similar bond distances and bond angles and have the geometry of the azapyrrole of the free base structure. This gives the macrocycle a symmetry closely approaching D_{4h}. The Cu(II) ion substitution causes a general "squaring-up" and overall contraction of the central core region of the free base macrocycle. This is accomplished by the movement of the pyrrole rings. The individual pyrrole rings are planar to ±0.004 Å whereas the porphine macrocycle is only planar to ±0.05 Å. In the case of the latter, the pyrroles are tilted slightly with respect to each other (3.7°).

Among other derivatives in our continuing study of the structures of porphyrin molecules, we have been interested in determining the effect of a metallo substitution upon the porphine macrocycle when the substitution was expected intrinsically to lead only to minor perturbations of the original system. With the recent advent of a precise description of the geometry of the free base macrocycle,[1] such a plan became feasible. Therefore, to this end, we have focused our attention upon the Cu(II) state. The ionic radius of Cu(II) was expected to be nearly optimal for undistorted accommodation within the central porphine core;[2] moreover, the ion favors square planar coordination and this should minimize geometrical perturbations on the macrocyclic system with substitution. In this way, we hoped to isolate the effect of chemical bond formation from other extraneous factors and to establish a base from which more complicated metallo substitutions might be better understood (different ionic radii, different electronic spin states, more complicated coordination geometries).[3]

(1) P. W. Codding and A. Tulinsky, *J. Amer. Chem. Soc.*, **94**, 4151 (1972).
(2) J. L. Hoard, *Science*, **174**, 1295 (1971).

Experimental Section

Twinned crystals of copper α,β,γ,δ-tetra-*n*-propylporphine (CuTPrP)[4] in the form of purple needles were prepared by allowing methanol to diffuse into a saturated solution of the compound in toluene. Various other solvents and other conditions also gave twinned crystals in the form of hexagonal prisms. However, some of these crystals could be cleaved into two nontwinned crystal fragments. A suitable nontwinned crystal fragment with approximate dimensions 0.05 × 0.075 × 0.5 mm was used for the X-ray studies.

Preliminary X-ray diffraction measurements showed the crystal system to be monoclinic and systematic absences of reflections showed the space group to be $P2_1/c$. The lattice parameters were obtained from diffractometer measurements by the least-squares fit of the angular coordinates of 12 reflections in the range $45° < 2\theta < 80°$ [$a = 5.010$ (7), $b = 11.55$ (4), $c = 22.50$ (9) Å and $\beta = 99.1$ (2)°]. The calculated density of the crystal on the basis of two molecules per unit cell is 1.396 g cm^{-3} and the observed density measured by flotation in aqueous silver nitrate solution is 1.39 g cm^{-3}.

The intensity data collection was carried out with Cu Kα radiation using a Picker four-circle diffractometer controlled by a Digital Equipment Corp. (DEC) 4K PDP-8 computer (FACS-I system)

(3) *E.g.*, square pyramidal chloromanganese tetraphenylporphine; B. M. L. Chen, Ph.D. Thesis, Michigan State University, 1970.
(4) We would like to thank Dr. Alan D. Adler of the New England Institute, for kindly supplying us with a sample of CuTPrP.