References

ALGIERI, A. A., LUKE, G. M., STANDRIDGE, R. T., BROWN, M., PARTYKA, R. A. & CRENSHAW, R. R. (1983). *J. Med. Chem.* **25**, 210.

BEURSKENS, P. T., BOSMAN, W. P., DOESBURG, H. M., GOULD, R. O., VAN DEN HARK, TH. E. M., PRICK, P. A. J., NOORDIK, J. H., BEURSKENS, G., PARTHASARATHI, V., BRUINS SLOTT, H. J. & HALTIWANGER, R. C. (1985). *DIRDIF*. Technical Report 1985. Crystallography Laboratory, Toernooiveld, 6525 Ed Nijmegen, The Netherlands.

BIRKNES, B. (1977). *Acta Cryst.* B**33**, 687–691.

BLACK, J. W., DUNCAN, W. A. M., DURANT, G. J., GANELLIN, C. R. & PARSONS, M. E. (1972). *Nature (London)*, **236**, 385–390.

BRADSHAW, J., BRITTAIN, R. T., CLITHEROW, J. W., DALY, M. J., JACK, D., PRICE, B. J. & STABLES, R. (1979). *Br. J. Pharmacol.* **66**, 464–465.

BRIMBLECOMBE, R. W., DUNCAN, W. A., DURANT, G. J., EMMETT, J. C., GANELLIN, C. R. & PARSONS, M. E. (1975). *J. Int. Med. Res.* **3**, 86–92.

BROWN, T. H., BLAKEMORE, R. C., DURANT, G. J., EMMETT, J. C., GANELLIN, C. R., PARSONS, M. E., RAWLINGS, D. A. & WALKER, T. F. (1988). *Eur. J. Med. Chem.* **23**, 53–62.

CARRUTHERS, J. R. & WATKIN, D. J. (1979). *Acta Cryst.* A**35**, 698–699.

CHATAR SINGH (1965). *Acta Cryst.* **19**, 861–864.

CLARK, G. R. & PALENIK, G. J. (1972). *J. Am. Chem. Soc.* **94**, 4005–4009.

COOPER, D. G., DURANT, G. J., GANELLIN, C. R., IFE, R. J., MEESON, M. L. & SACH, G. S. (1991). *Il Farmaco*, **46**(1), 3–20.

CROMER, D. T. & WABER, J. T. (1974). *International Tables for X-ray Crystallography*, Vol. IV, edited by J. A. IBERS & W. C. HAMILTON. Birmingham: Kynoch Press. (Present distributor Kluwer Academic Publishers, Dordrecht.)

DOMENICANO, A. & MURRAY-RUST, P. (1979). *Tetrahedron Lett.* **24**, 2818–2819.

EBNOTHER, A. & WEBER, H.-P. (1976). *Helv. Chim. Acta*, **59**, 2462–2468.

HALL, S. R., RASTON, C. L., SKELTON, B. W. & WHITE, A. H. (1981). *J. Chem. Soc. Perkin Trans.* 2, pp. 1467–1472.

JAMES, M. N. G. & WILLIAMS, G. J. B. (1974*a*). *Can. J. Chem.* **52**, 1872–1879.

JAMES, M. N. G. & WILLIAMS, G. J. B. (1974*b*). *Can. J. Chem.* **52**, 1880–1888.

KAMENAR, B., PROUT, K. & GANELLIN, C. R. (1973). *J. Chem. Soc. Perkin Trans.* 2, pp. 1734–1738.

LUMMA, W. C. JR, ANDERSON, P. S., BALDWIN, J. J., BOLHOFER, W. A., HABECKER, C. N., HIRSHFIELD, J. M., PIETRUSZKIEWICZ, A. M., RANDALL, W. C., TORCHIANA, M. L., BRITCHER, S. F., CLINESCHMIDT, B. V., DENNY, G. H., HIRSCHMANN, R., HOFFMAN, J. M., PHILIPS, B. T. & STREETER, K. B. (1982). *J. Med. Chem.* **25**, 210–212.

MAIN, P., FISKE, S. J., HULL, S. E., LESSINGER, L., GERMAIN, G., DECLERCQ, J.-P. & WOOLFSON, M. M. (1980). *MULTAN*80. *A System of Computer Programs for the Automatic Solution of Crystal Structures from X-ray Diffraction Data*. Univs. of York, England, and Louvain, Belgium.

PEPINSKY, R., RATHLEV, J. & TURLEY, J. W. (1959). *Acta Cryst.* **11**, 295–292.

PRECIGOUX, G., BARRANS, Y., BUSETTA, P. & MARSAU, P. (1975). *Acta Cryst.* B**31**, 1497–1499.

PROUT, K., BURNS, K., WATKIN, D. J., COOPER, D. G., DURANT, G. J., GANELLIN, C. R. & SACH, G. S. (1993). *Acta Cryst.* B**49**, 547–559.

PROUT, K. BURNS, K., WATKIN, D. J., DURANT, G. J. & BROWN, T. H. (1993). *Acta Cryst.* C**49**, 44–46.

SCHILD, H. O. (1947). *Br. J. Pharmacol. Chemother.* **2**, 189–206.

SHARMA, B. D. & MCCONNELL, J. F. (1965). *Acta Cryst.* **19**, 797–806.

VINTER, J. G., DAVIS, A. & SAUNDERS, M. R. (1987). *J. Computer-Aided Mol. Des.* **1**, 31–53.

WALKER, N. & STUART, D. (1983). *Acta Cryst.* A**39**, 158–166.

WATKIN, D. J., CARRUTHERS, J. R. & BETTERIDGE, P. W. (1985). *CRYSTALS User Guide*. Chemical Crystallography Laboratory, Univ. of Oxford, England.

YANAGISAWA, I., HIRATA, Y. & ISHII, Y. (1984). *J. Med. Chem.* **27**, 849–857.

YELLIN, T. O., BUCK, S. H., GILMAN, D. J., JONES, D. F. & WARDLEWORTH, J. M. (1979). *Life Sci.* **25**, 2001–2009.

Acta Cryst. (1994). C**50**, 243–251

Structure of the Stacked Cyclic Oligoamides: 1,6-Diaza-2,7-cyclodecadione and 1,5,9-Triaza-2,6,10-cyclododecatrione. A Ring Model of the α-Helix

BY V. TERESHKO

Institute of Bioorganic Chemistry, Byelorussian Academy of Science, Zhodinskaya St 5/2, *Minsk* 220067, *Byelorussia*

AND J. M. MONSERRAT, J. PÉREZ-FOLCH, J. AYMAMÍ, I. FITA AND J. A. SUBIRANA*

Departament d'Enginyeria Química, ETS Enginyers Industrials de Barcelona, Universitat Politècnica de Catalunya, Av. Diagonal 647, 08028 *Barcelona, Spain*

(*Received* 1 *July* 1993; *accepted* 11 *October* 1993)

Abstract

The structure of two cyclic amides, comprised of methylene and peptide groups, is described. The cyclic amides form parallel columns of stacked hydrogen-bonded rings. The cyclic dimer of butyramide (1,6-diaza-2,7-cyclodecadione) (I), $C_8H_{14}N_2O_2$, forms monoclinic crystals, space group $P2_1/n$, $a = 4.874$ (2), $b = 11.714$ (4), $c = 8.088$ (2) Å,

* To whom correspondence should be addressed.

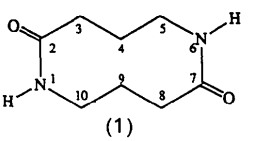
$\beta = 107.3\ (3)°$, $R = 0.125$ for 536 observed reflections, $I > 3\sigma(I)$, $Z = 2$, $D_x = 1.28\ g\ cm^{-3}$, $\lambda(Cu\ K\alpha) = 1.5418$ Å, $\mu = 7.26\ cm^{-1}$, $T = 298$ K. Extra peaks were found near the peptide groups, but the R factor could not be reduced by introducing additional parameters to account for disorder. The ten-membered ring skeleton adopts a centrosymmetric crown conformation which is rarely found in other ten-atom rings, but had been described for its isomer 1,5-diaza-6,10-cyclodecadione. A cyclic trimer of β-alanine (1,5,9-triaza-2,6,10-cyclododecatrione) (2), $C_9H_{15}N_3O_3$, is also studied. It forms trigonal crystals, $a = b = 13.74\ (2)$, $c = 4.82\ (1)$ Å, space group $R3m$, $R = 0.060$ for 161 observed reflections, $I > 2.5\sigma(I)$, $Z = 3$, $D_x = 1.35\ g\ cm^{-3}$. $\lambda(Cu\ K\alpha) = 1.5418$ Å, $\mu = 8.16\ cm^{-1}$, $T = 298$ K. The 12-atom ring has two conformations which are mirror images and co-exist in the crystal. They appear superimposed in the solved structure. The peptide groups are planar and the organization of the stacked rings is very similar to that of an α-helix.

Introduction

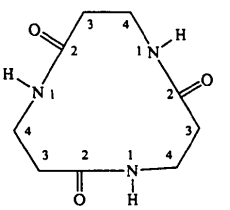
Cyclic oligamides have originated a great deal of interest owing to their biological activity (Blout, 1981), and to their presence as a by-product in the synthesis of industrial polyamides (Hermans, 1953). In particular, some natural cyclopeptides contain four α-amino acid residues and thus form 12-membered rings (Flippen & Karle, 1976; Rich, Kawai & Jasensky, 1983). However, owing to the strain imposed by ring closure, the peptide groups in such rings cannot maintain a planar conformation and deviate about 20° from the *trans* conformation, as has been theoretically predicted (Ramakrishnan & Sarathy, 1968) and experimentally reported (Flippen & Karle, 1976). In the cyclic tetrapeptide dihydrochlamydocin (Flippen & Karle, 1976), transannular hydrogen bonds are formed and the conformation of the ring deviates strongly from the conformation of the amino acid residues in an α-helix.

Inspection of the Cambridge Structural Database (Allen, Kennard & Taylor, 1983) shows that there are over 20 cyclic tetrapeptides which have been crystallized, but most of them contain proline, *N*-substituted amino acids and other unusual amino acids. Transannular hydrogen bonds and *cis* peptide groups are common. No example was found of a conformation which might be related to an α-helix.

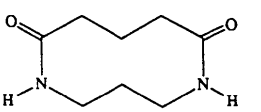
Currently, there is much interest in understanding, theoretically (Zhu, Chen, King & Evans, 1992) and experimentally (Scholtz & Baldwin, 1992), the molecular features which stabilize the α-helix in polypeptides and proteins. In our laboratory, alternative models have been studied for the α-helix, in which additional methylene groups have been introduced into the main chain (Fernández-Santín, Aymamí, Rodríguez-Galán, Muñoz-Guerra & Subirana, 1984; Fernández-Santín *et al.*, 1987; Puiggalí, Muñoz-Guerra, Rodríguez-Galán, Alegre & Subirana, 1988; Bella, Alemán, Fernández-Santín, Alegre & Subirana, 1992). However, there are very few adequate low-molecular-weight models for the α-helix (Parthasarathy, Chaturvedi & Go, 1990). Many cyclic peptides have been studied as models for β-turns, but no low-molecular-weight ring models or even helix templates for the α-helix are currently available (Rizo & Gierasch, 1992). Therefore, we decided to study a trimer of β-alanine [cyclo(β-alanine)$_3$ (2)], which is the same size as a cyclic tetrapeptide, in order to determine whether this compound will form a stack of hydrogen-bonded rings similar in external appearance to a continuous α-helix and with a similar density of hydrogen bonds.



(1)



(2)



(3)

The structure of a dimer of butyramide [cyclo(butyramide) dimer (1)] is also presented. Its structure is very similar to that found for an isomer (3) with an inverted peptide group (Srikrishnan & Dunitz, 1975).

The work reported here may be considered as a contribution towards an understanding of the conformation of medium-sized rings made from several methylene and peptide units. Our results, therefore, are also discussed under this perspective. The structures determined for this work will also be compared with those of other cycloamides, cyclopeptides and ring compounds.

Experimental

The cyclo(butyramide) dimer (2) was synthesized as described by Brozek, Roda & Králicek (1988), who gave us a sample of the product for this study. Crystals of the butyramide dimer suitable for X-ray diffraction were obtained in water/*N*-methylformamide by the vapour-diffusion method.

Table 1. *Crystallographic data*

	Cyclo(butyramide) dimer	Cyclo(β-alanine)$_3$
Crystal size (mm)	0.08 × 0.08 × 0.52	0.2 × 0.2 × 15.0
Molecular formula	$C_8H_{14}N_2O_2$	$C_9H_{15}N_3O_3$
Crystal system	Monoclinic	Trigonal
Space group	$P2_1/n$	$R3m$ (hexagonal axis)
Cell		
a (Å)	4.874 (2)	13.74 (2)
b (Å)	11.714 (4)	13.74 (2)
c (Å)	8.088 (2)	4.82 (1)
β (°)	107.3 (3)	–
V (Å^3)	440.8 (4)	788 (4)
Z (molecules/cell)	2	3
Asymmetric unit	1/2 molecule	1/6 molecule
D_x (g cm^{-3})	1.28	1.35
Range of data		
$2\theta_{max}$ (°)	138	120
h	−5–5	−13–3
k	−14–14	−15–15
l	−9–9	−5–5
Collected reflections	3063	1549
Unique reflections	822	166
R_{int} $[\sum\|F^2 - (F^2)_{mean}\|/\sum F^2]$	0.052	0.027
$R(\sigma)$ $[\sum\sigma(F^2)/\sum F^2]$	0.052	0.015
Observed reflections	536 $[I > 3\sigma(I)]$	161 $[I > 2.5\sigma(I)]$
Number of refined parameters	55	38
R	0.125	0.060

The cyclic trimer of β-alanine was obtained by the active ester method, using *N*-hydroxysuccinimide and dicyclohexylcarbodiimide. The *N*-carbobenzoxy protected active ester was coupled with β-alanine and β-alanyl-β-alanine bromohydrate, in an aqueous/organic medium with a typical yield of 65%. Precursors were crystallized whenever possible and their identity and purity checked using thin-layer chromatography, infrared spectroscopy and melting-point determination. Removal of the *N*-protecting group was achieved by hydrogenation, in the presence of a palladium carbon catalyst, in 50% ethanol. Recrystallization from water/methanol (1:5) yielded the crystalline tripeptide, which was cyclized by the tetraethylpyrophosphite/diethylphosphite method (Rothe, Rothe, Brunig & Schwenke, 1959). The crude cyclotri-β-amino acid was dissolved in water and treated with an Amberlite MB-3 mixed-bed ion-exchange resin and subsequently purified by sublimation at 553 K and 1.3×10^{-2} Pa. The molecular weight and cyclic nature of the oligopeptide were confirmed by FAB-mass spectrometry [*m/e* observed for C(9)H(15)N(3)O(3) 214.5, predicted 213.24]. Extremely elongated crystals of cyclo(β-alanine)$_3$, reaching, in some cases, 100 mm in length, were grown from water/isopropanol.

X-ray intensity data were collected in both cases with an Enraf–Nonius CAD-4 diffractometer with Cu $K\alpha$ radiation and a graphite monochromator. Lattice parameters were determined from a least-squares analysis of the setting angles for 25 reflections in the range $30 < 2\theta < 60°$. Lorentz, decay, polarization and absorption corrections were applied. No significant decay was detected. No weights were assigned to the data. A MicroVAX 2000 computer was used for all calculations.

Table 2. *Atomic coordinates with e.s.d.'s in parentheses and equivalent isotropic thermal parameters* (Å^2) *for the cyclo(butyramide) dimer*

$$B_{eq} = (8\pi^2/3)\sum_i\sum_j U_{ij} a_i^* a_j^* \mathbf{a}_i.\mathbf{a}_j.$$

	x	y	z	B_{eq}
C(2)*	0.480 (1)	0.4090 (5)	0.636 (1)	3.6
O(2)	0.2256 (9)	0.3899 (4)	0.6188 (8)	5.0
N(1)	0.607 (1)	0.3710 (4)	0.5228 (9)	3.7
C(10)	0.456 (2)	0.3251 (6)	0.357 (1)	3.8
C(9)	0.456 (2)	0.4009 (7)	0.208 (1)	4.7
C(3)	0.663 (1)	0.4782 (6)	0.787 (1)	3.6
H(1)	0.815 (1)	0.3875 (4)	0.5436 (9)	6.3

* Atomic coordinates of the other half of the molecule are obtained by the symmetry operation $1 - x$, $1 - y$, $1 - z$. All the H atoms were included in the refinement with fixed isotropic temperature factors $U_{11} = 0.08$ Å^2. Coordinates for H atoms and U_{ij} thermal parameters for all atoms are given as supplementary material. Here and in Tables 3 and 5, the atoms are numbered according to the IUPAC convention, as given in the scheme. From a crystallographic point of view, the C(9)—C(10)—N(1)—C(2)—C(3) sequence is the mirror image of the equivalent C(4)—C(5)—N(6)—C(7)—C(8) sequence.

Table 3. *Bond lengths* (Å) *and bond angles* (°) *with e.s.d.'s in parentheses for the cyclo(butyramide) dimer*

O(2)—C(2)	1.225 (7)	C(10)—N(1)	1.430 (9)
H(1)—N(1)	1.00	C(9)—C(10)	1.49 (1)
N(1)—C(2)	1.324 (9)	C(8)—C(9)	1.54 (1)
C(2)—C(3)	1.52 (1)		

N(1)—C(2)—O(2)	121.6 (7)	C(2)—N(1)—C(10)	124.2 (6)
H(1)—N(1)—C(2)	118.7 (4)	C(9)—C(10)—N(1)	114.2 (6)
C(3)—C(2)—O(2)	121.5 (7)	C(8)—C(9)—C(10)	116.1 (6)
C(10)—N(1)—H(1)	116.2 (4)	C(2)—C(3)—C(4)	112.2 (6)
C(3)—C(2)—N(1)	116.9 (6)		

Crystal data for the two compounds are given in Table 1. Koyama & Dunitz (1972) have already determined the unit cell of butyramide crystals, but they could not collect diffraction data suitable for calculating the molecular structure.

Structure analysis of the cyclo(butyramide) dimer

The structure was solved by direct methods using the *SHELXS*86 computer program package (Sheldrick, 1986) and refined by a full-matrix least-squares procedure (*SHELX*76; Sheldrick, 1976). An *E* map revealed all the non-H atoms. Cycles of refinement and difference Fourier syntheses showed all the H atoms in the structure. The H atom bonded to the N atom was placed in a position found in the difference Fourier map. The remaining H atoms were included at calculated positions. All the H atoms were refined with geometrical constraints (riding model). Anisotropic full-matrix refinement

Table 4. *Interatomic distances* (Å) *and bond angles* (°) *associated with intermolecular hydrogen bonds*

	H(1)···O(2)	N(1)···O(2)	N(1)—H(1)···O(2)	Symmetry code
Cyclo(butyramide) dimer	1.91	2.89	167	$x+1, y, z$
Cyclo(β-alanine)$_3$	1.92	2.99	160	$x, y, z+1$
Cyclo(β-alanine)$_3$	1.92	2.83	135	$y-x, y, z+1$

Table 5. *Torsion angles* (°) *of the cyclo(butyramide) dimer and related ten-atom rings*

	Cyclo(butyramide) dimer	Isomer*	Cyclodecane†
C(10)—N(1)—C(2)—C(3)	−166.8 (ω)	−163	−152.2
N(1)—C(2)—C(3)—C(4)	120.1 (ψ)	134	56.2
C(2)—C(3)—C(4)—C(5)	−64.6	−68	65.8
C(3)—C(4)—C(5)—N(6)	55.8	67	−67.0
C(4)—C(5)—N(6)—C(7)	−108.2	−98	−54.1
C(5)—N(6)—C(7)—C(8)	166.8	163	152.2
N(6)—C(7)—C(8)—C(9)	−120.1	−136	−56.2
C(7)—C(8)—C(9)—C(10)	64.6	69	−65.8
C(8)—C(9)—C(10)—N(1)	−55.8	−63	67.0
C(9)—C(10)—N(1)—C(2)	108.2 (φ)	94	54.1
C(10)—N(1)—C(2)—O(2)	12.7	14	-
C(4)—C(3)—C(2)—O(2)	−59.5	−43	-

* Equivalent angles determined by Srikrishnan & Dunitz (1975) for 1,5-diaza-6,10-cyclodecadione.

† Values determined by Shenhav & Schaeffer (1981).

for non-H atoms and isotropic for H atoms converged to a standard agreement factor, $R = 0.125$ for 536 reflections with $I > 3.0\sigma(I)$. At this step, a difference Fourier map revealed three peaks with heights of 0.87, 0.47 and 0.42 e Å^{-3}. Except for these peaks, the maximum and minimum heights in the difference Fourier map were 0.34 and −0.35 e Å^{-3}, respectively.

The difference peaks mentioned above were found near the peptide group indicating that some disorder or alternative conformation might be present, although we were unable to introduce such features in the refined model. The presence of these anomalous peaks may explain the rather poor R factor (12.5%) of our molecular model.

The atomic coordinates,* bond lengths and angles, and hydrogen-bond parameters are given in Tables 2, 3 and 4, respectively. The atoms are numbered according to the IUPAC convention, as shown in the scheme. The torsion angles are given in Table 5 and are compared with other related structures. A stereoview of the molecules is shown in Fig. 1 and a view of the unit cell is presented in Fig. 2. The molecules form infinite stacks, which are hydrogen bonded, along the a axis.

* Lists of structure factors, anisotropic thermal parameters and H-atom parameters have been deposited with the British Library Document Supply Centre as Supplementary Publication No. SUP 71540 (19 pp.). Copies may be obtained through The Technical Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

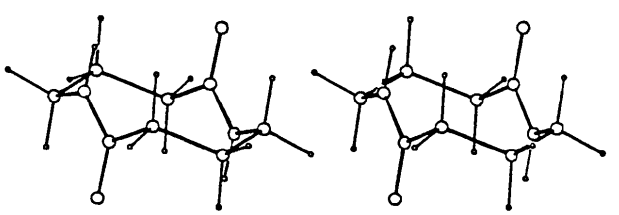


Fig. 1. Stereoview of the cyclo(butyramide) dimer.

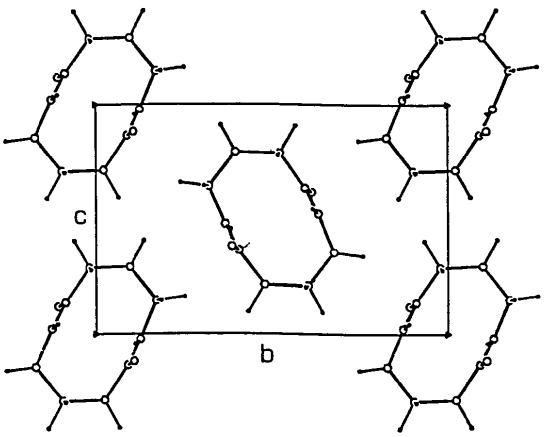


Fig. 2. Packing of cyclo(butyramide) dimers, looking down the a axis. The rings form infinite columns of hydrogen-bonded rings stacked in the a direction.

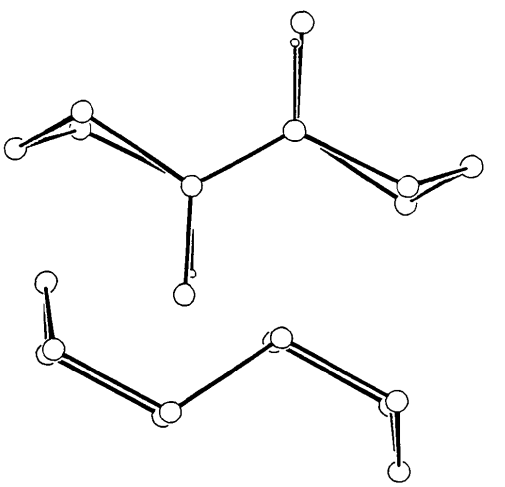


Fig. 3. Lateral view of the cyclo(butyramide) dimer showing its crown conformation compared with the diamond-lattice conformation of cyclodecane. Methylene H atoms are not shown.

In Fig. 3 we compare the lateral view of the molecule with that of cyclodecane. It is obvious that they have a different shape. The cyclodecane conformation is also favoured in other ten-atom rings (Wiberg, Waldron, Schulte & Saunders, 1991). On the other hand, compound (3), studied by Srikrishnan & Dunitz (1975), has a conformation very similar to the cyclic dimer presented here, as is obvious from Table 5. Such a conformation was unexpected for (3), because from its structural formula it should have a mirror plane (Srikrishnan & Dunitz, 1975). Instead, it adopts an approximately centrosymmetric conformation similar to that found in the cyclo(butyramide) dimer we studied.

On projection the cyclic diamide rings have a rather elongated shape, as shown in Fig. 2. In this conformation the peptide groups on opposite sides of the ring are very close [the distance between N(1) and C(7) is 2.85 Å] and are oriented in opposite directions, a fact which should favour their dipole interaction. Such a close interaction may also explain the rather high deviation of the peptide angle ω from 180°.

On the other hand, the cyclodecane and related rings are more circular. The distance between opposite methylene residues is 3.29 Å, which is significantly larger than the equivalent value of 2.85 Å reported above for the diamide rings. In this way, the repulsion between methylene H atoms in close contact at either side of the rings is reduced.

Structure analysis of cyclo(β-alanine)$_3$

Data collection was carried out for the whole diffraction sphere. The actual crystal system and space group were defined after data reduction and structure solution in different space groups, as described in detail below. Cell dimensions and the resulting space group are given in Table 1, together with other experimental parameters. This compound had already been crystallized by White, Morrow, Cox, Drey & Lowbridge (1982). They obtained crystals with an identical unit cell, but did not determine the molecular structure.

The structure was first solved in the $R3$ space group by direct methods using the *SHELXS*86 computer program package (Sheldrick, 1986) and refined by a full-matrix least-squares procedure (*SHELX*76; Sheldrick, 1976). Cell dimensions and the systematic absence of reflections with $-h+k+l \neq 3n$ indicated that the space group should be rhombohedral with one monomer of β-alanine in the asymmetric unit. The E map, calculated for the best solution in the $R3$ space group, revealed four peaks bonded to each other. Together with their symmetrical images relative to the three-fold axis, these peaks composed one 12-membered ring. However, some peaks (including the peak with maximum height) were not among them and could not be O atoms. At the same time, the O atom did not appear in this map. The partial structure expansion method was used in order to improve the pattern obtained. After this procedure the O atom did not appear, but two possible orientations of the four-atom monomer were found. For these orientations only the positions of two atoms were different. The other two atoms remained in the same place. For both orientations the ring was completed by rotational symmetry. All located positions were considered as the positions for C atoms with occupancy factors equal to 0.5 or 1.0. Four cycles of isotropic refinement resulted in decreasing the R factor to 0.20 and a difference Fourier synthesis showed two possible positions of the O atom. The position of the N atom in the ring was then determined. It was found that two enantiomeric monomers co-exist in the asymmetric unit, which may be related by mirror symmetry $y-x,y,z$. Therefore, we concluded that the actual space group should be $R3m$. The symmetry of the experimental X-ray data allowed such a transformation. Thus, the asymmetric unit contains four non-H atoms: one methylene atom with full occupancy and three atoms of the amide group each with half occupancy. After several cycles of anisotropic least-squares refinement in the $R3m$ space group, the R factor decreased to 0.10. The difference Fourier synthesis showed the H atom bonded to the N atom, which is placed on a mirror plane. This H atom was included in the refinement with half occupancy and a fixed isotropic temperature factor. Due to the presence of two superimposed conformations in the asymmetric unit, four peaks corresponding to the methylene H atoms (each with half occupancy) should appear in the difference electron density map. However, the difference map revealed only one peak which could be considered as the H atom bonded to the C(4) atom. It was found in the equatorial plane of the molecule, a position not expected for an sp^3 hybridization of the C(4) atom. This H atom occurs at a position expected for sp^2 hybridization of the C(4)/C(3) atom, but most likely it represents an average position of the equatorial methylene H atoms from the two superimposed enantiomeric monomers which co-exist in the asymmetric unit. In view of this situation, two independent sets of refinement were carried out. First, the peak found in the difference Fourier map was included in the refinement as a fictitious H atom, H(4), with full occupancy and a fixed isotropic temperature factor. Anisotropic full-matrix refinement for non-H atoms and isotropic for H atoms converged to a standard agreement factor $R = 0.060$ for 161 reflections with $I > 2.5\sigma(I)$. The maximum and minimum heights in the final

Table 6. *Atomic coordinates with e.s.d.'s in parentheses, site occupancy factors and equivalent isotropic thermal parameters* (Å^2) *for cyclo(β-alanine)*$_3$

$B_{eq} = (8\pi^2/3)\sum_i\sum_j U_{ij}a_i^*a_j^*\mathbf{a}_i.\mathbf{a}_j$.

	x	y	z	s.o.f.	B_{eq}
C(4)*	−0.2182 (4)	−0.1633 (4)	0.1469	1.0	5.3
N(1)	−0.1221 (6)	−0.1644 (6)	0.206 (2)	0.5	3.1
C(2)	−0.0460 (7)	−0.1705 (7)	0.037 (2)	0.5	3.2
O(2)	−0.0570 (6)	−0.1788 (7)	−0.209 (2)	0.5	5.5
H(1)†	−0.078 (2)	−0.157 (4)	0.407 (3)	1.0	4.7
H(4)	−0.2997 (4)	−0.2284 (4)	0.1711	0.5	4.7

* In order to fix the origin in the space group *R3m*, the *z* coordinate of the C(4) atom was not allowed to change during refinement. The position of the C(3) atom of the β-alanine monomer and the atomic coordinates of its mirror image are obtained by the mirror symmetry $y-x, y, z$.

† The H(1) atom is bonded to the N atoms of two superimposed monomers related by a mirror plane, as shown in Fig. 4. It is at a special position on the mirror plane. The ring is completed by the symmetry operations $-y, x-y, z$ and $y-x, -x, z$.

Table 7. *Bond lengths* (Å) *and bond angles* (°) *with e.s.d.'s in parentheses for cyclo(β-alanine)*$_3$

C(3)—C(4)	1.49 (1)	C(2)—C(3)	1.438 (9)
N(1)—C(4)	1.357 (8)	O(2)—C(2)	1.199 (9)
C(2)—N(1)	1.360 (9)	H(1)—N(1)	1.12 (1)
		H(4)—C(4)	1.03 (1)

C(3)—C(4)—N(1)	119.8 (7)	C(2)—N(1)—H(1)	97.3 (7)
C(2)—N(1)—C(4)	131.2 (5)	N(1)—C(4)—H(4)	127.3 (4)
N(1)—C(2)—C(3)	121.5 (7)	H(1)—N(1)—C(4)	131.5 (8)
C(2)—C(3)—C(4)	121.1 (7)	C(3)—C(4)—H(4)	108.9 (8)
O(2)—C(2)—N(1)	122.2 (8)	H(3)—C(3)—C(2)	127.0 (8)
O(2)—C(2)—C(3)	116.2 (8)		

difference Fourier map were 0.21 and −0.18 e Å^{-3}, respectively.

An alternative refinement was carried out with all the methylene H atoms at their calculated positions. Because the C(4) and C(3) atoms are mirror images, the H atoms bonded to them were included in the refinement with half occupancy. Their isotropic temperature factors U_{11} were fixed and equal to 0.08 Å^2. An agreement factor $R = 0.069$ was then found. However, the difference Fourier map revealed six closely connected peaks with an average height 0.27 e Å^{-3}. These peaks appeared near the position found before and were assigned to the fictitious H(4) atom. Refinement of the isotropic temperature factors of these methylene H atoms neither improved the difference map nor reduced the R factor (R = 0.068). The coordinates of all these methylene H atoms are given as supplementary material.

The atomic coordinates of the asymmetric unit and bond lengths and angles for this structure are given in Tables 6 and 7. The hydrogen-bond parameters are given in Table 4. Fig. 4 shows a projection of the unit cell and Fig. 5 a view of three stacked rings. The C(3) atom is the mirror image of the C(4) atom. Both atoms are crystallographically equivalent and represent the average of two chemically different methylene C atoms. The three β-alanyl residues which comprise one ring are generated by mirror-plane and threefold-axis symmetry. At each position it is possible to place rings with either of the two mirror image conformations. However, when consecutive rings are mirror images, the hydrogen bond parameters are slightly less favourable, as shown in Table 4 (short N—O distance 2.83 Å; N—H···O angle 135°). It is likely that the crystal is organized as a random mixture (50/50) of the two mirror image molecules associated in long stacks of rings with identical conformation, with occasional changes between the two mirror images. The two types of stacked columns are shown as stereoviews in Fig. 6.

The structure of the asymmetric unit and the unit cell can be best understood from the schematic diagram shown in Fig. 5. The asymmetric unit is made of a methylene C atom [C(4)] to which is attached one N atom [N(1)] bonded to a carbonyl group [C(2)O(2)]. The asymmetric unit is completed with the peptide H atom [H(1)] and the fictitious methylene H atom [H(4)]. All of these atoms, except H(4), are indicated by arrows in Fig. 5. A mirror plane placed between the two methylene groups generates two complete β-alanine residues superimposed, shown one in black and another one in white in Fig. 5. The trigonal axis generates the complete superimposed rings (white and black in Fig. 5), which are mirror images of each other and have an occupancy factor of 50%.

The positions of the H(1) atoms attached to the N atoms deserve a special consideration. They have been assumed to be exactly on the mirror plane. This is not necessarily so and more likely they are slightly displaced from it. However, from the resolution of our data it is not reasonable to replace the single H atom used in the refinement by two symmetric half H atoms slightly displaced from the mirror plane.

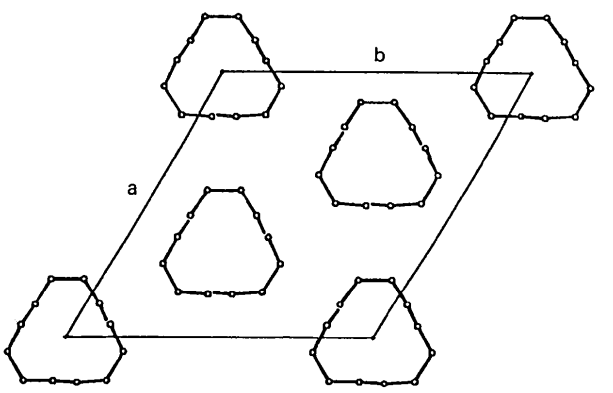


Fig. 4. Projection of the crystal structure of cyclo(β-alanine)$_3$ onto the *ab* plane. H atoms are not shown.

It is interesting to note that although the crystal contains a mixture of rings in two different conformations, all of them have the peptide groups oriented along the same direction. Thus, the macroscopic crystal has a polar structure, with opposite *N*-terminal and carbonyl-terminal ends.

The projection shown in Fig. 4 may give the wrong impression that there is a central hole in the structure. However, when the van der Waals radii are considered, the central hole is less than 1 Å in diameter. In fact, the calculated density (1.35 g cm^{-3}) is practically identical to the calculated density of crystalline poly(β-alanine), a fully extended polymer, which is 1.36 g cm^{-3} (Muñoz-Guerra, Fernández-Santín, Rodríguez-Galán & Subirana, 1985).

In Fig. 7 and Table 8 a comparison of the structure of cyclo(β-alanine)$_3$ with the polypeptide α-helix and related polymers is presented. The approximate triangular shape of the ring compares well with the smaller 3_{10}-helix, but from the point of view of size it is closer to the projection of the α-helix, which has 10.8 atoms per turn on projection. On the other hand, the projected α-helix has an approximately square shape, whereas cyclo(β-alanine)$_3$ has a triangular shape.

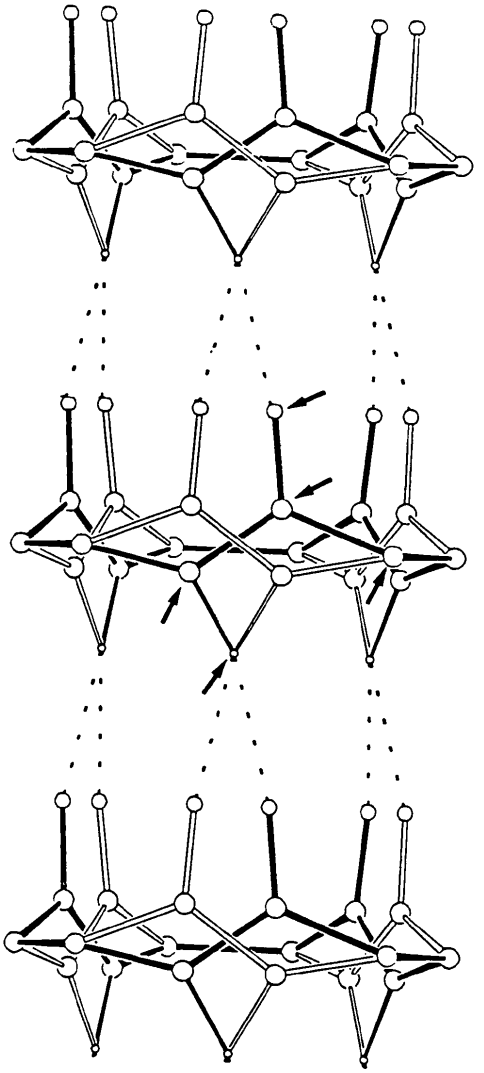


Fig. 5. Detail of one column of cyclo(β-alanine)$_3$. Each position in the column may be occupied by one type of molecule (in black) or by its mirror image (in white). Hydrogen bonding among identical molecules appears to be favoured although black and white molecules may also be hydrogen bonded, with slightly distorted hydrogen bonds (Table 4). Methylene H atoms are not shown. The arrows indicate the atoms present in one asymmetric unit. Due to the presence of two conformations, the chemically distinct atoms C(3) and C(4) appear superimposed in the asymmetric unit.

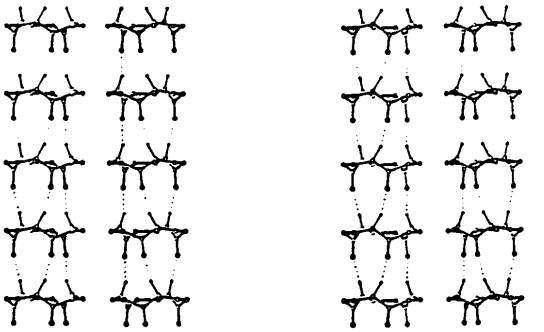


Fig. 6. Stereoview showing columns of cyclo(β-alanine)$_3$ in its two mirror image conformations. Each column closely resembles an α-helix (either right or left handed) in its diameter, in the number of hydrogen bonds and in the polar orientation of the peptide groups. Methylene H atoms are not shown.

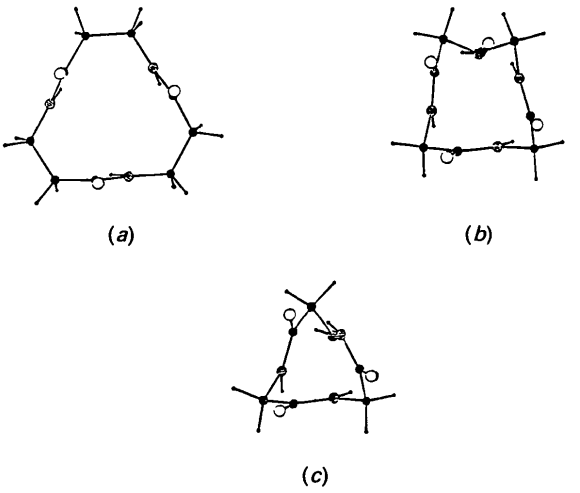


Fig. 7. (*a*) The molecular structure of cyclo(β-alanine)$_3$. It is compared with (*b*) the projection of an α-helix (four residues) and (*c*) the projection of a 3_{10}-helix. Coordinates are taken from Benedetti *et al.*, (1991). C atoms are black, O atoms white and N atoms are hatched.

Table 8. *Comparison of torsion angles (°) of cyclo(β-alanine)$_3$, the α-helix and related compounds*

	Cyclo(β-alanine)$_3$	α-helix*	3_{10}-helix	Dodecatriene	Cyclododecane oxime	Poly(α-isobutyl D-aspartate)
φ	−100.6	−62	−71	−116	−70	−148
ω	176.1	180	180	178	161	180
ψ	−96.6	−41	−18	−116	−70	−129
ξ	39.3	-	-	63		62

* The values for the α- and 3_{10}-helices have been taken from a statistical study in proteins (Benedetti *et al.*, 1991). They cannot be directly compared with cyclo(β-alanine)$_3$ due to the different number of methylene groups. Only one of the two possible conformations of *cyclo*(β-alanine)$_3$ is shown. Its mirror image has identical angles with opposite signs. The values for dodecatriene correspond to *trans,trans,trans*-1,5,9-dodecatriene, as reported by Immirzi & Allegra (1967). The average values are given, since individual angles vary slightly at different points of the ring. The values for cyclododecane oxime are the average of the four different sets of similar angles at each side of the approximately square ring (Groth, 1979). Similar values are found in other cyclododecane derivatives (Groth, 1969, 1975, 1980). Poly(α-isobutyl D-aspartate) is a polymer of β-alanine with side chains made of isobutyl ester groups. The conformational angles have been taken from model 2*R* (Fernández-Santín *et al.*, 1987), with all signs changed for an adequate comparison.

Discussion

The presence of peptide groups in the two cyclic molecules we have studied has a strong influence on their conformation. Both rings acquire a unique conformation which is only rarely found in other rings of the same size.

In the case of ten-atom rings, most compounds (Wiberg, Waldron, Schulte & Saunders, 1991) adopt a diamond-lattice conformation, practically identical to that found in cyclodecane, shown in Fig. 3 and in Table 5. The crown conformation found by us is different, as is also shown in Fig. 3. A similar conformation was predicted by Dale (1973) to be a local minimum for cyclodecane, with a slightly higher energy than the standard conformation. The presence of peptide groups at either side of the ring may favour this conformation, which allows the two peptide groups to be at a shorter distance. Nevertheless, the peptide groups deviate significantly from a planar conformation ($\omega = 166.8°$), probably due to the strain imposed by ring closure. NMR studies have confirmed that the same conformation is found in solution (Winkler & Leutert, 1982).

Cyclo(β-alanine)$_3$ has a unique conformation which is not usually found in other 12-membered rings. Only in the case of *trans,trans,trans*-1,5,9-dodecatriene was a similar conformation found by Immirzi & Allegra (1967). This compound has double bonds placed at the same positions as the peptide groups of cyclo(β-alanine)$_3$.

The conformation of cyclo(β-alanine)$_3$ has been studied by molecular mechanics calculations (White, Morrow, Cox, Drey & Lowbridge, 1982). These authors found a global minimum with no conformational relationship to the structure we found. It is likely that the formation of hydrogen-bonded columns of molecules has a determining influence on the stability of the conformation found by us, whereas in solution different conformations might be found. Also, the theoretical studies of Dale (1973) did not predict any local energy minimum for a cyclododecane with a structure like the one we found. On the other hand, he found that part of the rings of cycloundecane and cyclotridecane could have a shape similar to two units of β-alanine in the cyclic trimer described here.

The results obtained with cyclo(β-alanine)$_3$ show some puzzling features. For example, a single methylene H atom [H(4)] was found at an unexpected equatorial position. At the same time, inspection of Table 7 shows that all interatomic distances in the ring are shorter than expected, except the peptide bond which is slightly longer. These observations might be due to the co-existence of two mirror images in the cyclo(β-alanine)$_3$ crystals, but it can not be excluded that other conformations among those predicted by White, Morrow, Cox, Drey & Lowbridge (1982) also contribute to the conformational variability in the crystal.

A good ring model of the α-helix would be a cyclic tetrapeptide, made from L-amino acids, containing 12 atoms. Such compounds have never been crystallized. Theoretically, it has been shown (Go & Scheraga, 1970) that the planarity of the peptide bond cannot be maintained in cyclic compounds made with four amino acids. In fact, cyclo(glycine)$_4$ was studied using NMR and shown to have distorted peptide bonds (Grathwohl, Tun-Kyi, Bundi, Schwyzer & Wüthrich, 1975). Most 12-atom rings which have been crystallized (Groth, 1969, 1975, 1979, 1980) have a square shape similar to the projection of the α-helix shown in Fig. 7*b*, with a torsion angle of about 160° in the central bond on each side of the square. The values for one of the structures are given in Table 8. Such a deviation from 180° is too large for a stable peptide bond, a fact which may explain why cyclic tetrapeptides from L-amino acids have never been crystallized. On the other hand, there are some studies of cyclic tetrapeptides with unusual amino acids (Flippen & Karle, 1976; Rich, Kawai & Jasensky, 1983) in which the peptide bond

adopts a transoid conformation ($\omega = 160°$ approx.). Furthermore, the shape of these rings is rather complex and intramolecular hydrogen bonds are formed across the ring.

Another interesting feature of cyclo(β-alanine)$_3$ is that it has three planar peptide groups in the *trans* conformation which are organized in long hydrogen-bonded columns with an appearance and hydrogen-bond density similar to an α-helix. As we discussed in the *Introduction*, no cyclic tetrapeptide made with standard α-amino acids has such a conformation, since the strain of ring closure prevents the four peptide groups being planar (Ramakrishnan & Sarathy, 1968).

Comparison of the structures presented in this paper with related cyclic molecules indicates that when peptide groups are present in a ring, it is necessary that the ring is a certain size in order to have the peptide groups in the *trans* conformation. Thus, cyclo(β-alanine)$_2$, an eight-atom ring, has its two peptide groups in the *cis* conformation (White & Guy, 1975). Adding two methylene residues to the ring, as in the cyclo(butyramide) dimer studied here, allows the peptide groups to approach the planar *trans* conformation. In the case of 12-atom rings, our work shows that they can accommodate three planar peptides in the *trans* conformation, but when four peptide groups are incorporated, they cannot be planar.

The β-alanine residue is also the basic building block of nylon 3, which forms a typical extended structure of parallel chains with intermolecular hydrogen bonds (Muñoz-Guerra, Fernández-Santín, Rodríguez-Galán & Subirana, 1985). However, when side chains are added, the polymer acquires a helical conformation similar to the α-helix (Fernández-Santín, Aymamí, Rodríguez-Galán, Muñoz-Guerra & Subirana, 1984; Fernández-Santín *et al.*, 1987). The conformational angles of such a polymer are also given in Table 8, where it is clear that they are very close to those found in cyclo(β-alanine)$_3$. The ring compound is thus a good model for the related helical polymer.

We are thankful to Dr J. Lloveras for his help in the collection of the diffraction data. This work has been supported in part by a DGICYT grant PB91-0588.

References

Allen, F. H., Kennard, O. & Taylor, R. (1983). *Acc. Chem. Res.* **16**, 46–153.

Bella, J., Alemán, C., Fernández-Santín, J. M., Alegre, C. & Subirana, J. A. (1992). *Macromolecules*, **25**, 5225–5230.

Benedetti, E., Di Blasio, B., Pavone, V., Pedone, C., Santini, A., Crisma, M. & Toniolo, C. (1991). *Molecular Conformation and Biological Interactions*, edited by P. Balaram & S. Ramaseshan, pp. 497–502. Bangalore: Indian Academy of Science.

Blout, E. K. (1981). *Biopolymers*, **20**, 1901–1912.

Brozek, J., Roda, J. & Králicek, J. (1988). *Makromol. Chem.* **189**, 1–7.

Dale, J. (1973). *Acta Chem. Scand.* **27**, 1115–1129.

Fernández-Santín, J. M., Aymamí, J., Rodríguez-Galán, A., Muñoz-Guerra, S. & Subirana, J. A. (1984). *Nature*, **311**, 53–54.

Fernández-Santín, J. M., Muñoz-Guerra, S., Rodríguez-Galán, A., Aymamí, J., Lloveras, J., Subirana, J. A., Giralt, E. & Ptak, M. (1987). *Macromolecules*, **20**, 62–68.

Flippen, J. L. & Karle, I. L. (1976). *Biopolymers*, **15**, 1081–1092.

Go, N. & Scheraga, H. A. (1970). *Macromolecules*, **3**, 178–187.

Grathwohl, C., Tun-Kyi, A., Bundi, A., Schwyzer, R. & Wüthrich, K. (1975). *Helv. Chim. Acta*, **58**, 415–423.

Groth, P. (1969). *Acta Chem. Scand.* **23**, 587–590.

Groth, P. (1975). *Acta Chem. Scand.* **29**, 840–845.

Groth, P. (1979). *Acta Chem. Scand.* **33**, 203–205, 503–509.

Groth, P. (1980). *Acta Chem. Scand.* **34**, 609–613.

Hermans, P. H. (1953). *Rec. Trav. Chim. Pays-Bas*, **72**, 798–803.

Immirzi, A. & Allegra, G. (1967). *Atti Acad. Naz. Lincei, Cl. Sci. Fis. Mat. Nat. Rend.* **43**, 338–349.

Koyama, G. & Dunitz, J. D. (1972). Unpublished results cited by Srikrishnan & Dunitz (1975).

Muñoz-Guerra, S., Fernández-Santín, J. M., Rodríguez-Galán, A. & Subirana, J. A. (1985). *J. Polym. Sci. Polym. Phys. Ed.* **23**, 733–742.

Parthasarathy, R., Chaturvedi, S. & Go, K. (1990). *Proc. Natl. Acad. Sci. USA*, **87**, 871–875.

Puiggalí, J., Muñoz-Guerra, S., Rodríguez-Galán, A., Alegre, C. & Subirana, J. A. (1988). *Makromol. Chem. Makromol. Symp.* **20/21**, 167–182.

Ramakrishnan, C. & Sarathy, K. P. (1968). *Biochim. Biophys. Acta*, **168**, 402–410.

Rich, D. H., Kawai, M. & Jasensky, R. D. (1983). *Int. J. Pept. Protein Res.* **21**, 35–42.

Rizo, J. & Gierasch, L. M. (1992). *Annu. Rev. Biochem.* **61**, 387–418.

Rothe, M., Rothe, I., Brunig, H. & Schwenke, K. (1959). *Angew. Chem.* **71**, 700.

Scholtz, J. M. & Baldwin, R. L. (1992). *Annu. Rev. Biophys. Biomol. Struct.* **21**, 95–118.

Sheldrick, G. M. (1976). *SHELX76. Program for Crystal Structure Determination.* Univ. of Cambridge, England.

Sheldrick, G. M. (1986). *SHELXS86. Program for the Solution of Crystal Structures.* Univ. of Göttingen, Germany.

Shenhav, H. & Schaefer, K. (1981). *Cryst. Struct. Commun.* **10**, 1181–1184.

Srikrishnan, T. & Dunitz, J. D. (1975). *Acta Cryst.* B**31**, 1372–1377.

White, D. N. J. & Guy, M. H. P. (1975). *J. Chem. Soc. Perkin Trans.* 2, pp. 43–46.

White, D. N. J., Morrow, C., Cox, P. J., Drey, C. N. C. & Lowbridge, J. (1982). *J. Chem. Soc. Perkin Trans.* 2, pp. 239–243.

Wiberg, K. B., Waldron, R. F., Schulte, G. & Saunders, M. (1991). *J. Am. Chem. Soc.* **113**, 971–977.

Winkler, T. & Leutert, T. (1982). *Helv. Chim. Acta*, **65**, 1760–1763.

Zhu, Y., Chen, C.-C., King, J. A. & Evans, L. B. (1992). *Biochemistry*, **31**, 10591–10601.