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Standard dimensions for *cis* N-methyl peptide unit and flexibility of *cis* peptide units

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Abstract. The mean dimensions of the *cis* N-methyl peptide unit have been arrived at by analysing the crystal structure data on compounds containing such units. These dimensions can be used as standard in conformational studies on cyclic peptides. While the bonds meeting at C are almost coplanar, those meeting at N show a slight pyramidal disposition. A comparison of the dimensions of the normal and N-methylated *cis* peptide units show that there are perceptible differences in the parameters connected with N. In addition, the flexibility of the *cis* peptide unit has been analysed by studying the distribution of the parameters in different classes of compounds such as cyclic di, tri and higher peptides. The salient features are: (i) The angle $C^{\alpha}CN$ in cyclic dipeptide and the angle $C^{\delta}NC^{\alpha}$ in higher peptides tend to be lower, when the peptide unit is associated with a prolyl residue; (ii) in cyclic tripeptides the internal angles *viz.,* $C^{\alpha}CN$ and CNC^{α} are significantly larger thereby increasing the intra-annular space; (iii) the bond C^{α}-C is distinctly shorter when it occurs in cyclic dipeptides. The results lead to the conclusion that the *cis* peptide unit takes up a *need-based* flexibility in its dimension.

Keywords. ***c**is* peptide units; dimension of *cis* peptide unit; N-methylation in *cis* peptide unit; *cis* peptide units in cyclic peptides; flexibility of *cis* peptide unit; dimensions of N-methylated *cis* peptide unit.

Introduction

In theoretical conformational calculations as well as model building studies it is necessary to use a set of standard dimensions for the peptide units. These dimensions are invariably obtained by an analysis of crystal structure data on simple peptides. By this method, the dimensions of the *trans* peptide unit were first arrived at by Corey and Pauling (1953). These values, known as P-C peptide dimensions, are being widely used to this day. With the availability of more data from crystal structures on peptides, other workers also analysed these data and obtained, the dimensions of the *trans* peptide unit (Marsh and Donohue, 1967; Ramachandran *et al.,* 1974; Ashida and Kakudo, 1974; Benedetti, 1977). These revised dimensions are not very different from the P-C peptide dimensions.

A similar attempt has also been made to arrive at a definite geometry for the *cis* peptide unit, since it is found to occur quite frequently in cyclic peptides. Ramachandran and Venkatachalam (1968) suggested a set of dimensions for the *cis* peptide unit from an analysis of the structures of cyclic dipeptides, more commonly known as diketopiparazines. Later Benedetti (1977) and Kolaskar and Sarathy (1980) have also arrived at the dimensions of the *cis* peptide unit from the then available crystal structure information. Table 1 gives the dimensions of the *cis* peptide unit obtained by these different workers. It is clear from the table that the dimensions

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Table 1. Dimensions of the *cis* peptide unit obtained by different workers.

Parameter	Ramachandran and Venkatachalam (1968)	Benedetti (1977)	Kolaskar and Sarathy (1980)
Bond length (Å):			
C^{α}-C	1·53	1·51	1·51
C=O	1·24	1·23	1·24
C-N	1·32	1·33	1·34
N-C^{α}	1·47	1·46	1·46
N-H	1·00	1·00	—
Bond angle (°):			
C^{α}-C-N	118·0	118·4	118·1
C^{α}-C=O	119·0	118·6	119·1
O=C-N	123·0	123·0	122·4
C-N-C^{α}	126·0	126·5	125·3
C-N-H	121·0	119·8	—
H-N-C^{α}	113·0	113·7	—

(except perhaps for the bond length C^{α}-C) do not vary significantly and any one of them can be used as standard.

Similarly, Jayati Mitra and Ramakrishnan (1980) have proposed a set of standard dimensions for the ester unit from crystal structure data on compounds containing such units. These are. particularly useful for conformational studies on cyclic depsipeptides which contain ester units (Jayati Mitra, 1978).

Methods

It is well known that *cis* peptide units are invariably associated with either prolyl residues or N-substituted residues (such as sarcosyl), the exception being diketo-piparazines, where the *cis* peptide unit occurs because of the geometrical necessity of ring closure.

A *cis* N-methyl peptide unit, by definition, is a *cis* peptide unit in which the hydrogen atom bonded to the nitrogen is substituted by the bulkier group, $-CH_3$. Such N-methylated peptide units are formed when the compound contains unusual amino acids such as sarcosine (N-methylglycine) or in general, NX amino acid, where X is any group other than a hydrogen atom. It is quite probable that such a substitution might bring about some changes in the dimensions of the peptide unit. A cursory examination reveals that the dimensions of the *cis* N-methyl peptide unit are slightly different from those of an unsubstituted *cis* peptide unit.

Mention has earlier been made of the standard dimensions of the *cis* peptide unit proposed by Ramachandran and Venkatachalam (1968). During the course of a conformational study on cyclic tripeptides (Ramakrishnan *et al.,* 1987) it was found that the normal Standard dimensions of the *cis* peptide unit showed variation especially in bond angles. There seems to be a *need-based* requirement for the *cis* peptide unit to expand its internal angles. To confirm that this feature is only in the case of cyclic tripeptides, the data from the crystal structures of cyclic tripeptides alone have been analysed and the dimensions derived are compared with those obtained from other cyclic peptides.

Available data on N-methylated peptide units have established that:

(i) N-Methylated peptide units often occur in *cis* configuration (Stewart and Siddal, 1970; Kolaskar and Sarathy, 1980).

(ii) CN-Methylation leads to steric constraints (Tonelli, 1976; Manavalan and Momany, 1980).

(iii) Hydrogen bonding is prevented on N-substitution and the basicity of the carbonyl group also increases.

The present analysis has thus been done to look at the following aspects:

(i) To get a set of standard dimensions for the *cis* N-methyl peptide unit which will be useful for conformational studies on cyclic peptides.

(ii) To examine the extent of rigidity (or flexibility) of the *cis* peptide units in different orders of cyclic peptides.

For the present analysis data from 60 cyclic peptides containing *cis* peptide units have been used. The names of these compounds as well as the literature citation are given in table 2 and the entries have been arranged according to the order of cyclic peptide namely, cyclic di-, tri- and higher peptides*.

Standard dimensions for the *cis* N-methyl peptide unit

To arrive at a set of dimensions, the weighted mean of each of the bond lengths and bond angles of the *cis* N-methyl peptide unit is computed from the crystal structure data. This is obviously more appropriate than the ordinary arithmetic mean and this approach has been used in earlier studies from our group (Jayati Mitra, 1978; Jayati Mitra and Ramakrishnan, 1980). The weightage used is dependent on the standard deviations of the reported parameters. The weighted mean is calculated using the formula:

$$\text{Weighted mean} = \sum_{i=1}^{N} \left(\frac{P_i}{\sigma_i^2}\right) \Big/ \sum_{i=1}^{N} \left(\frac{1}{\sigma_i^2}\right).$$

Where, P_i is the parameter involved, σ_i is the associated standard deviation and N is the number of examples. Wherever standard deviations have not been explicitly reported, they are calculated using standard formulae (Stout and Jensen, 1968 for bond lengths and Darlow, 1960 for bond angles).

The parameters evaluated are the 5 bond lengths: C^{α}-C, C = O, C-N, N-C^{α} and N-C^{δ} and the 6 bond angles: $C^{\alpha}CN$, $C^{\alpha}CO$, OCN, CNC, CNC^{δ} and $C^{\delta}NC^{\alpha}$. Here the carbon atom attached to N is called C^{δ} taking the analogy from the imino acid proline.

Data from 10 crystal structures containing 29 *cis* N-methyl peptide units occurring in cyclic tetra and higher peptides have been collected and used for obtaining the weighted mean values of the bond lengths and bond angles of the *cis* N-methyl peptide unit. The weighted mean and the standard deviations are given in column 2 of table 3 and the unit drawn with these dimensions is shown in figure 1. These

*The complete list of bond lengths and bond angles of the *cis* peptide unit occurring in these structures are not given here and a table containing these details can be had from the authors on request.

Table 2. Compounds used in the present analysis.

Sl No.	Compound	Reference
1.	c(Sar-Sar)	Groth (1969)
2.	c(Gly-Tyr)	Webb and Lin (1971)
3.	c(Pro-Gly)	Von Dreele (1975)
4.	c(Gly-Gly)	Degeilh and Marsh (1959)
5.	c(Pro-Leu)	Karle (1972)
6.	c(Gly-Tyr) W	Lin and Webb (1973)
7.	c(Ser-Tyr) W	Lin and Webb (1973)
8.	c(Thr-His)	Cotrait *et al.* (1976)
9.	c(Pro-D-Phe)	Ramani *et al.* (1976)
10.	c(N-me-Ala-Ala)	Filhol and Timmins (1976)
11.	$c(N\text{-me-Ala})_2$	Benedetti *et al.* (1976)
12.	$c(N\text{-me-Val})_2$	Benedetti *et al.* (1976)
13.	c(N-me-Val-N-me-D-Val)	Benedetti *et al.* (1976)
14.	$c(Sar)_2$ $LiClO_4$	Takahashi *et al.* (1977)
15.	c(D-Ala-Ala)	Sletten (1970)
16.	$c(Ala)_2$	Sletten (1970)
17.	c(His-Asp)	Ramani *et al.* (1978)
18.	c(Ser-His) W	Cotrait and Ptak (1978)
19.	$c(Ser)_2$	Fava *et al.* (1980)
20.	c(Met-Gly)	Bressen *et al.* (1982)
21.	c(N-ac-Phe-D-Pro)	Cerrini *et al.* (1984)
22.	c(Gly-Tyr)	Morris *et al.* (1974)
23.	c(Pro-Ala)	Cotrait and Leroy (1979)
24.	$c(Pro)_2$	Benedetti *et al.* (1976)
25.	c(Phe-Pro)	Mazza *et al.* (1984)
26.	c(His-Met)	Bressen *et al.* (1984)
27.	c(N-pyr-Phe-D-Pro)	Calcagni *et al.* (1985)
28.	c(Met-Gly) $Ag^{(+)}$	Valle and Ettore (1983)
29.	$c(Sar)_3$	Groth (1976a)
30.	$c(Pro)_3$	Druyan *et al.* (1976)
31.	c(Pro-D-Pro-Pro)	Bats and Fuess (1980)
32.	$c(BzlGly_2\text{-Pro})$	Bats and Fuess (1980)
33.	$c(BzlGly\text{-}Pro_2)$	Bats and Fuess (1982)
34.	c(BzlGly-D-Pro-Pro)	Kessler *et al.* (1983a)
35.	$c(NitroBzlGly\text{-}Pro_2)$	Kessler *et al.* (1983b)
36.	$c(L\text{-}2\text{-}HyiVal\text{-}Pro_2)$	Pinnen *et al.* (1985)
37.	$c(D\text{-}2\text{-}HyiVal\text{-}Pro_2)$	Pinnen *et al.* (1985)
38.	$c(Pro_2\text{-Hypro})$	Kartha and Ambady (1975)
39.	c(Leu-me-Phe-Gly-me-Ala)	Swepston *et al.* (1981)
40.	$c(Pro\text{-}Sar)_2$	Ueno and Shimizu (1983)
41.	c(Ala-Pro-Phe-Pro)	Chiang *et al.* (1982)
42.	$c(Ala\text{-}Sar_3)$	Declerecq *et al.* (1975)
43.	$c(Gly\text{-}Sar_3)$	Declerecq *et al.* (1975)
44.	$c(Sar)_4$	Groth (1970)
45.	$c(Pro\text{-}Val)_2$	Ueda *et al.* (1984)
46.	$c(Sar)_5$	Groth (1973a)
47.	$c(Ala\text{-}Sar_4)$	Groth (1974)
48.	$c(Sar)_6$	Groth (1977)
49.	$c(Gly\text{-}Pro\text{-}Pro)_2$	Czugler *et al.* (1982)
50.	Roseotoxin B	Springer *et al.* (1984)
51.	$c(Phe\text{-}Pro\text{-}D\text{-}Ala)_2$	Kartha *et al.* (1984)
52.	c(Pro-Pro-Gly-Pro-Leu-Gly)	Nakashima *et al.* (1984)
53.	$c(Gly\text{-}Pro\text{-}Pro)_2$ $Mg^{(2+)}$	Karle and Karle (1981)
54.	Ilamycin B_1	Iitaka *et al.* (1974)

Table 2 (Contd.)

Sl No.	Compound	Reference
55.	$c(Sar)_7$	Groth (1975)
56.	$c(Sar)_8$	Groth (1973b)
57.	$c(Pro\text{-}Sar)_4$	Shimizu *et al.* (1983)
58.	$c(Sar)_{10}$	Groth (1976b)
59.	Antamanide	Karle (1974)
60.	Antamanide [Phe^4, Val^6]	Karle and Duesler (1977)

Table 3. Dimensions of the *cis* peptide unit obtained from different cyclic peptides.

Parameter	*cis* N-methyl peptide unit*	Cyclic dipeptides**	Cyclic tri peptides	Cyclic higher peptides
Bond length (Å)				
C^α-C	1·53 (0·008)	1·51 (0·018)	1·53 (0·019)	1·53 (0·009)
C=O	1·23 (0·006)	1·23 (0·010)	1·23 (0·017)	1·23 (0·008)
C-N	1·35 (0·001)	1·33 (0·016)	1·34 (0·019)	1·35 (0·010)
N-C^α	1·45 (0·005)	1·46 (0·019)	1·47 (0·018)	1·46 (0·009)
N-C^δ	1·46 (0·010)	1·45 (0·008)	1·47 (0·009)	1·47 (0·019)
Bond angle (°)				
C^α-C-N	118·5 (1·0)	117·5 (2·0)	120·1 (3·0)	118·0 (1·7)
C^α-C=O	119·7 (0·9)	119·4 (1·9)	118·5 (2·9)	120·2 (1·3)
O=C-N	121·8 (0·6)	123·0 (0·8)	121·2 (1·2)	121·7 (1·2)
C-N-C^α	124·0 (0·9)	125·0 (2·0)	127·9 (2·2)	125·0 (1·9)
C-N-C^δ	119·1 (0·7)	121·4 (2·6)	119·2 (1·7)	121·5 (1·3)
C^δ-N-C^α	116·5 (0·7)	114·8 (2·7)	112·2 (2·6)	114·9 (2·3)

* Weighted Mean.

** The examples listed as entries 1–28 in table 2 have been used for obtaining the parameters not involving C^δ and compounds 1,3,5,9,10-14,21,23–25,27 have been used for the parameters involving C^δ as these alone are relevant.

Numbers in parentheses are standard deviations.

dimensions have been proposed as the standard dimensions that can be used in studies involving such units.

Results and discussion

The data on the dimensions of the *cis* N-methyl peptide units collected from crystal structures can be examined for possible dispersion by representing them in the form of histograms. The histograms for the distribution of bond lengths and bond angles are shown in figures 2 and 3, respectively. The interval chosen for the histograms are 0·02 Å for bond lengths and 1° for bond angles.

From the histograms in figures 2 and 3, the following aspects can be observed:

(i) The histograms for bond lengths show that the values have a greater spread for N-C^δ (figure 2e) than for the other bond lengths. This aspect is also reflected in the value of the standard deviation which is largest for N - C^δ (column 2 of table 3).

(ii) In general the bond lengths show a narrower distribution than the bond

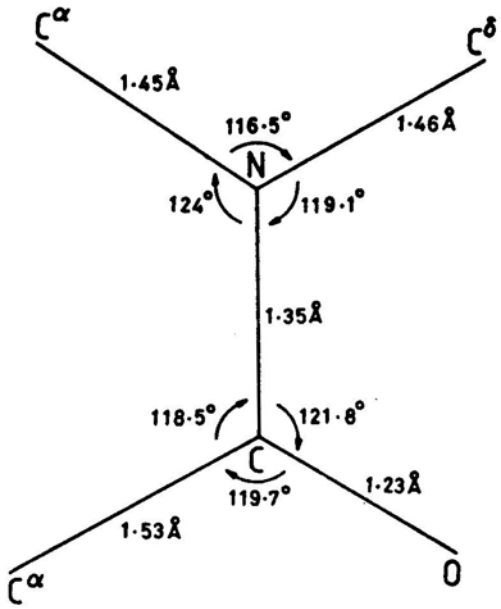

Figure 1. Dimensions of the *cis* N-methyl peptide unit.

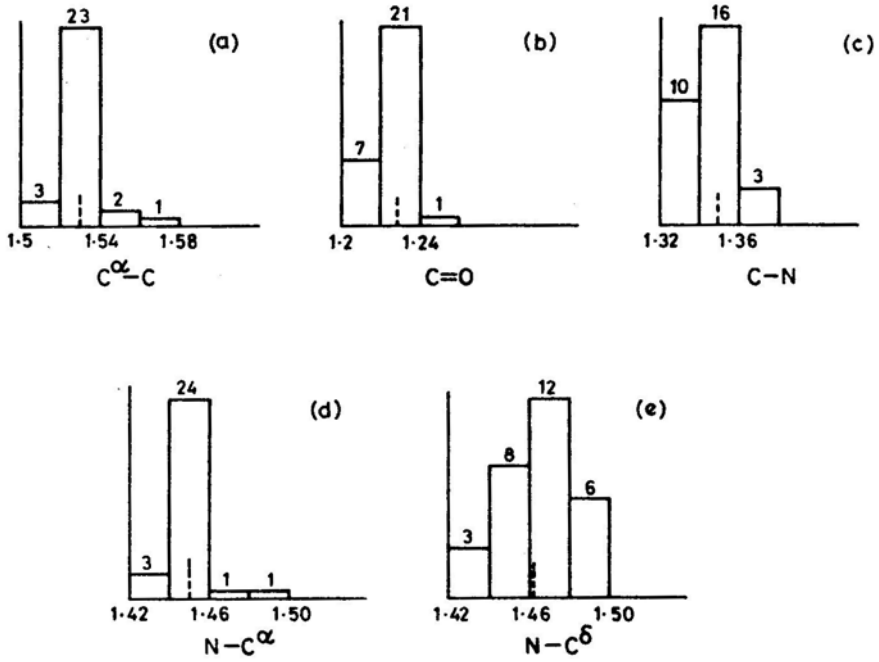

Figure 2. Distribution of bond lengths of the *cis* N-methyl peptide unit from crystal structures.

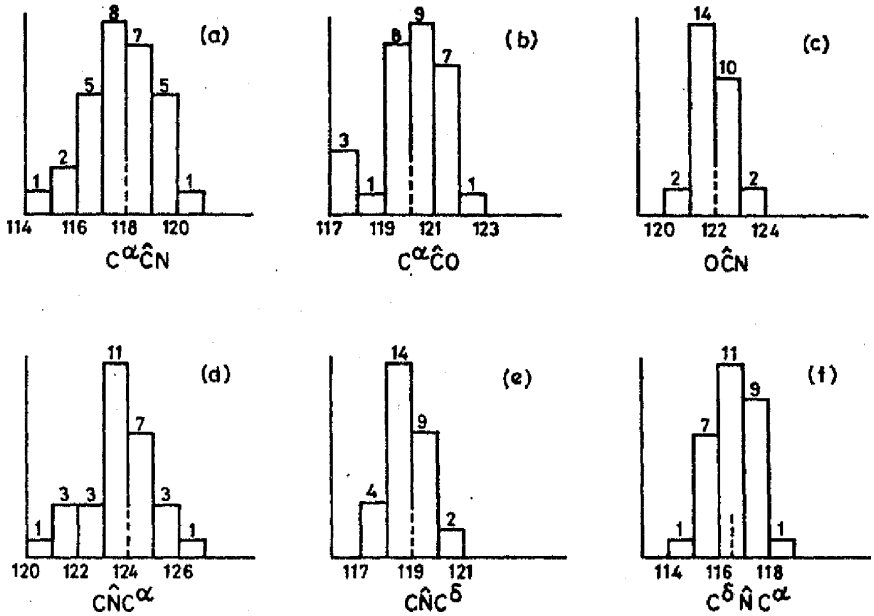

Figure 3. Distribution of bond angles of the *cis* N-methyl peptide unit from crystal structures.

angles. This is naturally to be expected because of the fact that the force constant for bond length distortion is much higher than that for bond angle distortion.

(iii) The sum of the bond angles at C as well as at N as calculated from the weighted mean are 360° and 359·6°, respectively. This indicates a very slight pyramidal disposition of the bonds at the atom N. In fact, if the sum of the angles at C are computed for the individual examples used, they are seen to lie within a narrow range between 359·8° and 360° indicating that the bonds meeting at C are almost coplanar. On the other hand, the sum of the bond angles at N show a distribution ranging from 356·6° to 360°. In one of the examples, $c(Sar)_6$ (Groth, 1977), this value is 354·5°, considerably far from the range mentioned above. Hence this value is omitted in the calculation of the mean and standard deviation. The mean value is 359·3° and the standard deviation is 1°. The corresponding figures for the sum of the angles at C are 359·96° and 0·07°.

Comparison with normal cis peptide unit

In order to study the effect of N-methylation, a comparison between the peptide dimensions of the normal and N-methylated *cis* peptide units can be done. A comparison of the figures given in column 2 of table 1 and the weighted mean given in column 2 of table 3 shows that the bond length C^{α}-C is not different while the bond length C = O is slightly shorter. The angles at the carbonyl carbon are also not affected significantly. On the other hand, the bonds associated with nitrogen, *viz.* C-N and N-C^{α}, show a perceptible difference. While the bond length C-N shows an increase of 0·03 Å, the bond N-C^{α} is shortened by 0·02 Å on methylation. In

a similar way, the angles CNC^{α} and CMC^{δ} (CNH in the normal peptide unit) are smaller by 2° and the angle $C^{\delta}NC^{\alpha}$ (HNC^{α} in the normal peptide unit) is larger. The presence of a carbon atom in the place of the hydrogen atom appears to have brought about some alterations in the average dimensions.

The sum of the angles around carbon is 360°, indicating a perfect planarity at that atom, similar to the situation in a normal *cis* peptide unit. However, there seems to be a slight nonplanarity at N, as evidenced by a value of 359·6° for the sum of the angles around that atom. In a normal *cis* peptide unit, this sum is 360° indicating perfect planarity. That this may perhaps be an artifact of rounding off of values cannot be entirely ruled out. However, on carrying out an analysis of the *cis* peptide unit in which the atom N is unsubstituted, the sum of the angles around this atom is found to be 359·92°, indicating near-planarity. Thus the substitution by a heavier group at N brings about a pyramidal disposition of the bonds, which however is extremely small.

From the above it is clear that the substitution by a methyl group (or for that matter any bulky group) is accompanied by changes in the basic dimensions of the *cis* peptide unit particularly in the bond lengths and bond angles involving the nitrogen atom. A possible reason can be sought in the difference in the electrical nature of the bonds N-H and $N\text{-}C^{\delta}$, as well as in the stronger interaction between the $C^{\alpha}_2 \ldots C^{\delta}$ atoms compared to that between the $C^{\alpha}_2 \ldots H$ atoms in the normal peptide unit.

If the above proposition is true, then a similar effect could be expected to be observed for the *trans* peptide unit also. Jayati Mitra (1978) has analysed such *trans* N-methyl examples in connection with studies on cyclic hexadepsipeptides. The data were taken from 8 crystal structures. Subsequently, some more crystal structures appeared and a fresh analysis was done using data from 14 crystal structures. The resultant weighted mean values of the various parameters of the *trans* N-methyl peptide unit are given in table 4 along with the P-C dimensions (Corey and Pauling, 1953). A comparison of the two values show that as in the case of the *cis* N-methyl peptide unit, the bond length C-N increases and this is accompanied by a slight

Table 4. Comparison of the dimensions of the *trans* peptide unit and *trans* N-methyl peptide unit.

Parameter	(P-C) *trans* peptide unit*	*trans* N-methyl peptide unit**
Bond length (Å)		
C^{α}-C	1·53	1·53
C=O	1·24	1·23
C-N	1·32	1·35
N-C^{α}	1·47	1·46
N-H (C^{δ})	1·00	1·47
Bond angle (°)		
C^{α}-C-N	114·0	116·0
C^{α}-C=O	121·0	121·0
O=C-N	125·0	123·0
C-N-C^{α}	123·0	118·0
C-N-H(C^{δ})	123·0	124·0
(C^{δ})H-N-C^{α}	114·0	118·0

* Corey and Pauling (1953). ** Jayati Mitra (1978).

decrease of bond length N-C^{α} The angles at the carbonyl carbon do show some variation but the changes are only small (2°). The distribution of the angles at N is however, considerably different in the two cases. The increase in the angle HNC^{α} on methylation ($C^{\delta}NC^{\alpha}$) is present in this case also and so is the decrease in CNC^{α}. The behaviour of the angle CNH (or CNC^{δ}) is however different from that in the *cis* N-methyl system.

For the present analysis, the examples have been chosen from cyclic tetra- and higher peptides only. The two other classes of compounds, *viz.,* cyclic dipeptides and cyclic tripeptides have not been included since in these cases it is believed that the peptide units can be distorted because of geometrical and stereochemical requirement in view of the small ring systems. The latter are particularly significant in that the necessity for additional intra-annular space brings about quite some *need-based* increase in the internal angles. Hence cyclic di- and tripeptides cannot be treated on par with the other cases of *cis* peptide units occurring in higher cyclic peptides, where the influence of stereochemistry on peptide dimensions is not that much.

Flexibility of the dimensions of the cis peptide unit

It has been mentioned earlier that there is a tendency for the internal angles $C^{\alpha}CN$ and CNC^{α} of the *cis* peptide unit to be larger when they occur in cyclic tripeptides. In order to examine this aspect in greater detail, the dimensions of the *cis* peptide unit as it occurs in cyclic di, tri and tetra and higher peptides are calculated from observed examples. For this purpose, no distinction has been made between the normal *cis* peptide unit (with N-H group) and the N-methylated unit or the prolyl residue.

Cyclic dipeptides

It is well known that a cyclic dipeptide can be formed only out of two *cis* peptide units. Clearly a combination of *cis* and *trans* peptide units cannot bring about the necessary ring closure because of their different end to end distances. Both from model building and on geometrical grounds it can be shown that cyclic dipeptides cannot be formed from two *trans* peptide units. Thus, this family of compounds offers plenty of data on *cis* peptide units.

Crystal structure data from 30 cyclic dipeptides have been used. There are 55 distinct peptide units that occur in these compounds (entries 1–28 of table 2). The 30 cyclic dipeptides must yield 60 examples of *cis* peptide units. However, in 5 cases, the molecule has some symmetry such as two-fold or inversion and hence only one set of distinct peptide dimensions are available for use. The distribution of the bond lengths and bond angles is shown as histograms in figures 4 and 5, respectively and the average dimensions of the *cis* peptide unit obtained from this class of cyclic peptides are given in column 3 of table 3.

While all the histograms in figure 4 show only one significant peak, some of the histograms in figure 5 (for bond angles) show a double peak. This is particularly apparent for the angle CNC^{δ} and $C^{\delta}NC^{\alpha}$ (figure 5e,f), In fact, the distribution for the angle $C^{\alpha}CN$ drawn at 1° intervals, shown in figure 6, exhibits two peaks: a small one at 114–115° and a larger one at 118–119°. These two peaks are separated by a clear

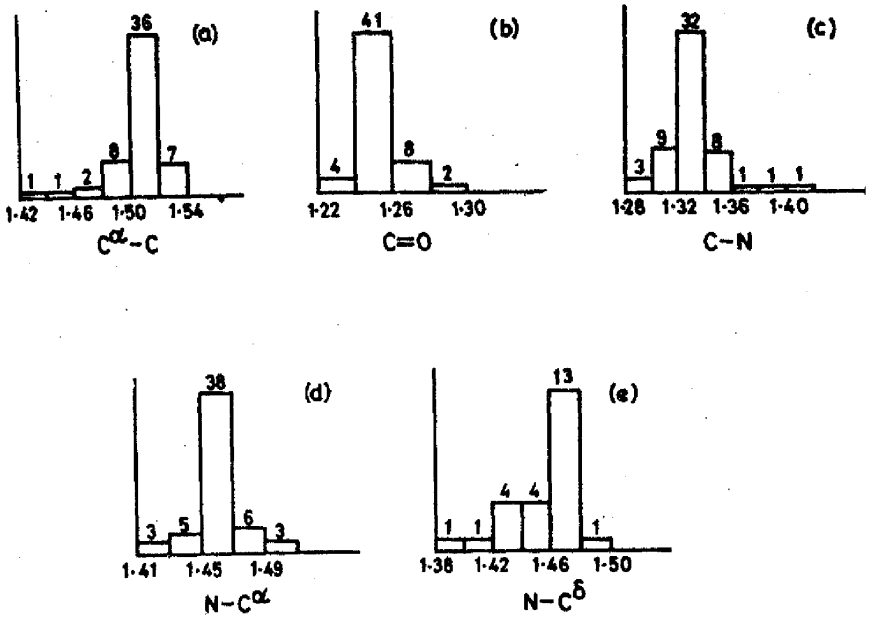

Figure 4. Distribution of bond lengths of the *cis* peptide unit in cyclic dipeptides.

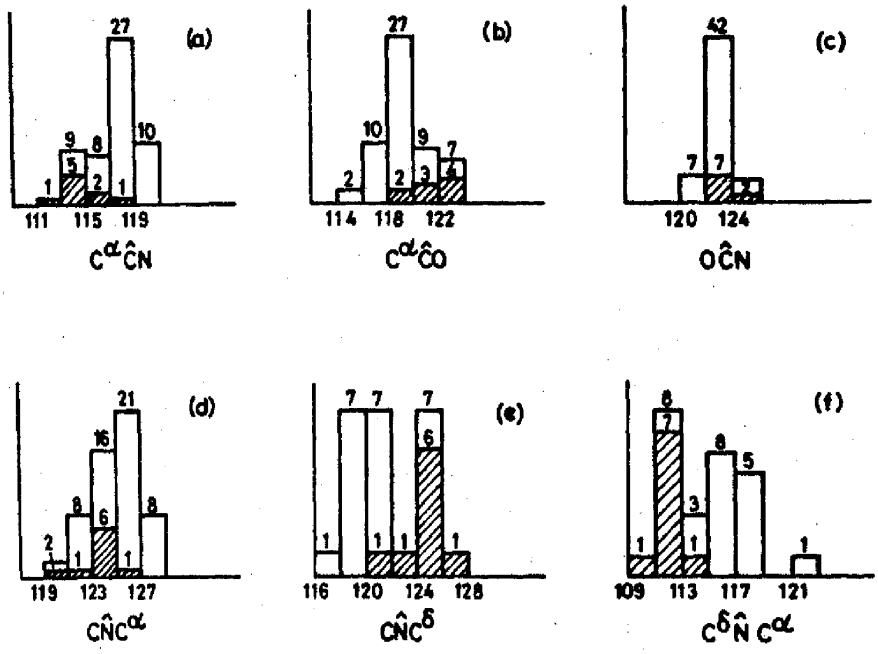

Figure 5. Distribution of bond angles of the *cis* peptide unit in cyclic dipeptides. Hatched regions correspond to *cis* peptide unit with a prolyl residue.

trough. A closer examination of the examples which constitute the small peak and its neighbourhood (*i.e.,* those examples where the angle $C^{\alpha}CN$ lies between 112 and 116°; there are 11 such examples) reveals that these are compounds which have a prolyl residue in the cyclic dipeptide. In fact, except for c(L-Pro-D-Phe), all the other prolyl

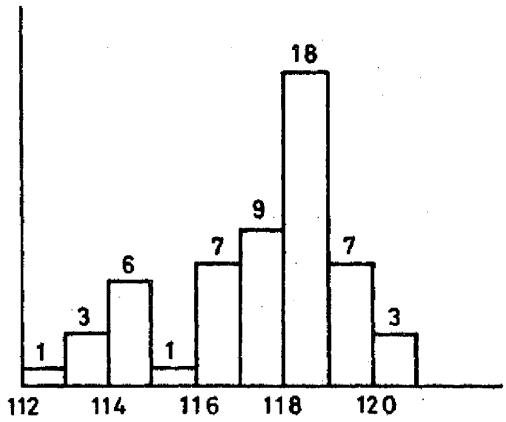

Figure 6. Distribution of bond angle $C^{\alpha}CN$ of the *cis* peptide unit in cyclic dipeptides, drawn at 1° intervals.

structures are accounted for. Hence, this must have been an effect of the prolyl residue in the cyclic dipeptide. The distribution of the angle $C^{\alpha}CN$ in cyclic tri and higher peptides (figures 10a and 8a) shows only one peak around 118° (which corresponds to the second peak in the present case). This is so in spite of the fact that there are prolyl residues in cyclic tri and higher peptides. Thus the smaller angle for $C^{\alpha}CN$ in cyclic dipeptides with prolyl residues must be a *need-based* requirement. It is perhaps pertinent to observe that the only exception c(L-Pro-D-Phe) has a D-residue along with the L-Pro in this cyclic peptide.

In order to examine whether there is a polarity of distribution between prolyl and non-prolyl residues in cyclic dipeptides in so far as the bond angles are concerned, the cases with the prolyl residue have been analysed separately and the distribution for these cases is shown as hatched regions in the histograms of figure 5. It is clearly seen that as in the case of $C^{\alpha}CN$, the two peaks of CNC^{δ} and $C^{\delta}NC^{\alpha}$ can definitely be attributed to cases with the prolyl residue and those without. These results can be interpreted to mean that the peptide dimensions, particularly the bond angles, show a flexibility depending upon whether the *cis* peptide unit is associated with a prolyl residue or not. A study has also been made to find out whether such a polarity in distribution exists for cyclic tri and higher peptides. The results indicate that while this is the case for $C^{\delta}NC^{\alpha}$ for both cyclic tri and higher peptides, it is not so for CNC^{δ}.Further detailed studies from a theoretical point of view are needed before more specific conclusions can be drawn.

Cyclic higher peptides

Cyclic higher peptides (tetra onwards) do not. have a stereochemical requirement for the peptide units to be in *cis* configuration. Still *cis* peptide units do occur when the compound contains N-methylated amino acids or prolyl residues, the only exception being dihydrotentoxin (Swepston *et al.,* 1981) in which the *cis* peptide occurs across Me-Phe-Gly and Me-Ala-Leu.

Crystal structures of 22 cyclic higher, peptides have been used (entries 39–60 of

table 2). The distribution of the bond lengths and bond angles is shown in figures 7 and 8, respectively and the average dimensions of the *cis* peptide unit obtained from this class of compounds are given in column 5 of table 3.

The parameters of the *cis* peptide occurring in Ilamycin B1 (Iitaka *et al.*, 1974) lie

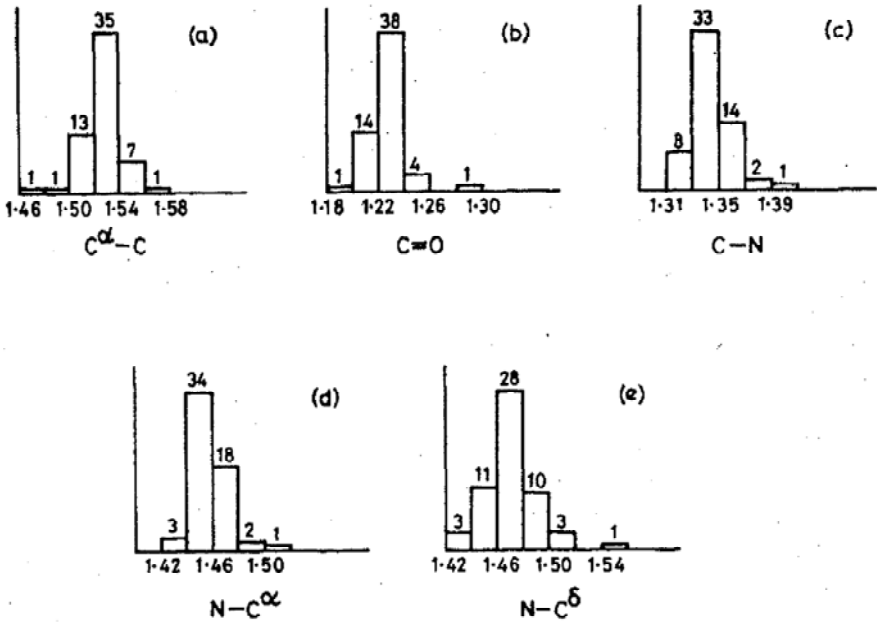

Figure 7. Distribution of bond lengths of the *cis* peptide unit in cyclic higher peptides.

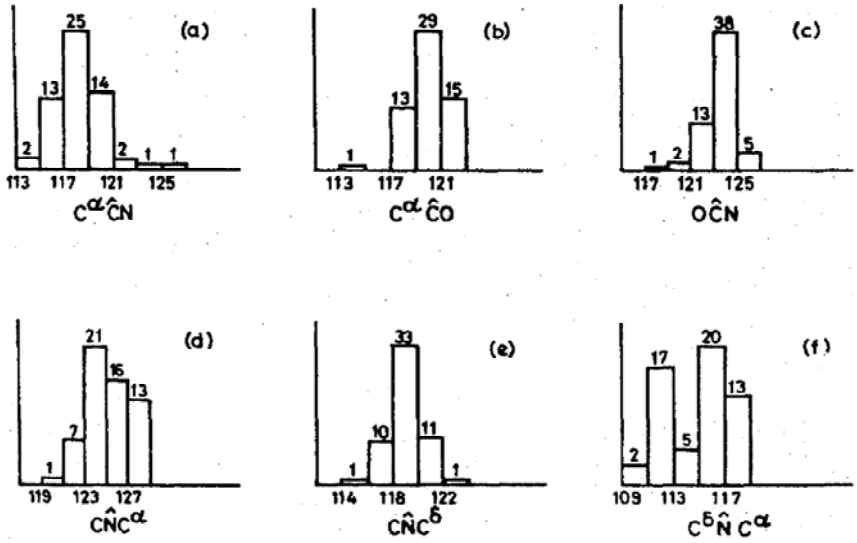

Figure 8. Distribution of bond angles of the *cis* peptide unit in cyclic higher peptides.

outside the general range of distribution for many parameters. Hence they have been excluded in the computation of the mean value. All the histograms show essentially one prominent peak, except that for $C^{\delta}NC^{\alpha}$, which shows two peaks: one at 111–113° and the other at 115–17°, separated by a trough. On a closer examination, it is found that the examples comprising the first peak contain the prolyl residue and those of the second peak do not.

Cyclic tripeptides

Cyclic tripeptides require a minimum of two *cis* peptide units for geometric ring closure (Ramakrishnan *et al.,* 1987). However, in many cases all the 3 peptide units are in *cis* configuration, and hence this family of compounds is again a rich source of data on *cis* peptide units. Crystal structures of 11 cyclic tripeptides have been solved so far (entries 29–8 in table 2) and there are 43 distinct peptide units in these. The 11 examples of cyclic tripeptides should yield only 33 *cis* peptide units. However, in the cases of $c(Sar)_3$ (Groth, 1976a), $c(Pro)_3$ (Druyan *et al.,* 1976), $c(Pro_2$-D-Pro)(Bats and Fuess, 1980) and c(BzlGly-D-Pro-L-Pro)(Kessler *et al.,* 1983a) there are two molecules in the asymmetric unit and the compound c(Nitro-zlGly-Pro_2) (Kessler *et al.,* 1983b) occurs as two different complexes. The compound c(L/D-2-Hyival-Pro_2) (Pinnen *et al.,* 1985) has only 2 *cis* peptide units. The distribution of bond lengths and bond angles is shown in figures 9 and 10, respectively and the average dimensions are given in column 4 of table 3.

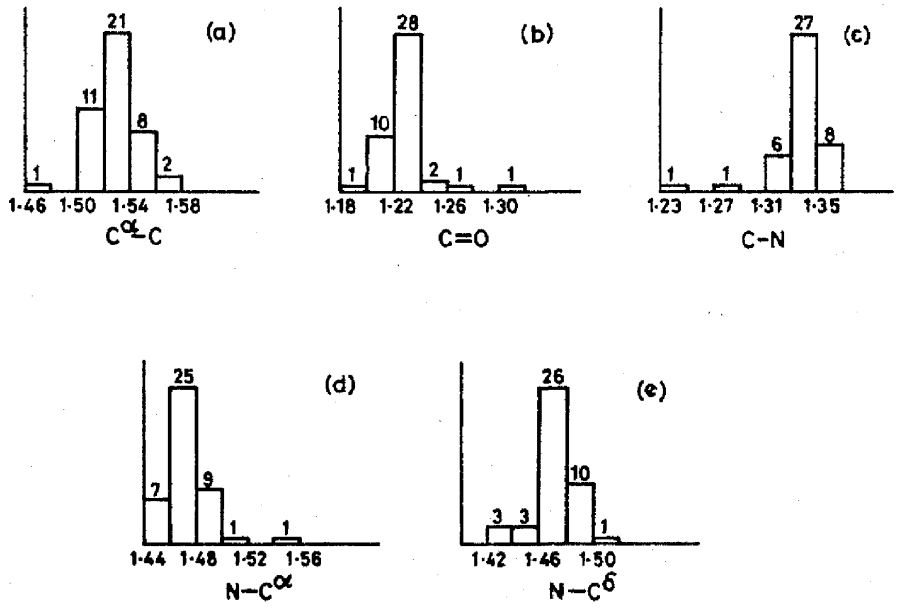

Figure 9. Distribution of bond lengths of the *cis* peptide unit in cyclic tripeptides.

All the histograms corresponding to bond lengths show only one peak. However, there are a few values in each of the histograms that lie way outside the ranges of dstribution (one value of C^{α}-C in the interval 1·46–1·48 Å, one of C=O in the

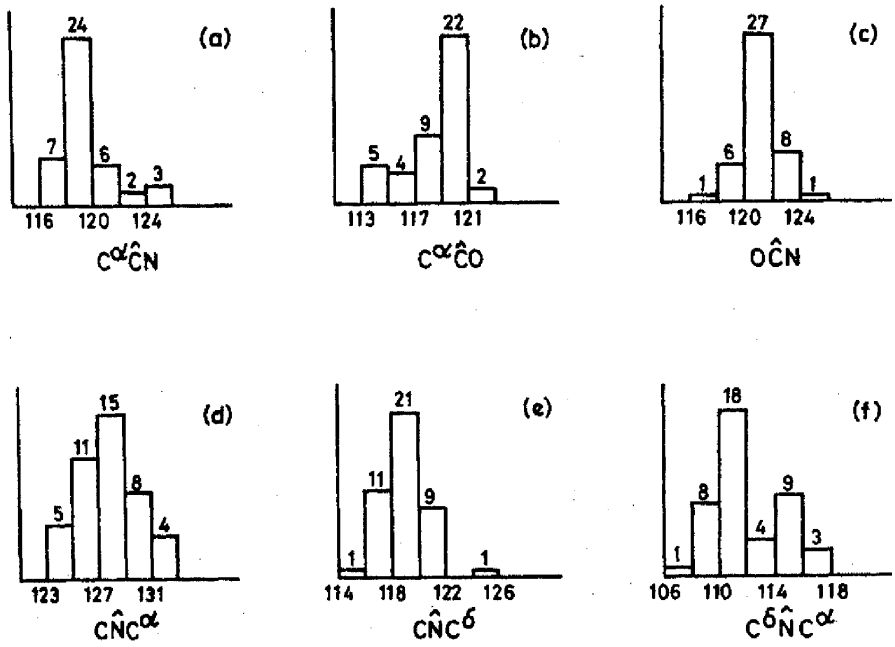

Figure 10. Distribution of bond angles of the *cis* peptide unit in cyclic tripeptides.

interval 1·30–·32 Å, etc.). Similarly, some of the bond angles also show appreciable deviation from the normal range (for example in the $CHCl_3$ complex of c(Nitro-Bzly-Pro2) (Kessler *et al.,* 1983b) the angle $C^{\alpha}CN$ (135°) and the angle $C^{\alpha}CO$ (105°) are quite far from the ranges of distribution (116–126° for $C^{\alpha}CN$ and 113–123° for $C^{\alpha}CO$). Hence such values are not included in the histograms and have also not been included in the calculation of mean and standard deviation. It is clear that in this structure, with exceptional values for the parameters, the peptide unit have undergone considerable distortion.

As in the case of higher cyclic peptides, here also the distribution of the angle C δNC^{α} shows two peaks, one at 110–112° and the other at 114–116°. As in the earlier case, the first peak is attributable to the examples that contain the prolyl residue and the second to examples that do not.

From the above it is clear that the angle $C^{\delta}NC^{\alpha}$ tends to be lower when the *cis* peptide unit is associated with a prolyl residue irrespective of the order of cyclic peptide.

By examining the mean values obtained in the different cases (table 3) for the various parameters the following salient features can be observed.

Bond lengths: The bond lengths in general do not show much deviation except C^{α}-C, which is 1·51 Å for cyclic dipeptides and 1·53 Å for cyclic tri and higher peptides. The value of 1·53 Å agrees well with that given by Ramachandran and Venkatachalam (1968) and the value 1·51 Å is the same as that given by Benedetti (1977) and Kolaskar and Sarathy (1980) (see table 1). It can also be seen from figures 4a, 7a and 9a that there exists a difference in the peak range values for this bond length (1·50–1·52 Å for cyclic dipeptides and 1·52–1·54 Å for the other two cases). Thus it is clear that there is a distinct shortening of the C^{α}-C bond when the *cis*

peptide unit occurs in cyclic dipeptides. The only other bond length which shows some variation is C-N and the change is only marginal; 1·33 Å for cyclic dipeptides, 1·34 Å for cyclic tripeptides and 1·35 Å for cyclic higher peptides.

Ramani and Venkatesan (1973) have analysed the effect of hydrogen bonds on the carboxyl groups occurring in amino acids and related compounds and have observed that the bond length C=O is in a way related to the strength of the hydrogen bonds received by it. In order to see whether there is any perceptible lengthening of the C=O in *cis* peptide units due to hydrogen bonding, the examples used in the present study were divided into two groups: (i) those C=O which are involved in hydrogen bonding and (ii) those in which the group is not hydrogen bonded. There are 33 examples of the former case and 125 examples of the latter. The distribution of bond length for the two cases is shown in figure 11. The average values (and standard deviations) are 1·234 Å (0·017) and 1·229 Å (0·014) for the hydrogen-bonded and non-hydrogen-bonded cases, respectively. Though the average value is slightly larger (0·005Å) when the group is hydrogen bonded, it is still well within the standard deviations. In both cases there is a broad peak between 1·21 and 1·25 Å. The peak in the interval 1·21–1·23 Å for the non hydrogen bonded examples shifts to 1·23–1·25Å in the other case. From the present study it is not possible to state convincingly that the C = O bond length undergoes an elongation on hydrogen bonding, though there is certainly no indication of a reverse trend.

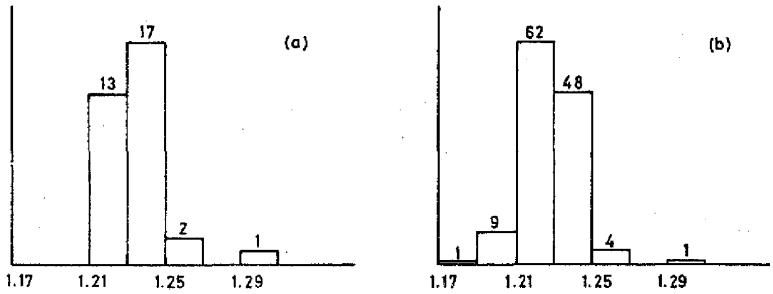

Figure 11. Distribution of bond length C = O of the *cis* peptide unit. (a), Examples in which C = O is hydrogen bonded; (b), examples in which C = O is not hydrogen bonded.

Bond angles: From the values given in table 4, it can be seen that the bond angles show a wider spread than the bond lengths. In particular, the two angles $C^{\alpha}CN$ and CNC^{α} show a marked increase in cyclic tripeptides compared to the situation in the other two classes of compounds. The mean value of $C^{\alpha}CN$ is 0·5° less for cyclic dipeptides and 1·7° more for cyclic tripeptides compared to cyclic higher peptides. For the cyclic dipeptides, the appropriate value for checking the planarity at N would be that obtained from only those examples which have a C^{δ} on the N atom. When this is done, the mean value of CNC^{α} works out to be 123·4° (1·6) and the sum of the 3 angles at N is 359·7°, indicating a pyramidal disposition of the bonds. The nonplanarity, however, is less pronounced in this case.

It will be interesting to find out the disposition of the bonds at N when it is unsubstituted (*viz.,* N-H). There are 33 such examples occurring in cyclic dipeptides and the hydrogen positions are reported for 14 of these only. Using this data, the sum of the angles at N, was computed. It shows an extremely narrow spread (359·9–

360°) with an average value very close to 360°. Though this indicates that the bonds meeting at N are more coplanar when there is no substitution, it must be viewed with some caution since in many cases the positions of the hydrogen atoms are not as reliable as those of the heavier atoms.

Conclusions

The results presented in this paper indicate that the *cis* peptide unit has a reasonable degree of flexibility to suit the system in which it occurs. The flexibility in bond angles is more pronounced than that in bond lengths. The dimensions of the unit show a more pronounced variation when it occurs with the imino acid proline. It is also very apparent that the *cis* peptide unit is a very flexible unit. The internal bond angles $C^{\alpha}CN$ and CNC^{α} either fold in or open out depending upon the geometry of ring closure as well as stereochemical requirements.

The case of cyclic tripeptides is particularly interesting. In these compounds the two internal angles $C^{\alpha}CN$ and CNC^{α} open out by as much as 2°. This is a special case of *need-based* requirement in that the intra-annular space has to increase in order that the structure as a whole may be stereochemically and energetically favourable. This aspect has been dealt with extensively elsewhere (Ramakrishnan *et al.,* 1987). Whether such a flexibility is a general feature of the peptide unit itself or only specific to *cis* peptide units can be ascertained by making a similar study of *trans* peptide units. Such a study is in progress.

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