# $^{13}$C NUCLEAR MAGNETIC RESONANCE STUDY OF CYCLODIPEPTIDES CONTAINING $^{13}$C ENRICHED HYDROPHOBIC AMINO ACIDS

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*Dedicated to Professor F. Šantavý on the occasion of his 65th birthday.*

The diastereoisomeric pairs of cyclodipeptides *cis*- and *trans*-cyclo(Ala-Ala), cyclo(Ala-Phe), cyclo(Val-Val) and cyclo(Leu-Leu) containing 85% $^{13}$C enriched amino-acid residues were synthesized and their $^{13}$C—$^{13}$C coupling constants were measured. The combination of $^{13}$C—$^{13}$C and $^{1}$H—$^{1}$H coupling constants enabled to estimate unequivocally the side chain conformation of the valine and leucine residues.

The cyclic dipeptides (substituted 2,5-piperazinediones) represent model substances for the study of relations between spectroscopic properties and geometric parameters of systems with two homoconjugated *cis*-amide groups. Their conformations have been studied by infrared spectroscopy[1-4], $^{1}$H and $^{13}$C-NMR spectroscopy[5-20], ORD and CD techniques[21-24], X-ray crystallography[25-33] and by quantum mechanical calculations[4,21,34-38]. As we are interested in the evaluation of the relationship between the coupling constants $^{13}$C—$^{13}$C and the conformation of the peptides, we have carried out a $^{13}$C-NMR study of $^{13}$C enriched cyclodipeptides. Our series contains six cyclodipeptides with aliphatic side chains of different bulkiness and two dipeptides with one side chain aromatic. Two compounds from this series have been yet subjected to $^{13}$C-NMR (natural abundance) studies: cyclo(L-Leu-L-Leu) (ref.[16,18])* and cyclo(L-Leu-D-Leu) (ref.[18]).

* The abbreviations follow the recommandations of IUPAC-IUB (ref.[39]). The prefixes *cis*- and *trans*-describe the relative configuration of substituents in positions 3 and 6 of the 2,5-piperazinedione system, *i.e. cis*-cyclo(Ala-Ala) means L-L cyclodipeptide, *trans*-cyclo(Ala-Ala) means L-D cyclodipeptide.

## EXPERIMENTAL

The cyclodipeptides were synthesized by the cyclization of the appropriate dipeptide methyl esters in methanol at laboratory temperature. Their melting points and optical rotations were in accord with the constants described in the literature. Their purity was checked by the thin layer chromatography in the system diisopropyl ether–chloroform–acetic acid (6 : 3 : 1, v/v/v). The amino acids enriched to 85% with the isotope $^{13}C$ were prepared in the Service de Biochimie, Centre d'Etudes Nucléaires, Saclay. $^{13}C$-NMR spectra were obtained on a Varian CFT-20 spectrometer operating in the pulsed Fourier transform mode with complete proton decoupling. The concentration of the samples (in hexadeuteriodimethyl sulfoxide) was about 0·1M, in the case of leucine derivatives the saturated solution in hexadeuteriodimethyl sulfoxide were used. The probe temperature was about 32°C, the samples were contained in 10 mm o.d. tubes. The maximum error for $^3J_{CC}$ is $\pm 0{\cdot}3$ Hz (for $^3J_{CC}$ of leucine containing compounds $\pm 0{\cdot}5$ Hz), for $^2J_{CC}$ is $\pm 0{\cdot}3$ Hz and for $^1J_{CC}$ is $\pm 1$ Hz. $^1H$-NMR spectra were measured on a CAMECA TSN 250 MHz spectrometer at 22°C at concentration described above. Rotamer populations were calculated from $^3J_{C^\alpha H-C^\beta H}$ and $^3J_{C'C^\gamma}$ coupling constants. The parameters obtained from the equation proposed by Cung and coworkers[40] for residues examined here: $^3J_{C^\alpha H-C^\beta H} = (5{\cdot}1 \pm 0{\cdot}4)\cos 2\Theta - (1{\cdot}8 \pm 0{\cdot}6)\cos\Theta + (7 \pm 0{\cdot}2)$ for $^3J_{C^\alpha H-C^\beta H}(\text{gauche}) = 3{\cdot}55$ Hz $\pm$ $\pm 0{\cdot}2$ Hz and $^3J_{C^\alpha H-C^\beta H}(trans) = 13{\cdot}9$ Hz $\pm 1$ Hz. For the coupling constants $^3J_{CC'}$ the set $^3J_{C'C^\gamma}$ (gauche) = 0·6 Hz and $^3J_{C'C^\gamma}(trans) = 3{\cdot}9$ Hz was used (the values obtained from theoretical calculations[41,42] and experimental data[43–46]). As the limits of error for the relationship $^3J_{C'C^\gamma}$ are not known, only the mean values of rotamer populations are given and the erorr may reach $\pm 0{\cdot}10$.

## RESULTS

The $^{13}C$ chemical shifts of the cyclodipeptides studied are listed in Table I. The shifts in the diastereoisomeric pairs of symmetric compounds are very similar. Only with the unsymmetrical compounds (diastereoisomeric pair cyclo(Ala-Phe)) the differences in the chemical shifts are more significant (for carbons $C^\beta$ of alanine residues the difference between *cis*- and *trans*-isomer reaches 1·3 ppm). With the compound cyclo(L-Leu-D-Leu) we have observed only one signal for each enriched carbon atom, indicating a symmetric arrangement of the molecule in solution, which is in variance with the results published in the literature[18]. The authors[18] observed two signals of equal intensity for each of the carbon atoms, which was explained as the manifestation of the magnetic nonequivalence of isobutyl side chains – one isobutyl group in the axial, the other one in the equatorial position of the 2,5-piperazinedione ring (boat form). When we reinvestigated their synthetic procedure we obtained the statistic mixture (1 : 1 : 2) of cyclo(L-Leu-L-Leu), cyclo(D-Leu-D-Leu) and cyclo(L-Leu-D-Leu), in other words 1 : 1 mixture of compounds *cis* and *trans* (proved by thin layer chromatography).

The observed $^{13}C$—$^{13}C$ coupling constants are listed in Table II. The differences in $^1J_{CC}$ are small throughout the whole series (for $^1J_{C'C^\alpha}$ less than 1 Hz, for $^1J_{C^\alpha C^\beta}$ less than 2 Hz). Thus they do not reveal great differences in the charges along the bonds and electron densities at the atoms involved and their interpretation in stereo-

chemical terms remains difficult. The two-bond coupling constants, $^2J_{CC}$ are small and they manifest themselves generally only by the broadening of the signals. The magnitude of the $^2J_{C'C^\beta}$, from the carbonyl to the $C^\beta$ is estimated to about 0·5 – 1·0 Hz

TABLE I

$^{13}$C Chemical Shifts in $^{13}$C Enriched Cyclodipeptides

| Compound[a] | Configuration | $C'^b$ | $C^{\alpha b}$ | $C^{\beta b}$ | $C^{\gamma 1 b}$ | $C^{\gamma 2 b}$ | $C^{\delta b}$ |
|---|---|---|---|---|---|---|---|
| Cyclo(**Ala**-Ala) | *cis* | 169·2 | 50·2 | 18·7 | — | — | — |
| | *trans* | 168·8 | 50·1 | 18·9 | — | — | — |
| Cyclo(**Ala**-Phe) | *cis* | 167·6 | 49·7 | 19·6 | — | — | — |
| | *trans* | 168·4 | 48·7 | 18·3 | — | — | — |
| Cyclo(**Val**-Val) | *cis* | 167·3 | 59·1 | 31·0 | 18·5 | 17·2 | — |
| | *trans* | 167·6 | 59·0 | 31·6 | 18·1 | 16·7 | — |
| Cyclo(**Leu**-Leu) | *cis* | 168·4 | 52·7 | 43·5 | [c] | | [c] |
| | *trans* | 168·7 | 52·4 | 41·2 | [c] | | [c] |

[a] In boldface — enriched residue; [b] in ppm, in relation to $\delta = 39{\cdot}60$ ppm for hexadeuteriodimethyl sulfoxide; [c] complicated multiplet not analysed.

TABLE II

Coupling Constants (Hz) in $^{13}$C Enriched Cyclodipeptides

| Compound[a] | Configuration | $^1J_{C'C^\alpha}$ | $^1J_{C^\alpha C^\beta}$ | $^1J_{C^\beta C^\gamma}$ | $^2J_{C'C^\beta}$ | $^3J_{C'C^{\gamma 1}}$ | $^3J_{C'C^{\gamma 2}}$ |
|---|---|---|---|---|---|---|---|
| Cyclo(**Ala**-Ala) | *cis* | 50·5 | 37·4 | — | 0·5—1·0 | — | — |
| | *trans* | 50·6 | 36·4 | — | 0·5—1·0 | — | — |
| Cyclo(**Ala**-Phe) | *cis* | 51·0 | 36·3 | — | 1·2 | — | — |
| | *trans* | 50·8 | 37·5 | — | 1·7 | — | — |
| Cyclo(**Val**-Val) | *cis*[b] | 50·3 | 35·5 | 34·9 | 0·5—1·0 | 2·9 | 0·5 |
| | *trans*[c] | 50·6 | 34·9 | 35·1 | 0·5—1·0 | 2·9 | 0·5 |
| Cyclo(**Leu**-Leu) | *cis* | 50·3 | 35·2 | [c] | 0·5—1·0 | 0·8 | — |
| | *trans* | 50·2 | 35·5 | [c] | 0·5—1·0 | 0·8 | — |

[a] In boldface — enriched residue; [b] coupling $^3J_{C^\alpha H - C^\beta H}$ is 3·1 Hz, measured on non-enriched compounds; [c] coupling $^3J_{C^\alpha H - C^\beta H}$ is 3·0 Hz, measured on non-enriched compounds.

except in the diastereoisomeric pair cyclo(Ala-Phe) where the two-bond coupling between the carbonyl carbon atom and the $C^{\beta}$ atom in the alanine residue manifests with the values of 1·2 and 1·7 Hz for the *cis*- and *trans*-isomer, respectively. In our series, the $^{3}J_{CC}$ coupling constants can be obtained for the compounds *cis*- and *trans*-cyclo(Val-Val) from the analysis of the $C^{\gamma 1}$, $C^{\gamma 2}$ signals presented in Figure 1. With the compounds containing leucine residue, the coupling $^{3}J_{C'C^{\gamma}}$ cannot be obtained from the signals of $C^{\gamma}$ due to their complexity and it was necessary to analyse the C′ region although the signal of C′ itself is broadened probably by the coupling with $C^{\beta}$. Therefore the precision of determining $^{3}J_{C'C^{\gamma}}$ is smaller in this particular case.

With the cyclo(Val-Val) diastereoisomers we measured also the $^{3}J_{C^{\alpha}H-C^{\beta}H}$ coupling constants (Table II). For the compounds containing leucine residue, the value of $^{3}J_{C^{\alpha}H-C^{\beta}H}$ have been reported[9].

## DISCUSSION

### cis- *and* trans-*Cyclo(Ala-Ala) and Cyclo(Ala-Phe)*

With the alanine containing cyclodipeptides, three-bond (vicinal) coupling constants are not available. However, also the two-bond (geminal) coupling constants are generally able to monitor the difference in mutual orientation of the carbon atoms involved (lit.[47] and references therein). For the diastereoisomeric pair *cis*- and *trans*-cyclo(Ala-Ala) the values of $^{2}J_{C'C^{\beta}}$ fall in the rang 0·5 – 1 Hz and we are not able to detect by means of these couplings the fine differences that should exist in these compounds. (In the crystalline state, the *trans*-compound is planar, the *cis*-com-

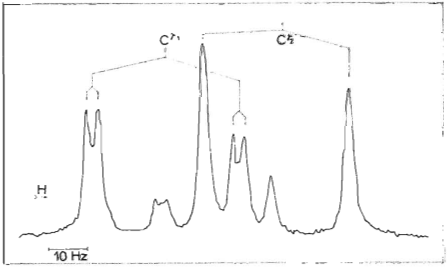


FIG. 1
The $C^{\gamma}$-Region in $^{13}C$-NMR Spectrum of the *cis*-Isomer Cyclo(L-Val-L-Val)

pound occurs in the twisted boat form[27]; in solution, the chirality of the boat of the *cis*-compound is probably reversed[23,37].) In case of phenylalanine containing compounds, cyclo(Ala-Phe), however, the coupling constants $^2J_{C'C^\beta}$(Ala) are different (1·2 and 1·7 Hz for *cis*- and *trans*-compound, respectively) which clearly indicates that the 2,5-piperazinedione ring is differently buckled in these two cyclodipeptides. As for significantly sharp manifestation of the coupling $C'C^\beta$ in alanine residue for the compounds with phenylalanine (*vs* compounds without phenylalanine), this may be due to the differences in the conformations of 2,5-piperazinedione rings.

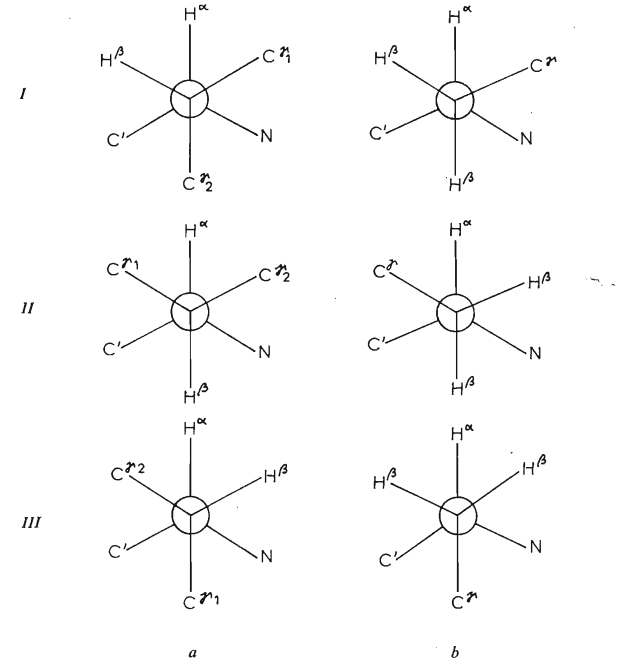


FIG. 2

The Staggered Side Chain Rotamers of the Valine Residue (*a*) and Leucine Residue (*b*)

cis- *and* trans-*Cyclo(Val-Val)*

The conformational analysis of amino acid side chains by means of the coupling constants $^3J_{C^\alpha H-C^\beta H}$ (and $^3J_{C'C^\gamma}$) is possible by two ways *i*) the calculation of populations of the three staggered conformers around the $C^\alpha—C^\beta$ bond (Fig. 2) by the method of Pachler[48], *ii*) the assumption of a unique conformation around the $C^\alpha—C^\beta$ bond – in this case "Karplus like" correlation coupling constant/dihedral angle for $H—C^\alpha—C^\beta—H$ is used[40]. At present state of knowledge we do not have the means of distinguishing these two possibilities and, therefore, we describe both approaches for each compound. In both approaches the additional informations inferred from $^3J_{C'C^\gamma}$ coupling constants are useful because they eliminate the ambiguities which are inherent to the estimation of the torsional angle $\chi^1$ from $^1H—^1H$ coupling constants only (*i.e.* the equality of the coupling constants for the conformations *I* and *III*, figure 2, and the multiplicity of the torsional angles corresponding to one value of $^3J_{C^\alpha H-C^\beta H}$).

*Rotamer populations methods*: In the case of *cis*-cyclo(Val-Val) the value $^3J_{C^\alpha H-C^\beta H}$ of 3·1 Hz corresponds to the almost exclusive populations of the rotamers *I* and/or *III* (Fig. 2) by referring to the relation of Cung and coworkers[40]. In the conformation *I* the torsional angle $C'C^\alpha C^\beta C^{\gamma 1}$ has the value 180° to which corresponding coupling constant $^3J_{C'C^{\gamma 1}}$ is 3·9 Hz; the other torsional angle $C'C^\alpha C^\beta C^{\gamma 2}$ in this conformation is 60°, to which corresponds 0·6 Hz for the coupling constant. In the conformation *III* both torsional angles are 60° and therefore the corresponding coupling constants $^3J_{C'C^{\gamma 1}}$ and $^3J_{C'C^{\gamma 2}}$ are about 0·6 Hz. From the value of the greater of the coupling constants $^3J_{C'C^\gamma}$ (2·9 Hz) we can now estimate unambiguously the population of the conformation *I*: $P_I = (2{\cdot}9-0{\cdot}6)/(3{\cdot}9-0{\cdot}6) = 0{\cdot}70$. Thus the rotamers *I* and *III* are populated as $I = 0{\cdot}70$ and $III = 0{\cdot}30$. In addition, the difference between $^3J_{C'C^{\gamma 1}}$ and $^3J_{C'C^{\gamma 2}}$ (Fig. 1) enables the unambiguous assignment of the methyl carbon atoms $C^{\gamma 1}$ and $C^{\gamma 2}$. $C^{\gamma 1}$ (low field, $\delta$ 18·5 ppm) is located mainly in *trans*-position with respect to the C' carbon atom since it shows larger coupling.

*Unique-conformation approach*: Using the relationship of Cung and coworkers[40], the coupling constants $^3J_{C^\alpha H-C^\beta H}$ of 3·1 Hz leads to two values of the torsional angle $\Theta$ ($H—C^\alpha—C^\beta—H$, Fig. 3), $\Theta = 65° \pm 5°$ and $\Theta = 110° \pm 5°$. On the other hand, the coupling constants $^3J_{C'C^{\gamma 1}}$ and $^3J_{C'C^{\gamma 2}}$ of 2·9 Hz and 0·5 Hz correspond[41–46] to the values for the torsional angle $\Theta'$ ($C'—C^\alpha—C^\beta—C^{\gamma 1}$) of $45° \pm 15°$ or $145° \pm 15°$ and for the torsional angle $\Theta''$ ($C'—C^\alpha—C^\beta—C^{\gamma 2}$) in the range from 70° to 110°. Then by comparing the $^1H—^1H$ and the $^{13}C—^{13}C$ data only the value of $\Theta = 110° \pm 5°$ and $\Theta' = 145° \pm 15°$ are mutually compatible and therefore only the value of $\chi^1 = -110°$ (Fig. 3) appears as a reasonable value for *cis*-cyclo(Val-Val).

With compound *trans*-cyclo(Val-Val), the coupling constants $^3J_{C^\alpha H-C^\beta H}$, $^3J_{C'C^{\gamma 1}}$ and $^3J_{C'C^{\gamma 2}}$ are practically the same as for *cis*-derivative. Therefore the conformation of the valine side chains will be the same in the *trans*- and *cis*-derivative.

cis- *and* trans-*Cyclo*(*Leu-Leu*)

*Rotamer population method*: For *cis*-cyclo(Leu-Leu) the value of 5·0 and 7·8 Hz for $^3J_{C^\alpha H-C^\beta H}$ have been reported[9], which corresponds to the fraction of population of *III* = 0·45 ± 0·06, and the remaining two conformers, *I* and *II*, are populated as 0·41 ± 0·06 and 0·14 ± 0·04. In an attempt to choose between *I* and *II* by using the set of $^3J_{C'C^\gamma}$ of 3·9 Hz, 0·6 Hz, 0·6 Hz for the conformations *I*, *II*, *III*, respectively, we estimate the "theoretical" value of $^3J_{C'C^\gamma}$ corresponding to the fraction of each conformer. We have obtained for the tentative assignment *I* = 0·41: $J_{theor}$ = 0·41 × × 3·9 + 0·59 × 0·6 = 1·9 (Hz) and for the alternative assignment *II* = 0·41 : : $J_{theor}$ = 0·14 × 3·9 + 0·86 × 0·6 = 1·1 (Hz). Then, since the experimental constant $^3J_{C'C^\gamma}$ 0·8 Hz is closer to the value of 1·1 Hz, we retained the attribution *I* = 0·14, *II* = 0·41 and *III* = 0·45.

For *trans*-cyclo(Leu-Leu) the values of $^3J_{C^\alpha H-C^\beta H}$ 5·6 and 6·6 Hz (lit.[9]) indicate the populations of conformers: *III* = 0·50 ± 0·07, *I* + *II* = 0·30 ± 0·05 and 0·20 ± ± 0·04. The "theoretical" value of $^3J_{C'C^\gamma}$ for the assignment *II* = 0·30 is 1·3 Hz and for *I* = 0·30 is 1·6 Hz. In this case, where there is a small difference in the populations of conformers *I* and *II*, the attribution is less certain because the difference in "theoretical" $^3J_{C'C^\gamma}$ approaches experimental error. Nevertheless, since $^3J_{C'C^\gamma}$ experimental is 0·8 Hz, we prefer the attribution *III* = 0·50, *I* = 0·20, *II* = 0·30.

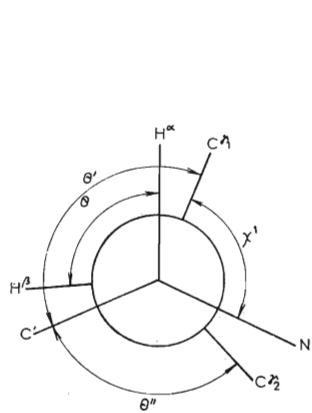


FIG. 3
Torsional Angles in the Valine Residue

*Unique-conformation approach*: The $^3J_{C^\alpha H - C^\beta H}$ coupling constants (5·0 + 7·8 Hz for *cis*-compound, 5·6 + 6·6 Hz for *trans*-compound) correspond to the values of dihedral angles $\Theta^1$ and $\Theta^2$ 115 – 120° and 130 – 138° for the *cis*-isomer, 118 – 125° and 125 – 130° for the *trans*-compound ($\Theta^1$, $\Theta^2$ are dihedral angles H—$C^\alpha$—$C^\beta$—$H^1$, H—$C^\alpha$—$C^\beta$—$H^2$, respectively). These values are not in contradiction to the $^{13}C$ data. In the conformation described above by $\Theta^1$, $\Theta^2$ the torsional angle C—$C^\beta$—$C^\alpha$—C' is near to 120°, to which corresponds the „theoretical" coupling constant $^3J_{C'C'}$ 1 Hz; experimental value is 0·8 Hz, In this conformation the atoms are nearly eclipsed which in general is considered as unfavourable. However, we do not exclude *a priori* this possibility because the global energy may be further lowered by the librations about the bonds.

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