J. Indian Inst. Sci. 63 (C), Apr. 1981 Pp. 81–103

Structural characteristics of prolyl residues—A review

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Received on March 31, 1981.

Abstract

The structural and conformational characteristics of prolyl residues are discussed on the basis of the available crystal structure data on peptides and proteins. The conformational flexibility of the pyrrolidine ring often leads to the apparent shortening of the bond lengths involving C^{β} and C^{γ}. The value of some angles in the residue vary substantially as a function of the nature and the geometrical disposition of the preceding residue. Almost all the X-Pro bonds in small linear peptides exist in the *trans* configuration. The pyrrolidine ring exists in C_s or C_2 conformation with C^{β} and/or C^{γ} deviating from the plane defined by the rest of the atoms, in all linear peptides of naturally occurring amino acids. Other ring conformations occur only in conditions of strain. The observed conformations in peptides suggest that the prolyl residue has a high propensity to assume the polyproline II structure. The residue has a high propensity to be at the second position in β-turns also. It occurs at the third position only under unusual circumstances and on rare occasions. A bend involving a *cis* X-Pro bond turns out to be an ideal structural feature for bringing about chain reversal in proteins. Prolyl residues occur extensively in β-turns in peptides containing D and unusual residues as well.

Key words: Prolyl residue, conformation, regular secondary structures, β-turns, unusual residues.

1. Introduction

As is well known, proline (along with its derivatives like hydroxy proline) is the only imino acid found in proteins. It occurs widely in different kinds of proteins. In particular, proline and hydroxyproline are abundant in the structural protein collagen[1]. A carbon atom, instead of a hydrogen atom in other residues, is attached to the main chain nitrogen atom in the prolyl residue; the side chain folds back and is linked to the nitrogen atom so that the α-carbon atom and the nitrogen atom form part of a five-membered ring. The conformational flexibility of the prolyl residue is, therefore, curtailed, thus making them uniquely important in determining and stabilising the conformation of proteins. It is also worth mentioning that *cis* peptide units have, so far, been observed in proteins only in X-Pro segments. Many such *cis* peptides are found to play, as will be shown later, a structurally important role. The fact that prolyl

residues can form *trans* and *cis* peptides with nearly equal ease has led to interesting suggestions regarding their involvement in the dynamics of peptide folding[2]. Prolyl residues play an important role in determining the conformation of biologically interesting peptides such as peptide antibiotics as well[3].

Proline containing peptides have been studied extensively using X-ray diffraction methods in an attempt to elucidate the geometrical basis of the unique structural importance, outlined above, of the prolyl residue. A brief survey of the known structural characteristics of prolyl residues is given in this review.

2. Nomenclature

Unless otherwise specified, the recommendations of the IUPAC–IUB Commission on Biochemical Nomenclature[4] have been followed in designating atoms and dihedral angles. The dihedral angles in relation to a prolyl residue are illustrated in Fig. 1. Amino acid residues are abbreviated in the usual manner (for example, proline is abbreviated to Pro). The abbreviations for unusual amino acids are indicated wherever relevant. Those used for protecting groups are as follows :

Benzyloxycarbonyl	Z
tertiary Butyloxycarbonyl	*t*-Boc
Amyloxycarbonyl	Aoc
Methylester	OMe
Methylamide	NHMe
N-Acetyl	N–Ac
Benzyl ester	OBzl
Pivaloyl	Piv.

3. Bond lengths and angles

The average bond lengths and angles in the prolyl residue have been calculated by many workers from the currently available crystal structure data[5–7]. As the latest calculation[6], based on information from more than 20 X-ray studies, was done only recently, it was not felt necessary to carry out an additional calculation incorporating the relevant X-ray results that have become available subsequently. Although the gross dimensions of proline remain nearly constant in salts, metal complexes and peptides, the finer details vary from structure to structure depending upon the immediate environment. The rather high standard deviations obtained in ref. 6 can be accounted for by these variations.

A feature that has been commented upon by De Tar and Luthra[6], as done by other workers also, is the variability of the dimensions, in particular the shortening of lengths, involving C^{β} and C^{γ}. This shortening, observed in many crystal structures[8–13], is of particular interest as it appears to be related to the conformational flexibility albeit limited, of the pyrrolidine ring. Most of the conformational possibilities of the ring

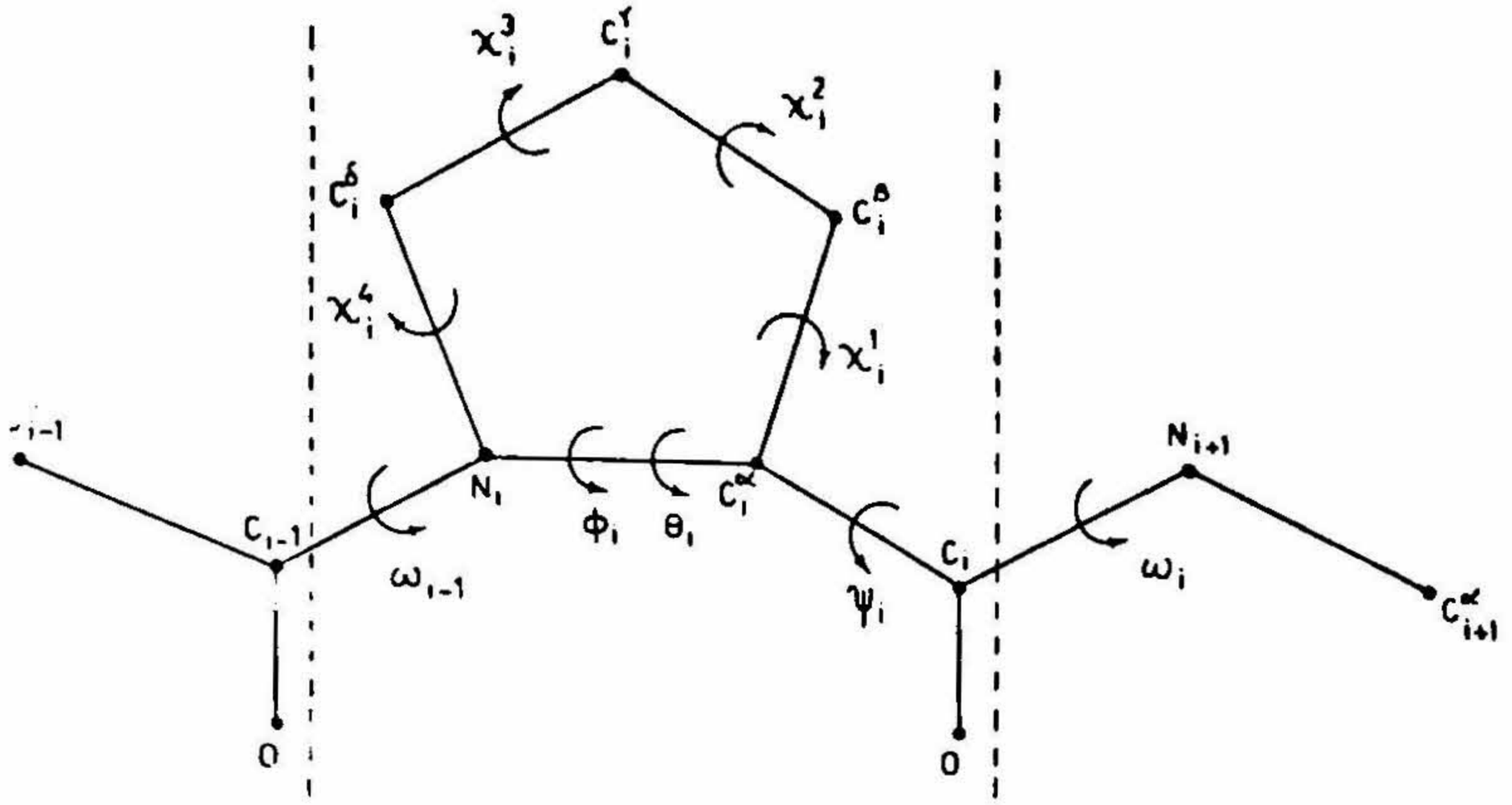

FIG. 1. Designation of the dihedral angles in a prolyl residue.

can be described as arising from the displacement of C^β, C^γ or both from the plane defined by the rest of the atoms[5]. These displacements can assume a continuous range of values, thus giving rise to the possibility of static disorder in a direction perpendicular to the plane of the ring. This disorder, along with thermal vibrations, could lead to highly anisotropic temperature factors for C^β and C^γ, and the consequent shortening of the C^β–C^γ bond. The example of Z-L-Ala-D-Phe-L-Pro H_2O[13] could be cited to illustrate the situation. In that structure, the B values along the principal axes of the thermal vibration ellipsoid of the C^γ atom are 20·2, 8·6 and 4·2 Å^2; the corresponding values for C^β are 10·8, 8·3 and 3·3 Å^2. In both the cases, the major axis is nearly perpendicular to the mean plane of the five-membered ring, the angle between the major axis and the plane normal being 4·6 and 24·2° for C^γ and C^β respectively. The observed C^β–C^γ length of 1·451 Å increased to 1·499 Å when corrected for correlated thermal vibrations[14].

Yet another feature worth mentioning is the opening out of C_{i-1}^α—C_{i-1}—N_i angle at the expense of O_{i-1}—C_{i-1}—N_i angle. The value of the former angle for a peptide group preceding proline is 118° as compared to the standard value[15] of 114°. As pointed out by Ashida and Kakudo[5], the steric repulsion between the C^α of the preceding residue and the C^δ in the prolyl side chain presumably causes this distortion. The value of this angle is further enhanced when a prolyl residue is preceded by a residue of α-aminoisobutyric acid (Aib)[16, 18], presumably to relieve the steric interactions between the two conformationally restrictive residues. A systematic enhancement of the C_{i-1}—N_i—C_i^δ angle is also observed when the concerned prolyl residue is preceded by another prolyl residue on the same side of the peptide chain or by an Aib residue[12, 16, 18]. This pheno-

menon can be accounted for by the steric interactions between adjacent conformationally restrictive side chains of Aib or Pro. Another feature often observed in prolyl residues occupying the second position in a Type I β-turn is the widening of the angle N_i—C—C^{α}_i. The average value of this angle in such a situation is 115° as against its normal value of 111° [19, 20].

4. Occurence of *cis* peptide units

Peptide bonds involving α-amino acids normally occur in the *trans* configuration in linear peptides and proteins. In fact, *cis* peptide bonds involving amino acids are observed only in constrained situations such as those in some cyclic peptides. X-Pro peptide bonds can, however, assume both the *cis* and the *trans* configurations. In peptides involving other amino acids, the α-carbon atom and the hydrogen atom in the NH group of the next residue are brought into close proximity when the peptide bond is *trans* whereas the two successive α-carbon atoms are brought into contact when it is *cis*. The *cis* configuration is, therefore, sterically unfavourable. In a prolyl residue, however, the δ-carbon atom is attached to the nitrogen atom instead of a hydrogen atom. Thus when a X-Pro bond is *cis*, the α-carbon atom of the prolyl residue and that of the preceding residue are in steric contact; when the bond is *trans* the δ-carbon atom of the prolyl residue is, instead, in steric contact with the α-carbon atom in the preceding residue. Consequently, both the isomers could be expected to have nearly the same energy on steric considerations alone[21]. Detailed energy calculations[22] confirm this expectation although the *cis* X-Pro bond was found to have slightly higher energy than the *trans* bond. Solution studies on model compounds are in conformity with the results of these theoretical calculations[22–26]. Both the *cis* and the *trans* X-Pro bonds occur in solution, but a larger proportion of the molecule has *trans* bonds.

The geometries of a number of X-Pro peptide bonds in small peptides and proteins have been determined by the X-ray diffraction method. As far as the structures of small linear peptides are concerned, all the X-Pro bonds except one exist in the *trans* configuration, the exception being the Gly–Pro bond in Z-Gly-Pro-Leu[26]. There are, however, a number of instances of *cis* 'peptide-like' X-Pro bonds (with an oxygen atom replacing the α-carbon atom in the preceding peptide group) occurring in small peptides when an amino terminal proline is protected by *t*-Boc, Aoc and Z groups[10, 12, 27–33]. The strain involved in cyclisation has an overriding influence in determining the isomeric state of peptide units in cyclic peptides and hence the intrinsic preference of the bond to be in the *cis* or the *trans* configuration is relatively unimportant as far as cyclic peptides are concerned. Of the 39 X-Pro bonds observed in cyclic peptides 15 are *trans* and 24 *cis*. X-Pro peptide bonds are found to occur in both the isomeric states in proteins with the *trans* form predominating. In the *cis* X-Pro bonds in proteins, the prolyl residue is almost invariably found to occur at the third position of a Type VI β-turn. This aspect of the problem is dealt with in some detail in a latter part when dealing with β-turns.

The energy barrier between the *cis* and the *trans* forms in a normal peptide is estimated to be of the order of 20 kcal/mol with an energy difference of over 2 kcal/mol[34]. The energy difference between the *cis* and the *trans* X-Pro bonds varies between 0·7 and 2·5 kcal/mol[35–38] with a barrier varying between 13 and 17 kcal/mol[35]. Thus the energy difference and the barrier between the *cis* and the *trans* configurations are small in X-Pro bonds compared to those in normal peptide bonds. Therefore not only does *cis* proline occur with significant population but *cis-trans* isomerism is also easier in X-Pro bonds. Such isomerism in Poly-L-proline and other oligomers of proline has been studied extensively by many workers using physico-chemical techniques[39–48]. It has also been suggested recently that *cis-trans* isomerism of X-Pro bonds plays an important role in protein folding[2].

5. Conformation of pyrrolidine ring

Conformational aspects of the five-membered pyrrolidine ring in the prolyl residue have been studied by many workers[5, 49]. The approach initiated by Ashida and Kakudo based on crystal structure data is perhaps more suitable than those of the others in describing in simple terms the geometry of the pyrrolidine ring.

The pyrrolidine ring is made up of a planar nitrogen atom and four tetrahedral carbon atoms. The ring cannot therefore be planar. The conformation of a non-planar five-membered ring can be conveniently described in terms of the displacement of one or two atoms from the plane defined by the rest of the atoms[50]. If four atoms lie nearly in a plane and the fifth atom deviates from this plane, the ring has an 'envelope' conformation with an approximate C_s symmetry. If, however, no set of four atoms lie in a plane, the conformation can be described in terms of the displacements (one positive and the other negative) of two adjacent atoms from the plane defined by the remaining three atoms. The ring then has a 'half-chair' conformation with an approximate C_2 symmetry. It must be mentioned that as the displacements (of one or two atoms) can assume a continuous range of values, instances occur when the conformation could be described as C_s or C_2.

In the case of prolyl residue, it is also useful to describe the geometry of the pyrrolidine ring in relation to that of the main chain. This can be conveniently done with reference to the carbonyl carbon atom in the residue which, owing to the tetrahedral nature of the α-carbon atom, is always displaced by more than an angstrom unit from the plane of the pyrrolidine ring. In a pyrrolidine ring with C_s symmetry the displaced atom is termed '*endo*' if it lies on the same side of the plane defined by the remaining four atoms as the carbonyl carbon atom; if it lies on the opposite side, it is described as '*exo*'. Thus if N, C^α, C^γ and C^δ lie in a plane and C^β is displaced towards that side of the plane where the carbonyl carbon is situated, then the conformation can be described as 'C_s–C^β *endo*'; likewise in a pyrrolidine ring with approximate C_2 symmetry, if C^β is displaced from the N–C^α–C^δ plane towards the side of the carbonyl carbon and C^γ towards the opposite side, the conformation is then described as C_2–C^β *endo*-C^γ *exo* (Fig. 2).

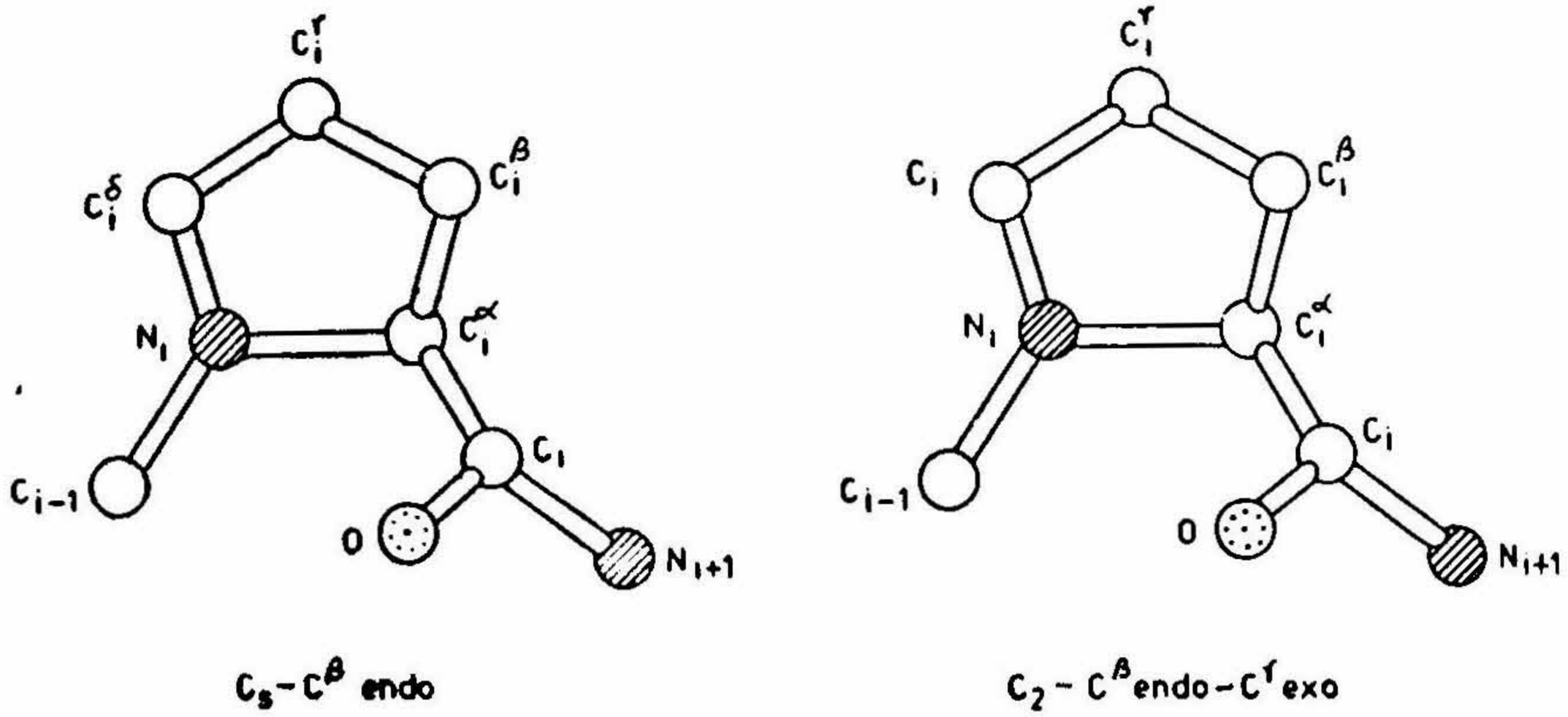

FIG. 2. Illustrations of typical C_s and C_2 puckerings in a pyrrolidine ring.

Among the four tetrahedral carbon atoms in the pyrrolidine ring, C^α and C^δ are attached to the planar nitrogen atom. Hence they have less freedom of movement compared to C^β and C^γ. Hence the conformations most frequently expected to occur are C_s–C^β *endo*, C_s–C^β *exo*, C_s–C^γ *endo*, C_s–C^γ *exo*, C_2–C^β *endo*-C^γ *exo* and C_2–C^β *exo*-C^γ *endo*. All these conformations have been observed in proline containing peptides. Indeed, these are the only conformations found to occur in linear peptides of naturally occurring amino acids. Other intrinsically less favourable conformations occur only when strains are introduced on account of cyclisation. Examples of such unusual conformations are C_s–C^α *endo*–C^β *exo* in cyclo triproline[51], cyclo (Pro-Pro-Hyp)[52] and Li^+ antamanide[53], C_2–N–*exo* C^δ *endo* in cyclo (Pro-Pro-Hyp)[52] and C_2–C^γ *exo*–C^δ *endo* in cyclo (L-Pro-Gly)[54] and Na^+ antamanide[11].

It may be mentioned that the pyrrolidine ring conformation can also be described in terms of the five ring dihedral angle, namely θ, χ^1, χ^2, χ^3 and χ^4,[49,55]. For example, the C_s conformation is characterised by the dihedral angle about the bond opposite to the displaced atom having a value close to zero and the other four angles having relatively large values, with alternate dihedral angles alternating in sign. The C_2 conformation as well as the directions of atomic displacements in C_s and C_2 conformations can also be described in terms of dihedral angles. A detailed account of this mode of description is not presented here.

Attempts have been made by many workers to correlate the ring conformation with the backbone conformation of proline containing peptides[56–61]. No universally accepted general principle appears to have emerged, to relate the ring conformation with the main chain conformation, from any of these studies.

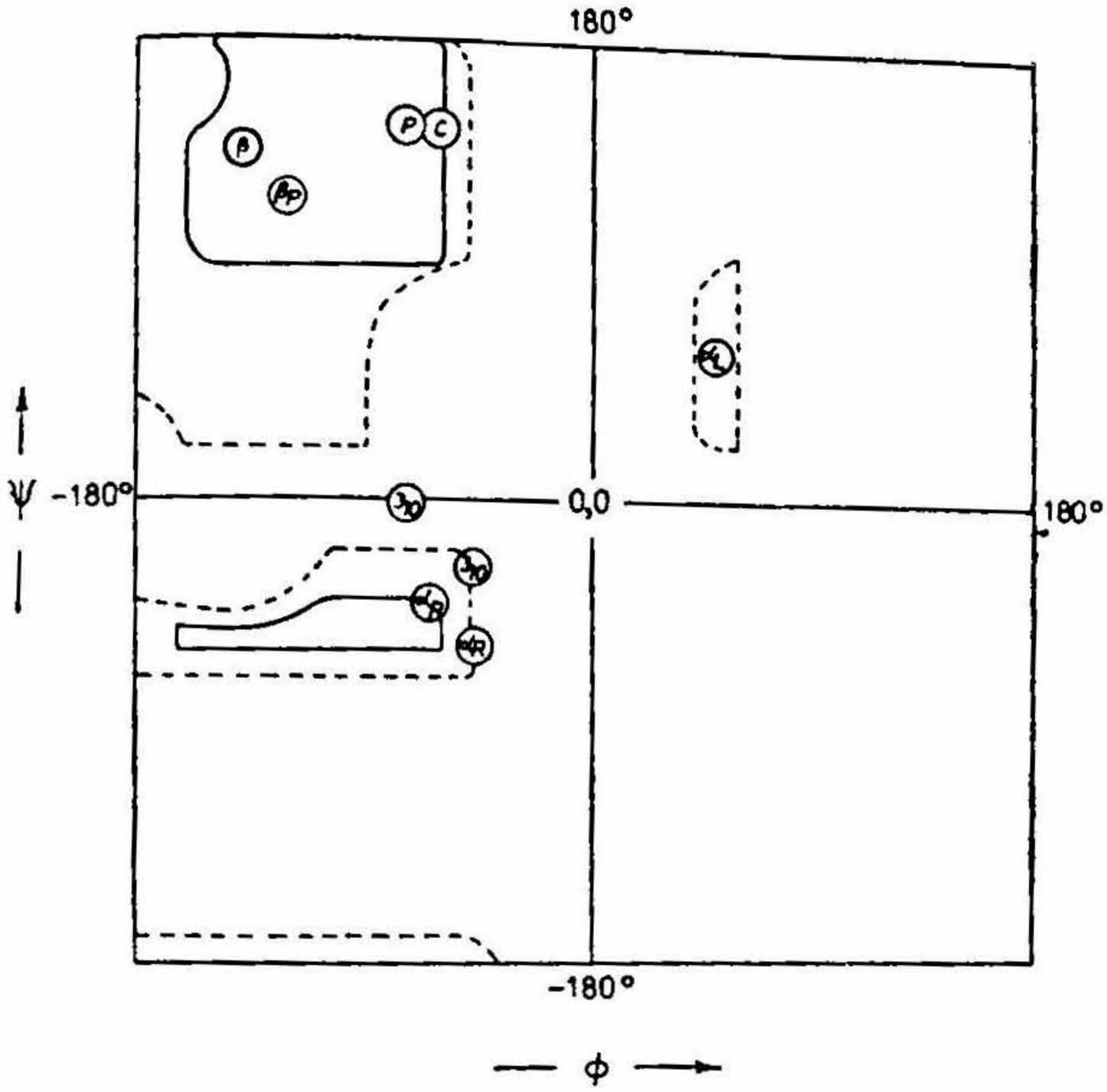

FIG. 3. Conformational map corresponding to L-alanine. The regions within broken lines are allowed for the extreme cases of contact distances.

6. Occurrence in regular secondary structures

It is well known that the main chain conformation of peptides and proteins are essentially defined by the dihedral angles ϕ and ψ[34]. The contact map showing the allowed regions in conformational space for two linked peptide groups with side chains corresponding to L-alanine is shown in Fig. 3. Regular structures are produced when the same or similar ϕ, ψ values occur repeatedly in successive residues in a peptide chain. Some of the such well characterised regular structures for peptides made up of L-residues are the right-handed α-helix ($\phi = -58°$, $\psi = -47°$), the 3_{10} helix ($\phi = -50°$, $\psi = -25°$), the β-structure ($\phi = -147°$, $\psi = 143°$ and $\phi = -119°$, $\psi = 114°$), the left-handed α-helix ($\phi = 58°$, $\psi = 47°$), collagen ($\phi = -67$ to $-76°$, $\psi = 156$ to $168°$) and polyproline structures [$\phi = -77°$, $\psi = 146°$ (PPII) and $\phi = -83°$, $\psi = 158°$ (PPI)]. The positions in the conformational space of these structures are also indicated in Fig. 3.

On account of steric restrictions, the value of ϕ for the L-Pro residue is confined to a range between $-40°$ and $-110°$. Therefore, as far as regular structures are concerned, L-prolyl residue can occur either in the region corresponding to right-handed

helices (α-helix or 3_{10} helix) or in the region corresponding to collagen and polyproline structures.

The α-helix is by far the most extensively studied secondary structure in proteins and polypeptides, and it is of interest to examine the occurrence of prolyl residue in it (the discussion which follows is applicable, with suitable obvious modifications, to the 3_{10} helix also). A perfect α-helix is stabilised by a set of hydrogen bonds between the carbonyl oxygen atom of the *i*th residue and the amino hydrogen of the $i + 4$th residue. Prolyl residues do not have amino hydrogen atoms and hence, on the basis of the requirement of hydrogen bond formation, can occur only in the first, second and third positions. Steric considerations also show that prolyl residues cannot occur in the middle of a stretch of α-helix[57]. Thus, although prolyl residues can occur at the first three positions of an α-helix, proline acts as a helix breaker at any other position. The detailed analysis carried out by Perutz and coworkers[62] on haemoglobin and myoglobin —two proteins with the highest helix content among those analysed so far by X-ray methods—also leads to the same conclusion. The analysis of Chou and Fasman[63] using a number of proteins confirm that proline is among the best helix breakers.

The richest source of proline is collagen, the structural protein found in connective tissues and tendons. In fact proline (~ 12) and its derivative hydroxyproline (~ 11%) accounts for a quarter of the amino acid content of the protein[64]. Among the monomeric units of proteins, proline is conformationally the least flexible. The most flexible amino acid is glycine which accounts for a third of the total number of amino acid residues in collagen. Thus, the appropriate combinations of the least flexible and the most flexible of amino acid residues, which between them constitute more than half of the amino acid content of the protein, lead to the unique structure of collagen. After several unsuccessful attempts by many workers[65–71], the structure was finally solved, in essence, in the fifties by Ramachandran and Kartha[72,73] and Rich and Crick[74]. The triple helical model proposed by these workers, which in essence has been universally accepted, and the subsequent work on collagen have led to a clear identification of the role of glycine, proline and hydroxyproline in stabilising the structure[75–79].

It is interesting to note that the synthetic homopolymer of L-proline, poly-L-proline, can assume a collagen-like folding[80,81]. Thus it would appear that the prolyl residue has an intrinsic propensity to be in the collagen fold. This poly-L-proline structure, with all *trans* peptide bonds, is known as poly-L-proline II (PPII). Another form of poly-L-proline, called poly-L-proline I (PPI), also exists. In this form, the peptide chain assumes a right-handed helical conformation with all peptide bonds in the *cis* configuration[82–83]. On account of the relatively small energy difference between *cis* and *trans* Pro-Pro peptide bonds mutarotation from one form to the other is also found to occur[39–41].

Solution studies indicate that a length of three to four residue is sufficient to enable a proline oligomer to assume the PPII conformation[84–86]. This indication is supported by the results of the X-ray analysis of *t*-amyloxycarbonyl triproline[12] and *t*-butyloxycarbonyl tetraproline benzyl ester[10]. The molecules assume PPII conformation in both

the structures. In fact the same tendency is retained even when the degree of polymerisation is reduced to the minimum possible value of two as in the crystal structure of benzyloxycarbonyl-L-prolyl-L-proline[29-30]. This is presumably an indication of the high propensity of prolyl residues to be in the PPII conformation.

7. Occurrence in β-turns in normal peptides and proteins

Until the late sixties and the early seventies attention was concentrated almost exclusively on helices and extended chains when considering the secondary structure in proteins and polypeptides. However, in 1968 the possibility of chain reversal involving a folded structure made up of three peptide units (or four amino acid residues) and stabilized by a $4 \rightarrow 1$ hydrogen bond was recognised. Three types of such β-turns have been described by Venkatachalam[87] with the following characteristic dihedral angles.

	ϕ_2	ψ_2	ϕ_3	ψ_3
Type I	−60	− 30	−90	0
Type II	−60	120	80	0
Type III	−60	− 30	−60	−30

These three β-turns are illustrated in Fig. 4 *a* to 4 *c*. Of these, the Type III turn corresponds to a turn in a 3_{10} helix. Three more β-turn conformations, which are mirror images (as far as the main chain is concerned) of the three types mentioned above, are also possible and are designated as Types I′, II′ and III′. There are obviously restrictions on the types of residues—L, D or Gly—that can occur at the second and third positions in these β-turns.

β-turns of the types described by Venkatachalam has since been observed in several proteins and peptides. The possible structural role of β-turns also began to be appreciated subsequently. β-turns involve the reversal of chain directions which are important in folding the linear polypeptides in proteins into globular shapes. It has also been suggested that β-turns might play a specific role in bringing together distant regions of the peptide chain in the course of protein folding[88]. In view of its structural importance, β-turns have recently been studied extensively.

It is interesting to note that the dihedral angles at the second residue are such that proline could occur at this position in all the three types of β-turns. Perhaps the best way to investigate the intrinsic propensity of prolyl residues to occur in β-turns is to examine the crystal structures of appropriate linear peptides containing proline along with other common amino acids (peptides containing unusual and D-amino acids are discussed later). It turns out that in every case when a proline is preceded by a *trans* peptide or a 'peptide-like' group and followed by two amino acid residues or an amino acid residue and an amide group, a β-turn is formed with the prolyl residue at the

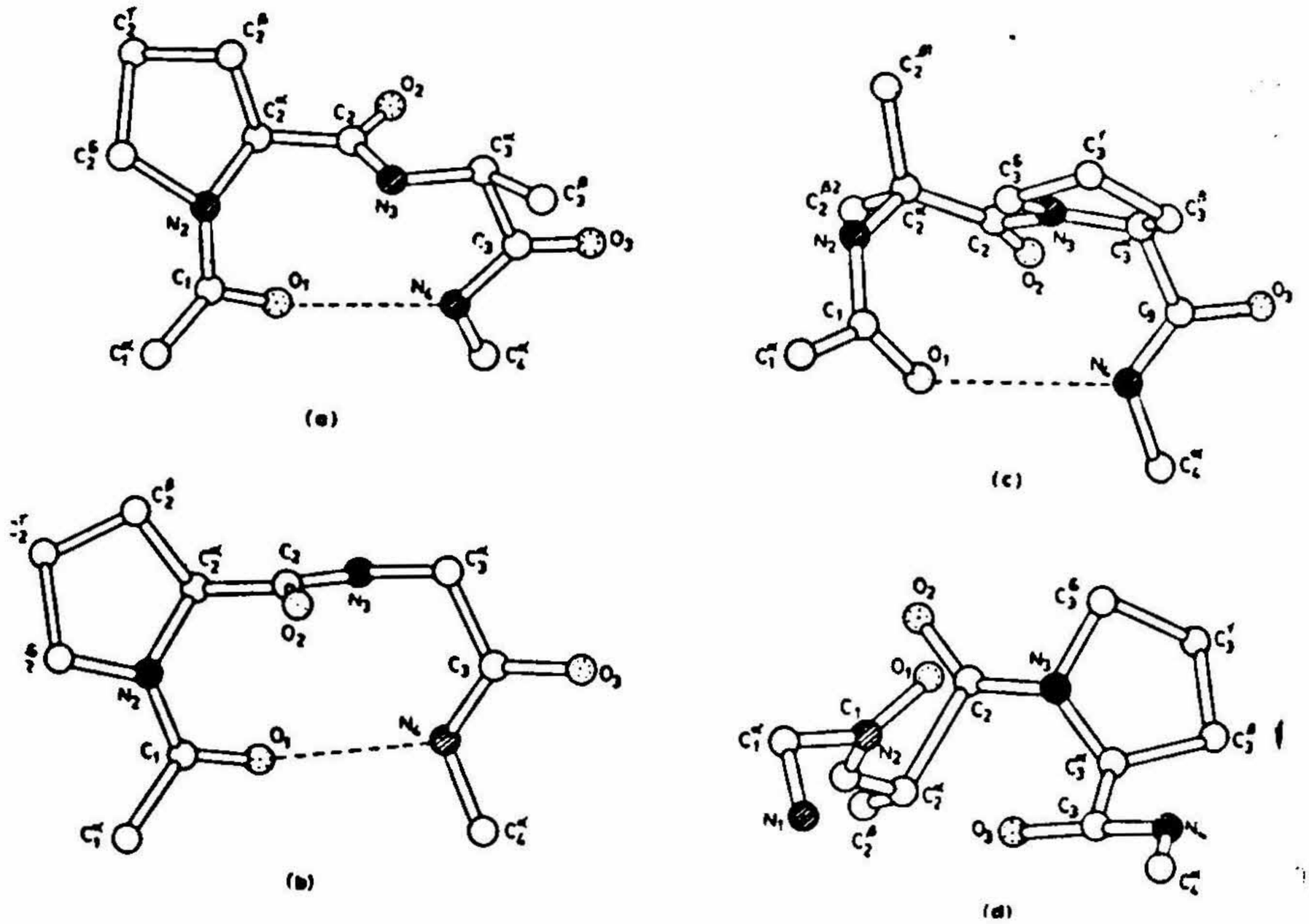

FIG. 4. Illustrations of the different types of β-turns. *a* : Type I ; *b* : Type II ; *c* : Type III ; *d* : Type VI, an open reverse turn. *a*, *b* and *c* show projections perpendicular to the mean plane of the first and the third peptide unit. *d* is projected normal to the pyrrolidine ring.

second position. A list of peptides in which these β-turns occur are given in Table I along with the relevant dihedral angles and bend type. Thus it would appear that the prolyl residue has a high intrinsic propensity to occur at the second position in β-turns provided it is flanked by chemical groups capable of participating in such turns. Among the thirteen bends listed in Table I ten are of Type I and three of Type II. Type III turns are yet to be observed in linear proline containing peptides made up of common amino acid residues.

Intrinsic structural propensities cannot often be estimated from the study of cyclic peptides on account of the strain introduced in cyclisation especially when the number of residues in the cycle is small as in several proline-containing diketopiperazines such as cyclo (L-Pro-L Leu)[89], cyclo (L-Pro-L-Phe)[10], cyclo (L-Pro-L-Pro)[91] and cyclic tripeptides such as cyclo (Pro-Pro-Pro)[51] and cyclo (Pro-Pro-Hyp)[52]. Among the comparatively large L-proline containing cyclic peptides two β-turns occur in antamanide[11] with proline in the first position while the remaining two prolyl residues do not take part in β-turns. None of the prolyl residues in cyclo (L-Pro-Gly)$_4$[92] participate in β-turns.

Table I

LL and LG β-turns in linear peptides with proline at second position

Sl. No.	Compound	Residue 2	Residue 3	ϕ_2	ψ_2	ϕ_3	ψ_3	Type
1.	N-Isobutyl-L-prolyl-[112] L-alanyl-isopropylamide	L-Pro	L-Ala	−59°	136°	66°	14°	II
2.	*t*-Boc-L-prolyl-L-leucyl-[19] glycine hydrate	L-Pro	L-Leu	−65	−21	−111	27	I
3.	(*o*-Br) Z-glycyl-L-prolyl-[113] L-leucyl glycyl-L-proline	L-Pro	L-Leu	−65	−27	−105	8	I
4.	(*p*-Br) Z-glycyl-L-prolyl-[9] L-leucyl glycine	L-Pro	L-Leu	−58	−33	−104	8	I
5.	N-Ac-L-Prolyl-L-lactyl-[108] methylamide	L-Pro	L-Lac	−55	−22	− 81	−11	I
6.	N-Ac-L-Prolyl glycyl-[114] L-phenylalanine	L-Pro	Gly	−59	128	81	− 6	II
7.	S-Bz-L-Cysteinyl-L-[115] prolyl-L-leucyl glycin-amide (Molecule A)	L-Pro	L-Leu	−70	−16	− 74	− 9	I
8.	S-Bz-L-Cysteinyl-[115] L-prolyl-L-leucyl glycin-amide (Molecule B)	L-Pro	L-Leu	−64	−28	− 71	−12	I
9.	Se-Bz-L-Cysteinyl-[116] L-prolyl-L-leucyl-glycinamide (Molecule A)	L-Pro	L-Leu	−70	−23	− 60	−21	I
10.	Se-Bz-L-Cystinyl-[116] L-prolyl-L-leucyl-glycinamide (Molecule B)	L-Pro	L-Leu	−67	−34	− 63	−16	I
11.	*t*-Boc-L-Prolyl-[116] L-prolyl glycinamide	L-Pro	Gly	−65	−23	− 89	6	I
12.	Piv-D-Prolyl-L-prolyl-[17, 20] L-alanyl-N-methylamide	L-Pro	L-Ala	−59	−23	− 88	0.	I
13.	Piv-L-Prolylglycyl-[117] isopropylamide	L-Pro	Gly	−61	137	84	−3	II

The situation in proteins is confused by the comparatively lower resolution of protein electron-density maps as well as the relatively greater influence of long range interactions. It is, therefore, useful, as pointed out by Lewis and coworkers[93], to somewhat relax the criteria for designating a given tetrapeptide sequence as a turn when dealing with proteins. Relaxing the definition of β-turns to include all tetrapeptide sequences involved in chain reversal with a C_i–C_{i+3} distance of less than 6 to 7 Å (more frequently

less than 6 Å), Lewis *et al* introduced four more types of β-turns with the following characteristics :

Type IV : Any of the β-turn Type I through III′ with angles ϕ_2, ψ_2, ϕ_3, ψ_3 differing by at least 40° from the typical values, for Types I through III′.

Type V : $\phi_2 \sim -80°$, $\psi_2 \sim 80°$, $\phi_3 \sim 80°$, $\psi_3 \sim 80°$.

Type VI : A *cis* proline at position 3 (open reverse turn).

Type VII : A kink in the protein chain created by $\psi_2 \simeq 180°$ and $|\phi_{i+2}| < 60°$ or $|\psi_{i+1}| < 60°$ and $\phi_{i+2} \simeq 180°$.

A critical analysis of these additional β-turn types, though useful, is perhaps unwarranted in the context of the present discussion. The Type VI β-turn is, however, of particular interest. Prolyl residue is the only one in proteins which can form a *cis* bond, and as can be seen from Fig. 4 *d* a β-turn, without hydrogen bond, involving a *cis* X-Pro bond with proline at the third position is an ideal structural feature for bringing about a chain reversal.

A number of workers have analysed protein structural data in terms of secondary structural characteristics[94–96]. The one carried out by Chou and Fasman[96] is typical and among the latest of these analyses. According to these authors, nearly 50% of the total number of prolyl residues occur in β-turn regions. An examination of the data provided by Chou and Fasman showed that of the 102 tetrapeptide β-turn sequences in which proline occurs, 19 were in position 1, 58 in position 2, 12 in position 3 and 13 in position 4. Of the 58 turns with Pro in second position, 31 were of Type I, 15 of Type II and 12 of Type III. Of the 19 with Pro at the first position, 9 were of Type I and 4 of Type III. Eight out of 12 with Pro in the third position were open reverse turns with *cis* Pro bond. Out of the 13 turns with Pro at the fourth position 7 were of Type I.

8. Proline in peptides containing D and unusual amino acids

In addition to proteins based on the common amino acids, lower organisms frequently produce small peptides with unusual structural features. Many of these peptides, often cyclic, contain D residues of common amino acids as well as several uncommon amino acid residues. For example, peptide antibiotics, gramicidin S and actinomycin, contain D residues (in addition to the unusual amino acids, ornithine in the former and sarcosin in the latter) whereas peptide antibiotics like alamethicin, suzukacillin, emmerimicins and antiamoebins contain the unusual amino acid α-amino-isobutyric acid (Aib). There are also peptide antibiotics which contain residues other than those of amino acids such as the residues of L-lactic acid and D-hydroxyisovaleric acid in valinomycin. Many of these antibiotics have been studied by X-ray diffraction methods ; several synthetic

model peptides containing D or unusual residues have also been analysed. A brief discussion of the conformational features of proline containing compounds among them is attempted in this section.

On the basis of theoretical considerations, Chandrasekharan *et al*[97] suggested the probability of the formation of β-turns in peptides with a L-residue at the second position and a D-residue at the third position (LD turn) or with a D residue at the second position and a L-residue at the third position (DI turn). For reasons which appear to be obvious in retrospect, LD turns should be of Type II and DL turns of Type II'. X-ray crystallographic studies have shown that this is indeed the case in most cases at least as far as proline-containing peptides are concerned, as can be seen from Table II which lists the relevant conformational parameters of proline containing LD and DL synthetic peptides (including cyclic peptides but excluding diketopiperazines and cyclic tripeptides) on which X-ray structural data are so far available. All of them except one contain LD or DL turns each involving a prolyl residue. All the LD turns are of Type II. The observed DL turns are of Type II'. Two D-Phe-L-Pro β-turns of Type II' occur in gramicidin S[98]. The two turns in actinomycin D, as determined from the X-ray analysis of its crystalline complex with deoxyguanosine[8], are unusual for oligopeptides in that both involve a *cis* peptide bond. One of them has a D–Val at the second position and L-Pro at the third position with a *cis* peptide bond connecting them. The other is a L-Pro-Sar turn, again with a *cis* peptide connecting them. It may be recalled that sarcosine has a methyl group attached to the amino nitrogen and hence an X-Sar peptide bond could easily assume the *cis* configuration. In fact *cis* X-Sar peptide bonds have been observed in the crystal structures of L-prolylsarcosine monohydrate and *t*-Boc-L-prolyl sarcosine benzyl ester also[99].

Among the compounds listed in Table II, c (Gly-L-Pro-Gly-D-Ala-L-Pro)[100] merits special mention. This is the only peptide in the group in which the D residue is not involved in a β-turn. Instead the cyclic peptide contains a Type II Pro-Gly turn. An unusual feature of the structure is the presence of a $3 \rightarrow 1$ hydrogen bonded 2_7 turn shown in Fig. 5, involving one of the prolyl residues. Thus, the prolyl residue, almost, by itself, is capable of initiating chain reversal on rare occasions.

A number of crystal structures of peptides containing the unusual optically neutral amino acid α-aminoisobutyric acid (Aib) has recently been analysed. Four of them contain proline as well. Two methyl groups, instead of a side chain and a hydrogen atom in the normal amino acids, are attached to the α-carbon atom in Aib. The presence of two methyl groups, one corresponding to the L configuration and the other to the D configuration, makes the residue very rigid with very little conformational mobility[101]. The residue can occupy only those regions in the conformational space which are allowed for both L and D residues. As shown in Fig. 6, these regions correspond to the right-handed and left-handed α-helices. Electron diffraction[102] and single crystal X-ray studies[104], however, indicated the propensity of Aib residues to be in the 3_{10} helical region although theoretical calculations have shown that it could assume a 3_{10},

Table II

β-turns in peptides containing D residues

Sl. No.	Compound	Residue 2	Residue 3	ϕ_2	ψ_2	ϕ_3	ψ_3	Type
1.	N-Isobutyl-L-prolyl-[118] D-alanyl isopropylamide	L-Pro	D-Ala	−62°	137°	96°	3°	II
2.	N-Ac-L-Prolyl-D-[118] alanyl-methylamide	L-Pro	D-Ala	−66	127	75	12	II
3.	c(Gly-L-Pro-D-Phe)$_3$[119]	L-Pro	D-Phe	−56	134	100	− 9	II
4.	c(Gly-L-Pro-D-Phe)$_3$[119]	L-Pro	D-Phe	−72	131	112	−19	II
5.	c(L-Ala-L-Pro-D-Phe)$_3$[120]	L-Pro	D-Phe	−60	122	78	9	II
6.	Pivaloyl-D-Alanyl-L-[121] prolyl-N-isopropylamide	D-Ala	L-Pro	60	−140	−89	9	II′
7.	c(Gly-L-Pro-D-Ala)$_3$[122]	L-Pro	D-Ala	−54	125	94	− 5	II
8.	c(Gly-L-Pro-Gly-D-Ala-[100] L-Pro)	L-Pro	Gly	−52	126	74	12	II
9.	N-Ac-L-Prolyl-D-lactyl-[109] methylamide	L-Pro	D-Lac	−62	140	91	− 8	II
10.	Piv-D-Prolyl-L-prolyl-[17, 20] L-alanyl-N-methylamide	D-Pro	L-Pro	59	−136	−59	−23	II′

an α-helical or a modified α-helical conformation[108]. As Aib is optically neutral, the helix could be right-handed or left-handed.

The peptides containing Aib as well as proline studied so far by X-ray methods are Z-Aib-Pro-NHMe[18], Z-Aib-Pro-Aib-Ala-OMe[18], *t*-Boc-Pro-Aib-Ala-Aib-OBzl[105] and Z-Aib-Pro-Aib-Pro-OMe[106]. All the β-turns except one occurring in these compounds are of Type III. The first of these compounds form an Aib-Pro β-turn. Z-Aib-Pro-Aib-Ala-OMe contains an incipient 3_{10} helix made up of two consecutive Type III turns, Aib-Pro and Pro-Aib. Indeed, these compounds provide the only examples of Type III turns in small peptides. This is not surprising in view of the propensity of Aib to be in 3_{10} helices and the preference of Pro to be in β-turns. As noted earlier, Aib residues can assume ϕ, ψ values corresponding to the left-handed or the right-handed conformations. They are in the right-handed conformation in the first three compounds whereas Aib assumes a left-handed conformation in the fourth. The prolyl residue often occurs in the third position of a β-turn in the Aib containing peptides. The other compounds in which proline occurs in the third position are gramicidin S[98] and Piv-D-Prolyl-L-prolyl-L-alanyl-N-methylamide[17, 20]. It is noteworthy that the compound Piv-D-prolyl-L-prolyl-L-alanyl-N-methylamide has an interesting compact conformation with consecutive DL and LL turns.

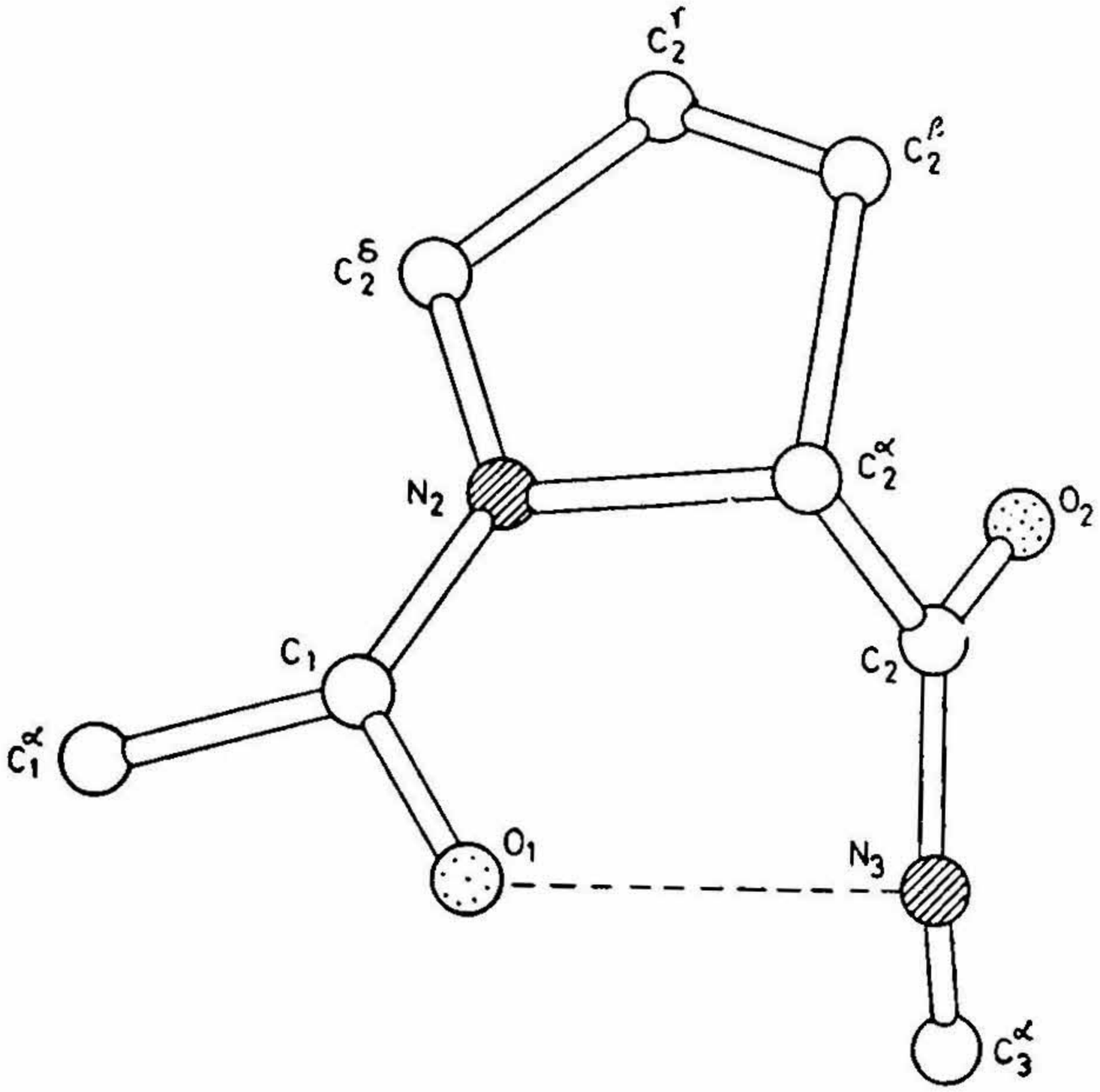

FIG. 5. Illustration of a 3 → 1 hydrogen bonded 2_7 turn.

Yet another proline containing peptide with unusual amino acid residues also present is the cyclic peptide antibiotic clamydocin with sequence cyclo (Aib-L-Phe-D-Pro-L–X) where X is a residue with a long side chain containing epoxide and ketone groups. The Aib residue is conformationally highly restrictive as far as the value of ϕ and ψ are concerned. The same is true about proline also in terms of restrictions in the value of ϕ. Consequently the molecule assumes a highly strained conformation with two 3→ 1 internal hydrogen bonds, one involving the Aib residue and the other the prolyl residue[107].

Depsipeptides with some peptide groups (–CO–NH) replaced by –CO–O– groupings are of considerable importance as many ionophoric antibiotics, typically valinomycin, belong to this category. In this context, the crystal structures of N-Ac-L-Pro-L-Lac-NHMe[108] and N–Ac-L-Pro-D-Lac-NHMe[109] are of some interest. These are the only two proline containing compounds of this type the crystal structures of which are available. A typical Type I turn is observed in N-Ac-L-Pro-L-Lac-NHMe whereas N-Ac-L-Pro-D-Lac-NHMe has a Type II turn. Thus the replacement of NH by 0 in the central peptide unit does not appear to affect the propensity of proline for the formation of β-turns. The conformation of prolinomycin, a peptide analog of valino-

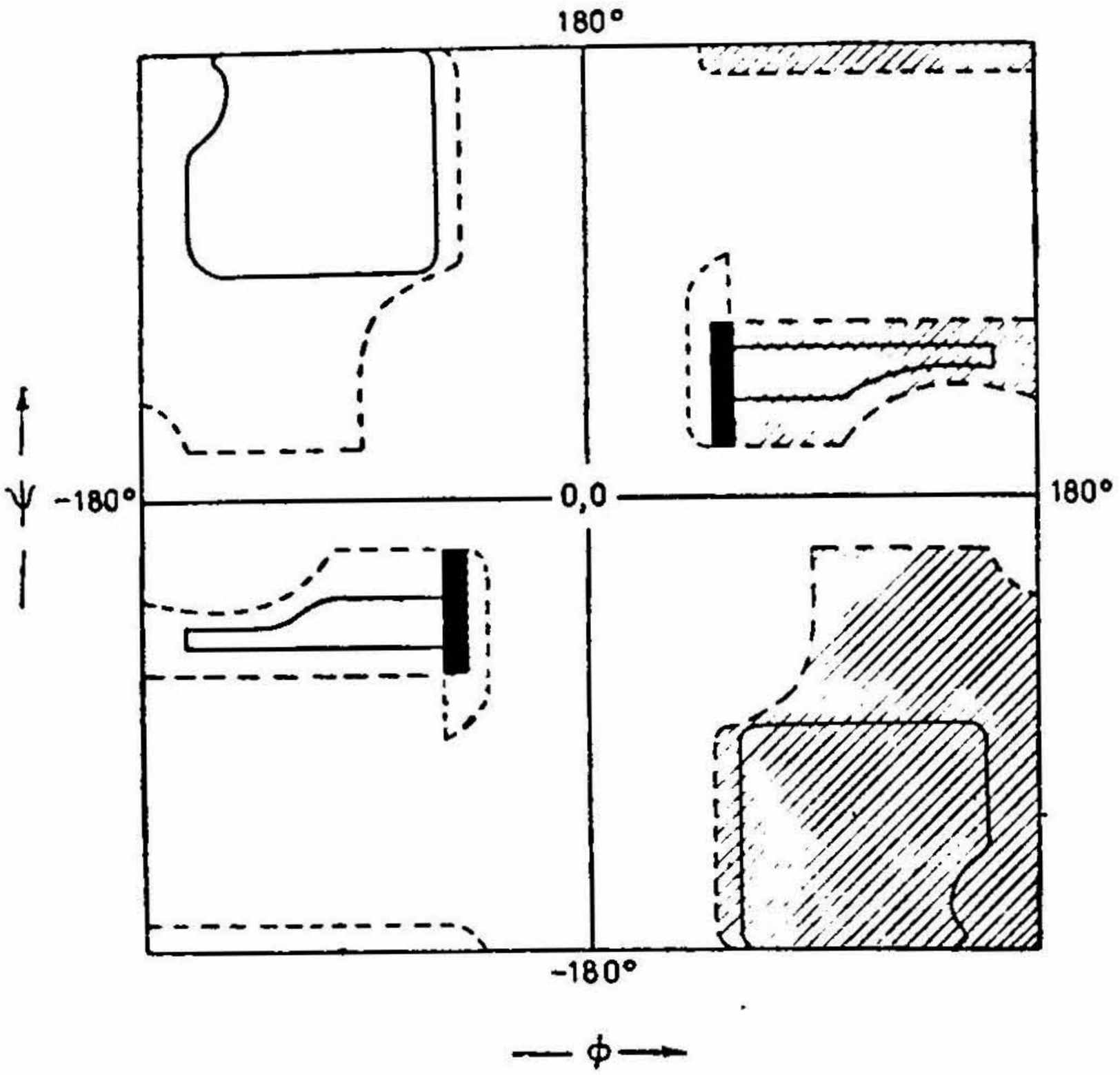

FIG. 6. Conformation map showing the allowed regions for Aib residue. The shaded regions are those allowed for D-alanine. The dark regions correspond to those allowed for Aib residue.

mycin, is also of some interest in this context. Prolinomycin is obtained by replacing the lactic acid and the hydroxyisovaleric acid residues in valinomycin by prolyl residues. The molecule of valinomycin in its potassium complex[110] is made up of a series of β-turns with an ester linkage (instead of peptide group in the conventional β-turn) in the middle position of each turn. These ester linkages are replaced by peptide linkages in prolinomycin. The prolinomycin molecule in its rubidium complex[111] still has nearly the same conformation and hydrogen bonding pattern as in the potassium complex of valinomycin.

Acknowledgements

The authors thank the University Grants Commission, India, and the Indian National Science Academy for financial support.

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