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CONFORMATION OF CYCLO(-L-PRO-D-LEU-D-TYR(ME)-L-ILE-) PREDICTED BY EMPIRICAL RULES FOR CYCLIC TETRAPEPTIDES WAS EVIDENCED BY ^{1}H- AND ^{13}C-NMR SPECTROSCOPY

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The predicted *cis-trans-cis-trans* backbone conformation of cyclo(-L-Pro-D-Leu-D-Tyr(Me)-L-Ile-) (2) has been evidenced by ^{1}H-NMR experiments. Peptide 2 and cyclo(-L-Pro-L-Leu-D-Tyr(Me)-L-Ile-) are the first diastereomeric peptides having *cis* (DD) and *trans* (LD) Leu-Tyr(Me) peptide bonds.

As phytotoxic activities of tentoxin[1] and AM-toxins[2] depend on their conformations, the sequence-conformation relationship of cyclic tetrapeptides has become an interesting problem. In this connection, we proposed empirical rules predicting conformations of cyclic tetrapeptides and cyclic tetradepsipeptides on the basis of reported X-ray crystallographic[3] and NMR spectroscopic[4] data. The rules can be summarized briefly as follows[5]: 1. A conformation allowing intramolecular hydrogen bonding, namely γ-turn, is preferred, and an ester bond always adopts *trans* conformation. 2. The carbonyl group acylating a D-amino acid residue is oriented to the upper side of the main ring, when the direction of the peptide bond is right-handed. 3. The carbonyl group acylating a D-proline or an *N*-alkylated D residue is oriented to the lower side of the main ring, forming a *cis* peptide bond. 4. A peptide ring having an LDDL configurational sequence adopts a backbone conformation with C_i symmetry. 5. Glycine behaves as a D residue.

Rules 1, 2, 3, and 5 are derived from ample examples cited.[3,4] However, the reported example for rule 4 was only dihydrotentoxin, cyclo(-L-Leu-D-MePhe-Gly-L-MeAla-),[6] and then the configurational sequence of dihydrotentoxin should be defined as LDGL where G denotes Gly, but not LDDL. Thus, in order to establish the usefulness of rule 4, it is interesting to synthesize a peptide carrying an LDDL sequence and to study whether the peptide adopts virtually the *cis-trans-cis-trans* backbone conformation with C_i symmetry.

Previously, we synthesized a cyclic tetrapeptide cyclo(-L-Pro-L-Leu-D-Tyr(Me)-L-Ile-) (1) as the simplified analog of a phytotoxin Cyl-2, cyclo(-L-Pip-Aeo-D-Tyr(Me)-L-Ile-) (Pip, pipecolic acid; Aeo, 2-amino-9,10-epoxy-8-oxodecanoic acid; Tyr(Me), *O*-methyltyrosine).[7] The ^{1}H-NMR spectrum of 1 was similar to that of Cyl-2, and the conformation of 1 was proposed to be unique *cis-trans-trans-trans*

backbone with a *cis* L-Ile-L-Pro peptide bond and with an intramolecular hydrogen bond between L-Pro CO and D-Tyr(Me) NH in $CDCl_3$ solution (Fig. 1).[7] These results are a definite evidence for rules 1, 2, and 3.

In the same line of study, we also synthesized cyclo(-L-Pro-D-Leu-D-Tyr(Me)-L-Ile-) (2), which is a D-Leu containing diastereomeric analog of peptide 1.[8] It is of note that the peptide 2 possesses the LDDL configurational sequence. If this peptide is in a *cis-trans-cis-trans* conformation, the rule should be strongly supported and the usefulness of whole the rules becomes considerable. Thus, we studied the conformation of cyclic peptide 2 using ^{1}H- and ^{13}C-NMR spectroscopy.

^{1}H- and ^{13}C-NMR spectra of 2 were recorded on a JEOL FX-200 spectrometer at 199.5 MHz and 49.9 MHz, respectively. Sample concentrations in dimethyl sulfoxide-d_6 and in $CDCl_3$ solution ranged from 0.01 to 0.05 M, sample tubes being degassed by freeze-thawing and sealing. Signals were assigned by mutual decoupling techniques. The ^{1}H-{^{1}H} NOE difference spectra were obtained by irradiation of the amide or αH proton signals and were gated during a waiting time of 30 s prior to each scan.

As intramolecular NOEs are very sensitive to interproton distances, NOE measurements have been used in conformational studies of peptides.[9] In the study on elucidating the conformation of peptide 1, positive NOEs were observed between D-Tyr(Me) NH and L-Leu αH and between L-Ile NH and D-Tyr(Me) αH, confirming *trans* conformation at L-Leu-D-Tyr(Me) and D-Tyr(Me)-L-Ile peptide bonds. No NOE was observed between Pro αH and L-Leu NH. All these results consisted in a *cis-trans-trans-trans* backbone conformation[10] as shown in Fig. 1. However, if peptide 2 is in a *cis-trans-cis-trans* backbone, the NOE between D-Tyr(Me) NH and D-Leu αH would disappear, and instead, an appreciable NOE should be observed between D-Tyr(Me) αH and D-Leu αH.

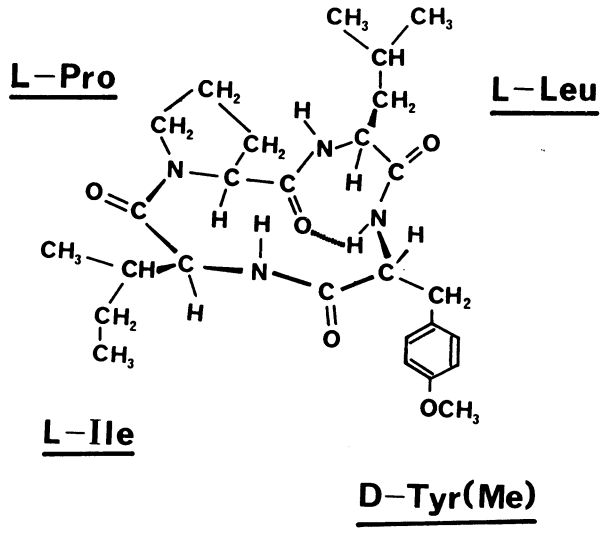

Fig. 1. Backbone conformation of cyclo(-L-Pro-L-Leu-D-Tyr(Me)-L-Ile-) (1).

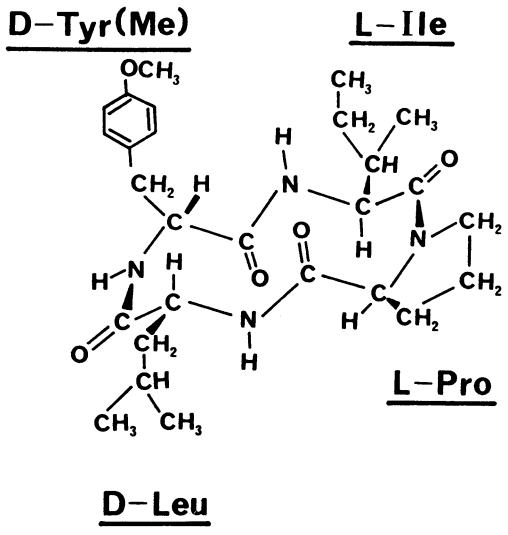

Fig. 3. Backbone conformation of cyclo(-L-Pro-D-Leu-D-Tyr(Me)-L-Ile-) (2).

^{1}H-NMR spectrum of 2 in dimethyl sulfoxide-d_6 showed one set of well-resolved signals, signifying the presence of a stable conformer. Fig. 2A shows the NOE difference spectrum of 2, αH proton of D-Tyr(Me) being saturated. Enhancement of D-Leu αH (15%) and L-Ile NH (5%) signals were observed. A similar enhancement was observed for D-Leu NH when αH of L-Pro was irradiated (Fig. 2B). These results clearly indicate the spacial proximity of three pairs of protons, namely between D-Tyr(Me) αH and D-Leu αH, between D-Tyr(Me) αH and D-Ile NH, and between L-Pro αH and D-Leu NH. In addition, ^{13}C-NMR spectra of 2 showed the *cis* conformation of L-Ile-L-Pro linkage.[8] Such a geometry is satisfied only when 2 adopts a *cis-trans-cis-trans* backbone conformation with C_i symmetry as shown in Fig. 3.

In regard to the effect of solvent change, the NH proton signals of 2 changed to unresolved multiplets when measured in $CDCl_3$. Furthermore, additional NH signals with low intensity emerged, suggesting the presence of interconverting conformers. Similarly, when peptide 1 was dissolved in dimethyl sulfoxide-d_6, all NH and some αH signals become very broad as compared to those measured in $CDCl_3$. These observations can be explained in terms of conformational stability. The *cis-trans-trans-trans* conformation of 1 with an intramolecular hydrogen bond should be stabilized in inert solvent, namely $CDCl_3$, and the *cis-trans-cis-trans* conformation of 2 should be favored in hydrogen-bond breaking solvent such as dimethyl

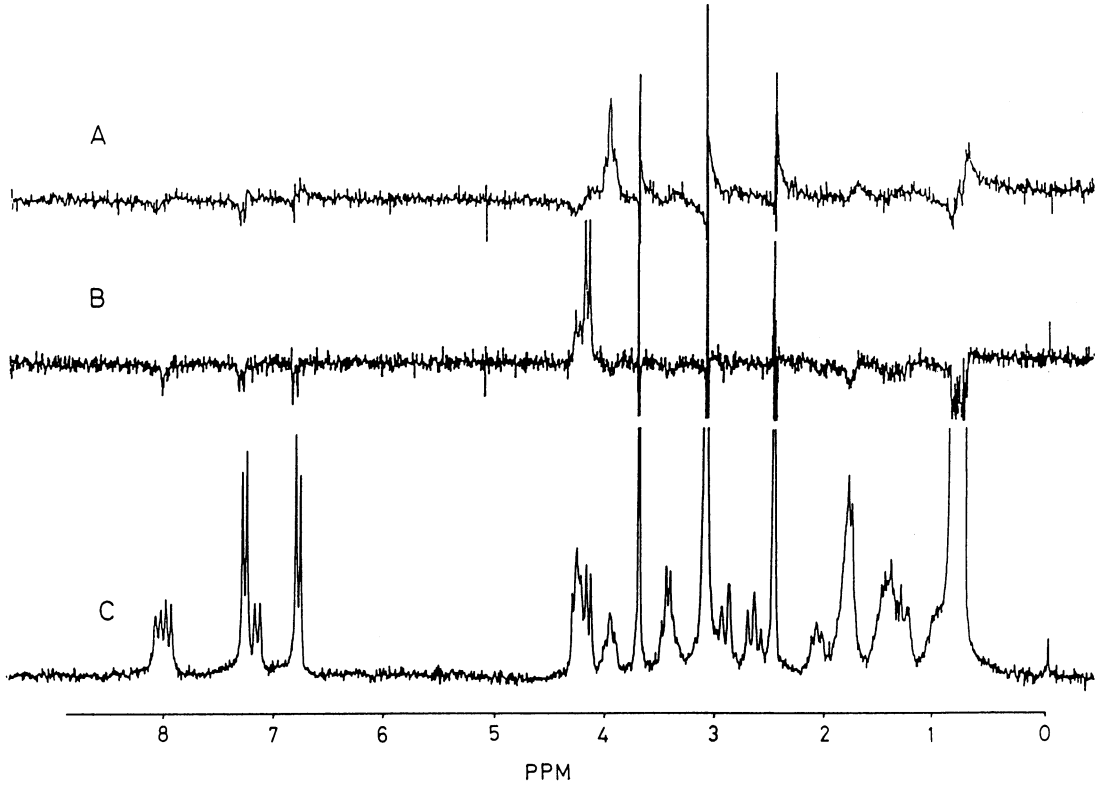

Fig. 2. 200 MHz NOE difference spectra of 2 in dimethyl sulfoxide-d_6 at 76°. A, D-Tyr(Me) αH was saturated. B, L-Pro αH was saturated. C, ^{1}H-NMR spectrum of 2 as a reference. Chemical shifts δ = 3.99 (D-Tyr(Me) αH), 4.18 (L-Pro αH), 4.29 (D-Leu + L-Ile αH), 7.20 (D-Tyr(Me) NH), 8.00 (D-Leu NH), and 8.10 (L-Ile NH).

sulfoxide-d_6. Loss of favorable solvent-peptide interactions would result in co-existence of conformers with similar conformation energies. These results can be explained sufficiently by rules 1 and 2. In addition to solvent effects, effects of side-chain variation might be considered in order to predict conformations precisely. The rules described above are undoubtedly the first approximation.

The present study shows that conversion of the configuration of a leucine residue from L to D results in formation of a *cis* bond in the Leu-D-Tyr(Me) amide bond. This conversion causes the cyclic tetrapeptide with an LDDL sequence to be in the *cis-trans-cis-trans* conformation, and thus pronounces the rule 4 appreciable. [L-Pro^1,L-Leu^2]Cyl-2 (1) and [L-Pro^1,D-Leu^2]Cyl-2 (2) are the first diastereomeric peptides having *trans* (LD) and *cis* (DD) secondary amide bonds, respectively. Conformational studies of these pair peptides clearly show that configurational sequences determine backbone conformations of cyclic tetrapeptides, as proposed by the rules described above.

Interestingly, [D-MeAla^1]tentoxin, a diastereomeric analog of tentoxin, possessed three isolable conformers.[11] Two of them showed comparable activities, but the third was inactive. Further studies must be needed to discuss conformation-activity relationships of the diastereomeric Cyl-2 analogs.

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