STRUCTURE AND CONFORMATION OF LINEAR PEPTIDES: XI—STRUCTURE OF GLYCYL-L-LEUCYL-L-TYROSINE

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ABSTRACT

The crystal structure of a tripeptide, glycyl-L-leucyl-L-tyrosine, has been determined. The crystals belong to the triclinic system, space group P1, with two crystallographically independent molecules in a unit cell of dimensions $a = 9.326(2)$, $b = 10.993(3)$, $c = 12.407(3)$A, $\alpha = 84.05(2)°$, $\beta = 83.91(2)$, and $\gamma = 80.03(2)$. The structure was solved by direct methods and refined to an R-index of 0.08. The two crystallographically independent molecules have essentially the same overall conformation except at the sidechain of leucyl residues. All the peptide units are trans and planar. The carboxyl group is planar and makes a dihedral angle of 30.2° and 36.7° respectively with the adjacent peptide unit in the two molecules. The peptide chains exist in an extended conformation, forming infinite anti-parallel β-sheet arrangement. Comparison of this structure with that of a copper complex of the peptide reveals that the molecular conformation is essentially independent of its environment.

INTRODUCTION

As a part of our studies on the structure and conformation of peptides, this paper describes the results of the X-ray crystallographic studies on a tripeptide, glycyl-L-leucyl-L-tyrosine.

EXPERIMENTAL

Crystals of the tripeptide were obtained by dissolving the commercial sample in a mixture of water and dimethyl sulfoxide (DMSO). The crystal data are summarized in table 1. The unit cell contains two crystallographically independent molecules of the peptide. Three-dimensional intensity

Table 1 *Crystal data for glycyl-L-leucyl-L-tyrosine*

Molecular formula	$C_{17}H_{25}N_3O_5$	
Molecular weight	351	
Crystal system	Triclinic	
Spacegroup	P1	
Unit cell	$a = 9.326(2)$ Å	$\alpha = 84.65(2)°$
	$b = 10.993(3)$	$\beta = 83.91(2)$
	$c = 12.407(3)$	$\gamma = 80.03(2)$
No. of molecules per cell (Z)	2	
Radiation used	CuKα ($\lambda = 1.5418$ Å)	
Density (measured)	1.22 g.cm^{-3}	
Density (calculated)	1.17 g.cm^{-3}	
Crystal size	$0.2 \times 0.2 \times 0.3$ mm	

Table 2 *Conformational features of glycyl-L-leucyl-L-tyrosine and its copper complex*

		Gly-Leu-Tyr (Present study) Molecule		Copper complex (Ref. 5) Molecule	
		A	B	A	B
Backbone conformation:					
ψ_1		170°	154°	180°	−176°
ω_1		177	−179	−173	−177
ϕ_2		−131	−146	−130	−131
ψ_2		148	134	139	142
ω_2		174	176	−177	177
ϕ_3		−146	−140	−149	−142
ψ_3		169	171	167	168
Side-chain conformation:					
	χ_1	−60	−176	−53	176
Leucine:	χ_{21}	169	151	174	153
	χ_{22}	−71	−86	−67	−92
	χ_1	65	58	54	58
Tyrosine:	χ_{21}	99	88	90	84
	χ_{22}	−90	−89	−79	−86
Hydrogen bonds linking peptide chains laterally:					
1N . . . 302′*		2.81A		2.82A	
2N . . . 20′*		2.86		2.80	
3N . . . 10′*		3.01		3.09	
1N′ . . . 302′		2.78		2.78	
2N* . . . 20′		3 04		2.85	
3N* . . . 10′		3 01		3.09	

* Refers to atoms of molecule B.

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data were collected on a Nonius CAD-4 diffractometer using CuK radiation. Out of a total of 5229 reflections collected with $\sin\theta/\lambda \leq 0.63$ Å^{-1}, 2620 had $I > 2\sigma$ and were used in the structure analysis. All the reflections were corrected for Lorentz and polarization effects and for absorption.

The structure was solved by direct methods using the computer program MULTAN[1]. The positions of the hydrogen atoms were determined from 3-D difference-Fourier calculations which also revealed five water molecules and two DMSO molecules as solvents of crystallization. One of the two DMSO molecules was found to be disordered. The disorder implied that the sulphur atom flipped between two alternative locations while the two methyl carbon atoms and the oxygen atom occupied the same sites.

The structure was refined by full-matrix least-squares procedures using the computer program LALS[2], with anisotropic temperature factors for the non-hydrogen atoms. The hydrogen atoms were included only in the SF calculations, but not refined. Convergence was reached when the average parameter shifts were less than 25% of the corresponding estimated standard deviations. The final R-index is 0.08. During the refinement, the function minimized was $\Sigma w(|F_0| - |F_0|)^2$, with the weights ($w$) assigned using the Hughes' weighting scheme. A list of the final parameters will be supplied on request.

DISCUSSION

Figure 1 shows the observed conformations for the two independent molecules. The e.s.ds in the bond lengths and bond angles are about 0.02 A and 0.9° respectively. The torsion angles[3] observed in the molecules are listed in table 2. The two molecules have essentially the same conformation except for the side-chain conformation at the leucyl residues. All the peptide units are trans and planar. The carboxyl group is also planar and makes a dihedral angle of 30.2° and 36.7° respectively with the adjacent peptide unit in the two independent molecules (A and B). The peptide chain length, measured from 1CA to 3CA, is 6.94 and 6.88 A respectively, in molecule A and B. The extended backbone conformation is thus characteristic of β-sheet structures.

Figure 2 shows the packing of the molecules viewed along a^*-direction. The two molecules A and B have their chain directions running parallel to the c-axis but anti-parallel to each other. The packing

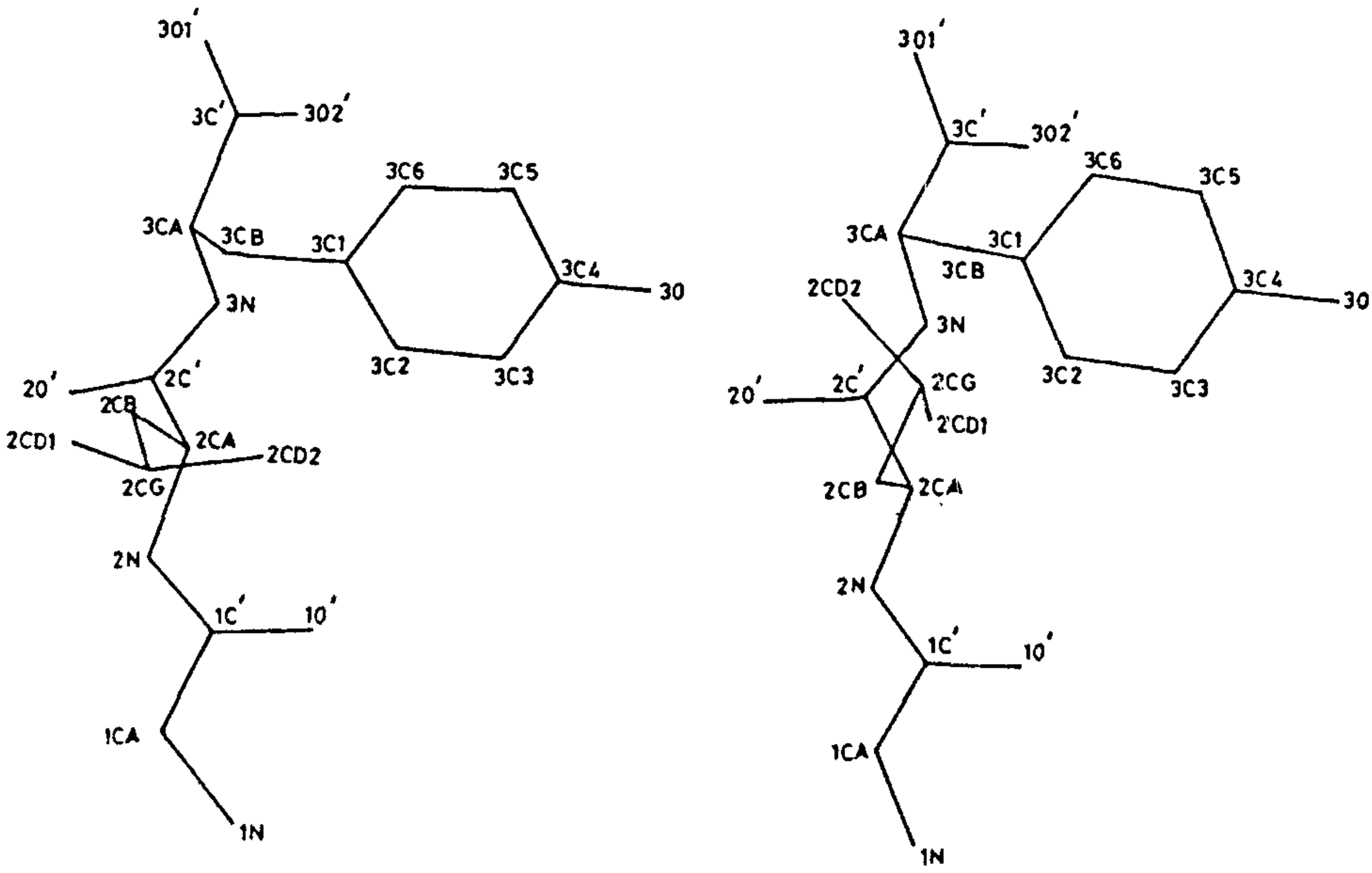

Figure 1. Observed conformation in the two independent molecules of glycyl-L-leucyl-L-tyrosine.

arrangement consists of the molecules being arranged 'head-to-tail' along the *c*-axis. Since the lattice is triclinic, the crystal packing gives rise to an infinite sheet of anti-parallel β-chains of the type ABABAB..... in the *ac*-plane, with lateral hydrogen bonds along the *a*-axis and 'head-to-tail' hydrogen bonds along the *c*-axis. The arrangement is very similar to that exhibited in the crystal structure of another tripeptide, L-alanyl-L-alanyl-L-alanine[4].

The observed molecular conformation and the dimeric anti-parallel association are very similar to those found in the crystal structure of the copper complex of this tripeptide[5], wherein also the asymmetric unit contains two crystallographically independent peptide molecules. Table 2 also lists the conformational parameters of the copper complex of gly-leu-tyr for comparison. Though copper complex crystallizes in a different spacegroup ($P\ 2_1\ 2_1\ 2_1$), the asymmetric unit contains two crystallographically independent molecules which have similar conformation and associate with each other dimerically in a manner similar to the uncomplexed form. The presence of the copper atoms merely results in coordination bonds replacing hydrogen bonds in one direction.

The fact that the chain conformation and dimeric association of molecules are similar in the two structures leads to the inference that the peptide sequence glycyl-leucyl-tyrosine has a dominant conformation, which is perturbed only slightly by the environment. It would indeed be interesting to

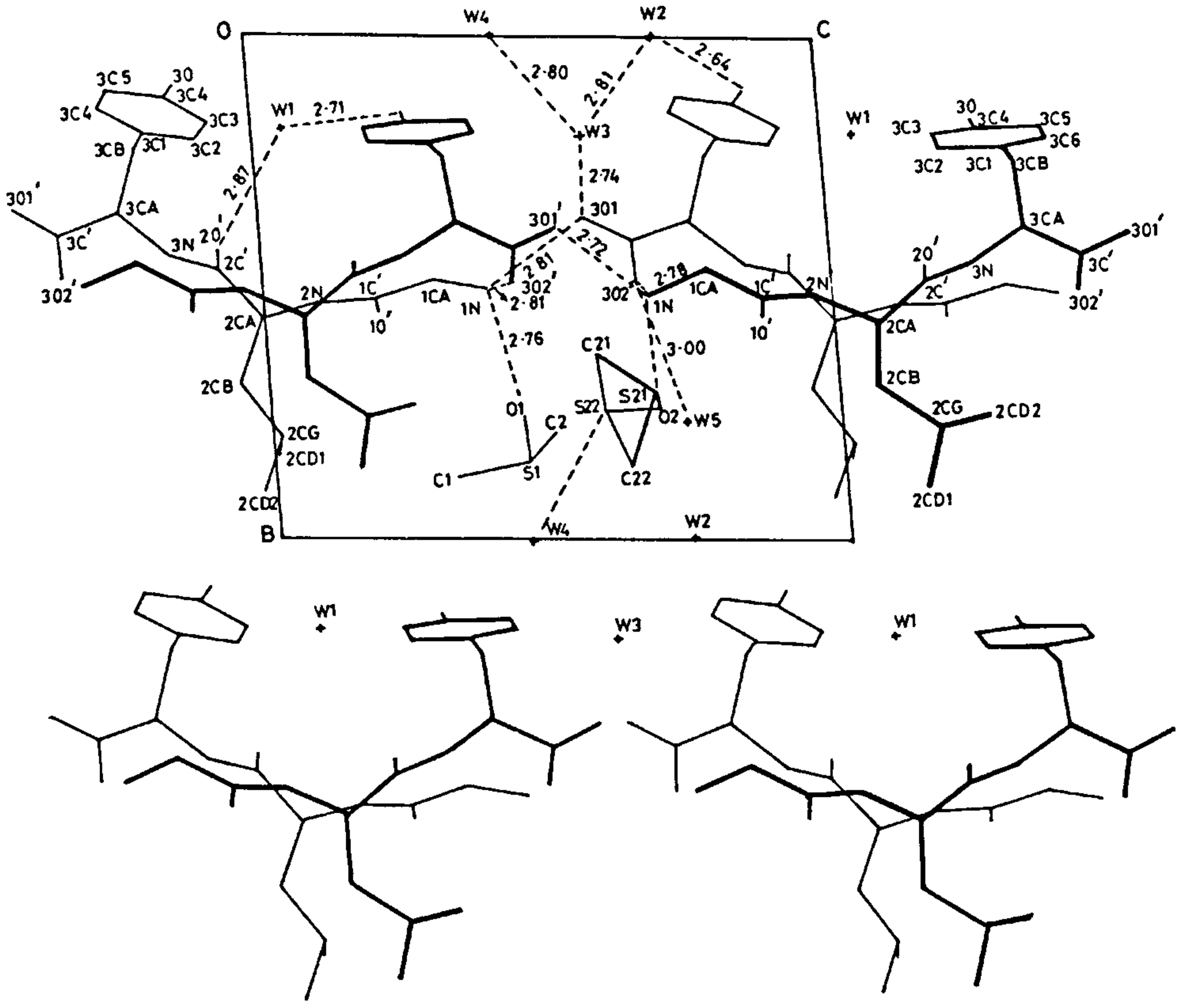

Figure 2. Crystal packing of the molecules of glycyl-L-leucyl-L-tyrosine, viewed along crystallographic a* direction.

perform solution studies on this peptide to establish the generality of this inference.

The sheets are nearly perpendicular to the *b*-axis and the separation between the sheets is equal to *b*. The sheets have all the tyrosyl sidechains sticking out on one side while the leucyl sidechains stick out on the other side. Since the sheets are related by lattice translation alone, crystal packing involves all the tyrosyl sidechains nestling against the leucyl sidechains. However, there are no contacts between these sidechains from adjacent sheets. Nor are there direct links via hydrogen-bonding between the sheets. The space between the sheets is filled by solvent molecules which presumably act as the glue between the sheets.

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