*Acta Cryst.* (1977). B33, 2080–2083

# Structure of a *cis*-Peptide Unit: Molecular Conformation of the Cyclic Disulphide L-Cysteinyl-L-cysteine

BY SANTE CAPASSO, CARLO MATTIA AND LELIO MAZZARELLA

*Istituto Chimico, Università di Napoli, Via Mezzocannone* 4, 80134 *Napoli, Italy*

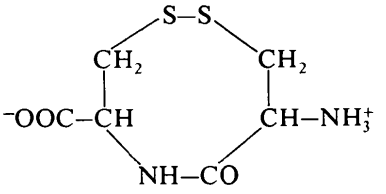
AND RAFFAELLA PULITI

*Laboratorio per la Chimica di Molecole di Interesse Biologico del CNR, Via Toiano* 2, *Arco Felice, Napoli, Italy*

(*Received* 10 *September* 1976; *accepted* 9 *December* 1976)

L-Cysteinyl-L-cysteine crystallizes in the orthorhombic space group $P2_12_12_1$ with $a = 8{\cdot}629$ (7), $b = 23{\cdot}500$ (9), $c = 5{\cdot}145$ (2) Å. The structure, solved by the heavy-atom method, was refined by least-squares calculations to an $R$ of 0·042, from 963 observed reflexions. The molecule contains a *cis*-peptide unit and a right-handed disulphide bridge with a torsion angle of 94° about the –S–S– bond. A comparison of the observed conformation with that predicted by energy calculations indicates that a better agreement should be obtained by using in the energy calculation a softened potential function for the H · · · H interactions.

In connexion with a research programme on the structure of cysteine-containing peptides, the cyclic disulphide L-cysteinyl-L-cysteine (I) has been synthesized in our laboratory and studied by X-ray methods.




(I)

The molecule can be constructed only with a '*cisoid*' peptide unit, and energy calculations have shown that the allowed conformations can be obtained only with a non-planar peptide unit (Ramachandran & Sasisekharan, 1968). Smaller distortions from planarity, however, are predicted if the bond angles at $\alpha$-C atoms are allowed to change even by a small amount (Chandrasekaran & Balasubramanian, 1969).

In the light of the above-quoted results and of the importance that theoretical predictions of molecular geometry have acquired in recent years, it appears of interest to compare the molecular features found in the solid state with those predicted by energy calculations.

## Experimental

### *Synthesis and crystallization*

The present compound was synthesized according to the procedure described by Izumiya & Greenstein (1954). This involves the preparation of *S*-benzyl-L-cysteinyl-*S*-benzyl-L-cysteine, removal of the benzyl groups and oxidation of the purified free dipeptide with a stream of air at $p$H = 6·5. The concentration of the dipeptide in the reaction mixture was kept at 0·15% to decrease the yield of polymeric materials (Wade, Winitz & Greenstein, 1956). The compound was purified by repeated crystallizations and was finally crystallized from water to obtain single crystals, suitable for X-ray analysis, in the form of well shaped long prisms.

### *Crystal data*

A single crystal, 0·06 × 0·07 × 0·6 mm, was selected for X-ray analysis. Preliminary photographic work showed that the crystals are orthorhombic; the systematic absences ($h00$ for $h$ odd, $0k0$ for $k$ odd and $00l$ for $l$ odd) uniquely determine the space group $P2_12_12_1$. Precise lattice constants were obtained from a least-squares refinement of the setting angles of 17 reflexions on a Siemens AED automatic diffractometer with Cu $K\alpha$ radiation ($\lambda = 1{\cdot}5418$ Å). The density, measured by the flotation method, indicated the presence of one

Table 1. *Crystal data*

| | |
|---|---|
| Formula $C_6H_{10}N_2O_3S_2.H_2O$ | $M_r = 240{\cdot}3$ |
| Space group $P2_12_12_1$ | $D_c = 1{\cdot}528$ g cm$^{-3}$ |
| $a = 8{\cdot}629 \pm 0{\cdot}007$ Å | $D_m = 1{\cdot}508$ |
| $b = 23{\cdot}500 \pm 0{\cdot}009$ | $F(000) = 504$ |
| $c = 5{\cdot}145 \pm 0{\cdot}002$ | $\mu$(Cu $K\alpha$) = 45·5 cm$^{-1}$ |
| $Z = 4$ | |
| Maximum $\sin\theta/\lambda$ | 0·58 Å$^{-1}$ |
| Number of measured reflexions | 1090 |
| Observed reflexions [$I > 2{\cdot}5\,\sigma(I)$] | 963 |

water molecule per independent unit. A summary of the crystal data is given in Table 1. For the intensity measurements the $\theta$–$2\theta$ scan technique was employed with a scan rate of 1·8° min$^{-1}$ and a scan range of (1·2 + 0·3 tan $\theta$)°; each reflexion was measured twice. The intensity of a standard reflexion was recorded every 20 reflexions to monitor crystal stability. Its intensity remained essentially constant throughout the run. 1090 independent reflexions were measured within the limit of sin $\theta/\lambda = 0{\cdot}58$ Å$^{-1}$; of these, 963 were considered observed [$I > 2{\cdot}5\ \sigma(I)$]. The data were corrected for Lorentz and polarization factors and for absorption (Busing & Levy, 1957).

*Structure determination and refinement*

The structure was solved through the location of the two non-equivalent S atoms from a sharpened Patterson function. The search for the expected S–S intramolecular vector, at about 2·05 Å from the origin, led to an unambiguous determination of the S atoms. The Patterson coordinates were refined by the block-diagonal least-squares method (unit weights and $B = 3$ Å$^2$) to an $R$ of 0·46, and used to calculate phases for the observed structure amplitudes. The subsequent Fourier synthesis showed all the remaining non-hydrogen atoms. Three cycles of least-squares refinement with anisotropic temperature factors for the non-hydrogen atoms reduced $R$ to 0·062.

The refinement was stopped at this point to calculate a difference Fourier map to locate the H atoms. All the H atoms appeared in the difference map with heights varying from 0·30 to 0·51 e Å$^{-3}$. The final parameters were obtained by a few more cycles of block-diagonal least-squares refinement. In the refinement, the H atoms did not move significantly from the original positions in the Fourier map. The weighting scheme $w^{-1} = 0{\cdot}30 + 0{\cdot}05F_o + 0{\cdot}00175F_o^2$ was derived from an analysis of $\langle \Delta F^2 \rangle$ as a function of $F_o$. At convergence, $R$ was 0·042 based on the observed reflexions.

In Table 2 the final fractional coordinates are reported with the corresponding standard deviations.* Atomic scattering factors for C, N, O and S atoms were taken from Cromer & Mann (1968) and for H atoms from Moore (1963). The corrections for the real and imaginary components of the anomalous dispersion for the S atoms ($\Delta f' = 0{\cdot}3$, $\Delta f'' = 0{\cdot}6$ for Cu $K\alpha$) were included in the last steps of refinement.

Table 2. *Final fractional coordinates* (×10$^4$, *for* H ×10$^3$) *and isotropic thermal parameters* (×10) *of hydrogen atoms with their standard deviations in parentheses*

| | $x$ | $y$ | $z$ | $B$ |
|---|---|---|---|---|
| S$^\gamma$(1) | 1407 (1) | 2008 (1) | 2071 (3) | |
| S$^\gamma$(2) | 3266 (1) | 2268 (1) | 4198 (4) | |
| C$^\beta$(1) | 601 (5) | 1409 (2) | 3825 (10) | |
| C$^\alpha$(1) | 1438 (4) | 840 (1) | 3379 (7) | |
| C(1′) | 2792 (4) | 762 (1) | 5250 (7) | |
| C$^\alpha$(2) | 4629 (4) | 1264 (2) | 2311 (8) | |
| C$^\beta$(2) | 4917 (6) | 1896 (2) | 2711 (9) | |
| C(2′) | 6150 (4) | 993 (2) | 1380 (8) | |
| N(1) | 328 (3) | 368 (1) | 3855 (7) | |
| N(2) | 4162 (3) | 987 (1) | 4699 (6) | |
| O(1) | 2551 (3) | 499 (1) | 7314 (5) | |
| O(2′) | 7101 (3) | 843 (1) | 3031 (6) | |
| O(2″) | 6319 (4) | 934 (2) | −1035 (6) | |
| $W$ | 1105 (4) | 291 (1) | 8670 (7) | |
| H$^{\beta 1}$(1) | 54 (8) | 145 (3) | 602 (14) | 41 (16) |
| H$^{\beta 2}$(1) | −38 (6) | 137 (2) | 284 (11) | 19 (12) |
| H$^\alpha$(1) | 181 (5) | 80 (2) | 168 (9) | 12 (9) |
| H$^\alpha$(2) | 387 (4) | 120 (2) | 119 (8) | 4 (7) |
| H$^{\beta 1}$(2) | 578 (8) | 202 (3) | 449 (15) | 47 (17) |
| H$^{\beta 2}$(2) | 530 (7) | 207 (3) | 100 (14) | 33 (16) |
| H$^1$(1) | 81 (5) | 87 (2) | 367 (9) | 4 (8) |
| H$^2$(1) | −14 (6) | 37 (2) | 537 (12) | 21 (12) |
| H$^3$(1) | −43 (5) | 42 (2) | 317 (10) | 16 (10) |
| H(2) | 488 (5) | 94 (2) | 560 (10) | 12 (9) |
| H$W$(1) | −212 (7) | 57 (2) | 863 (12) | 30 (13) |
| H$W$(2) | −148 (8) | 2 (3) | 895 (17) | 57 (21) |

## Results

Fig. 1 shows the overall conformation of the molecule, bond lengths and valence angles are reported in Table 3.

The results of the X-ray analysis confirm the earlier suggestion that the molecule contains a *cis*-peptide unit (Ramachandran & Sasisekharan, 1968). The atoms C$^\alpha$(1), C(1′), O(1), N(2), C$^\alpha$(2) and H(2) are coplanar to within ±0·04 Å; the torsion angle $\omega$ (Table 6) about the peptide bond is −7°.

* Lists of structure factors and anisotropic thermal parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 32390 (6 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

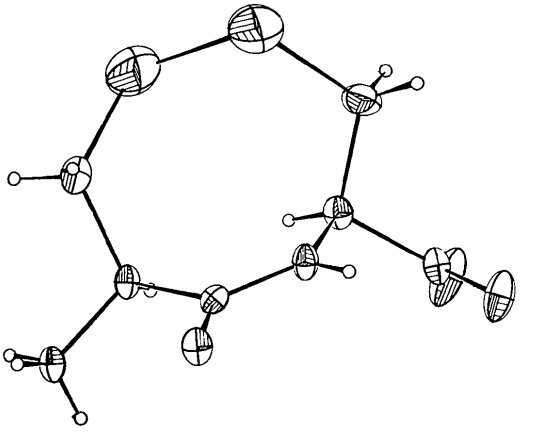


Fig. 1. View of molecule with thermal ellipsoids.

Although the position of $C^{\alpha}(2)$ and of the amide H atom might indicate a slight pyramidal character of the bonds meeting at the N atom (Winkler & Dunitz, 1971; Ramachandran, 1974), such distortion does not appear to be meaningful when compared with the accuracy of the H parameters (Table 4).

The bond parameters within the *cis*-peptide unit compare well with those found for a number of diketopiperazines and with the values suggested for a standard *cis*-peptide unit (Ramachandran & Venkatachalam, 1968).

The helical sense of the disulphide group in the molecule is right-handed; the torsion angle $C^{\beta}(1)$–$S^{\gamma}(1)$–$S^{\gamma}(2)$–$C^{\beta}(2)$ is +94°. A similar conformation has been found in the hexagonal (Oughton & Harrison, 1959) and tetragonal (Chaney & Steinrauf, 1974) forms of L-cystine, with dihedral angles of +106 and +69·3° respectively. The dimensions of the C–S–S–C fragment have been shown to be correlated with the torsion angle about the –S–S– bond (Jones, Bernal, Frey & Koetzle, 1974) and our values agree with those of compounds for which the torsion angle is close to ±90°.

The N–H bond lengths are somewhat shorter than those usually found in X-ray analysis; all the bond angles, however, involving H atoms are in the expected range. The structure is fully hydrogen bonded (Table 5). All three H atoms of the free amino group participate in hydrogen bonding with two water molecules [$N(1)\cdots W = 2{\cdot}776$, $N(1)^{i}\cdots W = 2{\cdot}952$ Å] and with the carbonyl O [$N(1)\cdots O(1)^{iv} = 2{\cdot}851$ Å]. The water molecule itself, as is found in many crystal structures of amino acids and peptides, mediates contacts among the acid and basic groups. It makes four approximately tetrahedral hydrogen bonds with two $-NH_3^+$ groups and with the O atoms of two carboxyl groups [$W\cdots O(2')^{iii} = 2{\cdot}818$, $W\cdots O(2'')^{ii} = 2{\cdot}692$ Å]. The amide N also makes one hydrogen bond to O(2″) [$N(2)\cdots O(2'')^{i} = 2{\cdot}884$ Å]. Of the two carboxyl O atoms, O(2″) is involved in two hydrogen bonds which lie almost in the plane of the carboxyl group, whereas O(2′) accepts only one in a direction almost normal to the carboxyl group. The different interactions of the two O atoms are also correlated with the values of bond angles and lengths of the carboxyl group; C(2′)–O(2″) is 0·03 Å longer than C(2′)–O(2′) and the angle $C^{\alpha}(2)$–C(2′)–O(2′) is slightly larger than $C^{\alpha}(2)$–C(2′)–O(2″) (Koetzle, Frey, Lehman & Hamilton, 1973).

The packing is illustrated in Fig. 2. All the hydrogen bonds occur in a double layer parallel to the *XZ* plane. The intermolecular distances are within the limits of the

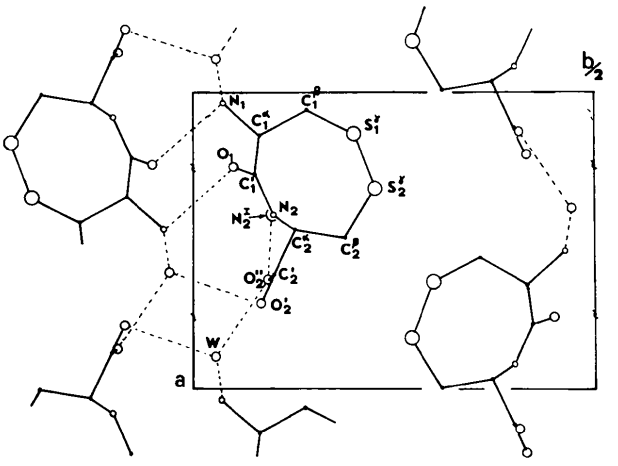



Fig. 2. Molecular packing projected on the *ab* plane: the hydrogen bonds are shown by dashed lines. [Note: $C_1^{\alpha} \equiv C^{\alpha}(1)$ *etc.*]

Table 5. *Hydrogen bonds*

| *C* | *D* | *A* | *D–A* | ∠*C D A* |
|---|---|---|---|---|
| | *W* | $O(2')^{iii}$ | 2·818 Å | |
| | *W* | $O(2'')^{ii}$ | 2·692 | |
| $C^{\alpha}(2)$–N(2) | | $O(2'')^{i}$ | 2·884 | 118·9° |
| C(1′)–N(2) | | $O(2'')^{i}$ | | 113·2 |
| $C^{\alpha}(1)$–N(1) | | $O(1)^{iv}$ | 2·851 | 94·2 |
| $C^{\alpha}(1)$–N(1) | | *W* | 2·776 | 119·0 |
| $C^{\alpha}(1)$–$N(1)^{i}$ | | *W* | 2·952 | 100·0 |

Symmetry code
(i) $x, y, z + 1$; (ii) $x - 1, y, z + 1$; (iii) $\frac{1}{2} - x, -y, \frac{1}{2} + z$; (iv) $\frac{1}{2} - x, -y, z - \frac{1}{2}$

Table 3. *Molecular dimensions*

The standard deviations are given in parentheses.

| | | | |
|---|---|---|---|
| $S^{\gamma}(1)$–$S^{\gamma}(2)$ | 2·036 (2) Å | $S^{\gamma}(1)$–$S^{\gamma}(2)$–$C^{\beta}(2)$ | 104·2 (1)° |
| $S^{\gamma}(1)$–$C^{\beta}(1)$ | 1·811 (5) | $C^{\beta}(1)$–$S^{\gamma}(1)$–$S^{\gamma}(2)$ | 105·5 (1) |
| $C^{\beta}(1)$–$C^{\alpha}(1)$ | 1·538 (6) | $C^{\alpha}(1)$–$C^{\beta}(1)$–$S^{\gamma}(1)$ | 115·0 (1) |
| $C^{\alpha}(1)$–N(1) | 1·485 (5) | C(1′)–$C^{\alpha}(1)$–$C^{\beta}(1)$ | 111·8 (2) |
| $C^{\alpha}(1)$–C(1′) | 1·525 (5) | N(2)–C(1′)–$C^{\alpha}(1)$ | 120·0 (1) |
| C(1′)–N(2) | 1·325 (5) | $C^{\alpha}(2)$–N(2)–C(1′) | 127·6 (2) |
| C(1′)–O(1) | 1·247 (4) | $C^{\beta}(2)$–$C^{\alpha}(2)$–N(2) | 111·8 (2) |
| N(2)–$C^{\alpha}(2)$ | 1·448 (5) | $S^{\gamma}(2)$–$C^{\beta}(2)$–$C^{\alpha}(2)$ | 113·2 (2) |
| $C^{\alpha}(2)$–C(2′) | 1·536 (5) | $C^{\beta}(1)$–$C^{\alpha}(1)$–N(1) | 108·8 (1) |
| C(2′)–O(2′) | 1·233 (5) | C(1′)–$C^{\alpha}(1)$–N(1) | 107·5 (1) |
| C(2′)–O(2″) | 1·259 (5) | $C^{\alpha}(1)$–C(1′)–O(1) | 118·0 (1) |
| $C^{\alpha}(2)$–$C^{\beta}(2)$ | 1·520 (6) | N(2)–C(1′)–O(1) | 121·9 (2) |
| $C^{\beta}(2)$–$S^{\gamma}(2)$ | 1·838 (5) | N(2)–$C^{\alpha}(2)$–C(2′) | 108·4 (1) |
| (C–H) mean | 1·03 (6) | $C^{\beta}(2)$–$C^{\alpha}(2)$–C(2′) | 108·0 (2) |
| N(2)–H(2) | 0·78 (5) | $C^{\alpha}(2)$–C(2′)–O(2′) | 118·2 (2) |
| [N(1)–H] mean | 0·81 (5) | $C^{\alpha}(2)$–C(2′)–O(2″) | 116·9 (2) |
| (*W*–H) mean | 0·90 (7) | O(2′)–C(2′)–O(2″) | 124·9 (2) |

Table 4. *Deviations* (Å) *from least-squares planes through the peptide group*

| | | | | | |
|---|---|---|---|---|---|
| $C^{\alpha}(2)$ | −0·029 | −0·109* | O(1) | −0·020 | 0·004 |
| C(1′) | −0·004 | −0·011 | N(2) | 0·044 | 0·004 |
| $C^{\alpha}(1)$ | 0·009 | 0·003 | H(2) | −0·001* | −0·041* |

* Atom not included in the calculation of the plane.

Table 6. *Experimental values of the relevant conformational parameters of cyclic* L-*cysteinyl*-L-*cysteine compared with those of the two lower-energy conformations as calculated by Chandrasekaran & Balasubramanian* (1969)

The values reported in columns I and II have been changed according to the IUPAC convention.

| Torsion angles* | Atoms involved | Present work | I | II |
|---|---|---|---|---|
| $\psi$ | N(1)–C$^{\alpha}$(1)–C(1′)–N(2) | 156° | 132° | 150° |
| $\omega$ | C$^{\alpha}$(1)–C(1′)–N(2)–C$^{\alpha}$(2) | −7 | −12 | 14 |
| $\varphi$ | C(1′)–N(2)–C$^{\alpha}$(2)–C(2′) | −129 | −150 | −138 |
| $\chi_1^1$ | N(1)–C$^{\alpha}$(1)–C$^{\beta}$(1)–S$^{\gamma}$(1) | −154 | 177 | −165 |
| $\chi_1^2$ | C$^{\alpha}$(1)–C$^{\beta}$(1)–S$^{\gamma}$(1)–S$^{\gamma}$(2) | −81 | 52 | −79 |
| $\chi_1^3$ | C$^{\beta}$(1)–S$^{\gamma}$(1)–S$^{\gamma}$(2)–C$^{\beta}$(2) | 94 | −98 | 100 |
| $\chi_2^2$ | C$^{\alpha}$(2)–C$^{\beta}$(2)–S$^{\gamma}$(2)–S$^{\gamma}$(1) | −48 | 75 | −51 |
| $\chi_2^1$ | N(2)–C$^{\alpha}$(2)–C$^{\beta}$(2)–S$^{\gamma}$(2) | −54 | −74 | −57 |

* IUPAC–IUB Commission on Biochemical Nomenclature (1970).

accepted van der Waals radii and the shortest contact between S atoms is 3·73 Å.

## Conclusion

The most stable conformations of cyclic L-cysteinyl-L-cysteine have been calculated by Chandrasekaran & Balasubramanian (1969). The authors reported two possible structures for the molecule represented by the conformational parameters listed in columns I and II in Table 6 [the dihedral angles have been changed from the original papers according to the convention of the IUPAC–IUB Commission on Biochemical Nomenclature (1970)]. The main difference between the two conformations lies in the opposite chirality of the disulphide bridge.

The X-ray analysis shows that the conformation II, which has a slightly higher calculated energy, is that found in the solid state. However, in the recently published structure of cyclo-L-cysteine (Mez, 1974), a molecule obtained by further cyclization of L-cysteinyl-L-cysteine (Kamber, 1971), a left-handed disulphide chirality is found and the torsion angles about the bonds C$^{\beta}$(1)–S, C$^{\beta}$(2)–S and S–S are close to those of conformation I.

The differences between the experimental and calculated conformation II deserve some further comment. The energy calculation performed with tetrahedral bond angles at $\alpha$-C atoms predicted a much larger deviation from planarity of the peptide group ($\omega \sim 30°$). If the above bond angles are allowed to refine in the energy calculations the two conformations I and II are obtained. The resulting peptide group is more planar; however, the deviation from planarity in conformation II ($\omega = 14°$) is opposite to that of the experimental conformation ($\omega = -7°$).

This discrepancy can hardly be ascribed to the constraints imposed by the packing forces and it is related, in our opinion, to the short contact (~2 Å) between the H atoms of the $\alpha$-C atoms.* By using in the energy calculations an H···H potential function minimized at a distance of 2·4 Å, this interaction gives a positive contribution to the energy, which can be partially decreased by a positive rotation about the C(1′)–N(2) bond. This rotation is not observed in the X-ray structure, suggesting that for the H···H interaction a softened potential function minimized at a distance of 2 Å could be more appropriate in this case. Based on other studies, similar suggestions have also appeared recently in the literature (Ramachandran, 1974; Jordan, 1973).

* The experimental positions of H(1) and H(2) give bond angles close to the tetrahedral values, but rather short bond lengths (on average 0·92 Å). If the bond lengths are corrected to the accepted value of 1·08 Å, the distance between the two H atoms changes from 2·02 to 1·85 Å.

## References


BUSING, W. R. & LEVY, H. A. (1957). *Acta Cryst.* **10**, 180–182.

CHANDRASEKARAN, R. & BALASUBRAMANIAN, R. (1969). *Biochim. Biophys. Acta*, **188**, 1–9.

CHANEY, M. O. & STEINRAUF, L. K. (1974). *Acta Cryst.* B**30**, 711–716.

CROMER, D. T. & MANN, J. B. (1968). *Acta Cryst.* A**24**, 321–324.

IUPAC–IUB COMMISSION ON BIOCHEMICAL NOMENCLATURE (1970). *J. Mol. Biol.* **52**, 1–17.

IZUMIYA, N. & GREENSTEIN, J. P. (1954). *Arch. Biochem. Biophys.* **52**, 203–216.

JONES, D. D., BERNAL, I., FREY, M. N. & KOETZLE, T. F. (1974). *Acta Cryst.* B**30**, 1220–1227.

JORDAN, F. (1973). *J. Theor. Biol.* **41**, 375–395.

KAMBER, B. (1971). *Helv. Chim. Acta*, **54**, 927–930.

KOETZLE, T. F., FREY, M. N., LEHMAN, M. S. & HAMILTON, W. C. (1973). *Acta Cryst.* B**29**, 2571–2575.

MEZ, H. C. (1974). *Cryst. Struct. Commun.* **3**, 657–660.

MOORE, F. H. (1963). *Acta Cryst.* **16**, 1169–1175.

OUGHTON, B. M. & HARRISON, P. M. (1959). *Acta Cryst.* **12**, 396–404.

RAMACHANDRAN, G. N. (1974). *Peptides, Polypeptides and Proteins*, Proc. Rehovot Symp., Israel, May 1974, edited by E. R. BLOUT, F. A. BOVEY, M. GOODMAN & N. LOTAN, pp. 14–34. New York: John Wiley.

RAMACHANDRAN, G. N. & SASISEKHARAN, V. (1968). *Advanc. Protein Chem.* **23**, 283–437.

RAMACHANDRAN, G. N. & VENKATACHALAM, C. M. (1968). *Biopolymers*, **6**, 1255–1262.

WADE, R., WINITZ, M. & GREENSTEIN, J. P. (1956). *J. Amer. Chem. Soc.* **78**, 373–377.

WINKLER, F. K. & DUNITZ, J. D. (1971). *J. Mol. Biol.* **59**, 169–182.