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SYNTHESIS AND CONFORMATION OF *cyclo*(-D- OR L-TYR(ME)-L-ILE-L-PRO-L-LEU-), DIASTEREOMERIC AND SIMPLIFIED ANALOGS OF CYL-2

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Diastereomeric cyclotetrapeptides, *cyclo*(-D- or L-Tyr(Me)-L-Ile-L-Pro-L-Leu-) (1D or 1L), which are simplified analogs of a phytotoxin Cyl-2, have been synthesized. A conformation with *trans-trans-cis-trans* peptide bonds,L-Ile-L-Pro bond being *cis*, is proposed for 1D on the basis of ^{1}H- and ^{13}C-NMR data.

Cyl-2 is a phytotoxin inhibiting the root growth of lettuce seedlings and its primary structure was shown to be *cyclo*(-D-Tyr(Me)-L-Ile-L-Pip-L-Aoe-): Tyr(Me), *O*-methyltyrosine; Pip, pipecolic acid; Aoe, 2-amino-8-oxo-9,10-epoxydecanoic acid.[1] As a preliminary study on the synthesis and conformation of Cyl-2, we synthesized simple analogs *cyclo*(-D-Tyr(Me)-L-Ile-L-Pro-L-Leu-) (1D) and *cyclo*(-L-Tyr(Me)-L-Ile-L-Pro-L-Leu-) (1L), which contain easily available amino acids such as L-Pro and L-Leu in place of L-Pip and L-Aoe. Here we report the results of the synthesis and NMR experiments on 1D and 1L, and propose a possible conformation of 1D with a *trans-trans-cis-trans* peptide backbone.

Boc-L-Ile-OH (25 mmol) was coupled with H-L-Pro-OMe (25 mmol) by the aid of dicyclohexylcarbodiimide and 1-hydroxybenzotriazole to afford oily Boc-L-Ile-L-Pro-OMe (94%), which was saponified with 1 M NaOH to give Boc-L-Ile-L-Pro-OH (2) (98%). A mixed anhydride prepared from 2 (20 mmol), isobutyl chloroformate, and triethylamine was coupled with H-L-Leu-OBzl to give Boc-L-Ile-L-Pro-L-Leu-OBzl (97%), which was treated with 0.1 M HCl in formic acid to afford H-L-Ile-L-Pro-L-Leu-OBzl·HCl (3) (93%). Compound 3 (7.0 mmol) was coupled with a similar mixed anhydride (7.7 mmol) prepared from Boc-D- or L-Tyr(Me)-OH to give oily Boc-D- or L-Tyr(Me)-L-Ile-L-Pro-L-Leu-OBzl (4D, 93%; 4L, 92%). Catalytic hydrogenation of 4D gave Boc-D-Tyr(Me)-L-Ile-L-Pro-L-Leu-OH (5D, 92%); mp 89-91°C; $[\alpha]_D^{20}$ -58.1°

(*c* 1, $CHCl_3$); R_f^1 (TLC with $CHCl_3$:MeOH = 5:1) 0.65. Found: C, 61.72; H, 8.22; N, 8.74%. Calcd for $C_{32}H_{50}O_8N_4 \cdot 1/4H_2O$: C, 61.66; H, 8.17; N, 8.99%. Compound 5L was obtained similarly from 4L (85%); mp 101-103°C; $[\alpha]_D^{20}$ -63.3° (*c* 1, $CHCl_3$); R_f^1 0.60. Found: C, 61.57; H, 8.21; N, 8.91%. Compound 5D (4 mmol) was treated with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide and N-hydroxysuccinimide to give succinimide ester 6D (90%).

Upon treatment of 6D (2.15 g, 3 mmol) with trifluoroacetic acid, the active ester trifluoroacetate was dissolved in dimethylformamide (30 ml) and the solution was stirred in pyridine (1000 ml) at room temperature. The reaction mixture was stirred for 2 days and evaporated to remain a solid residue, which was collected with methanol; yield of 1D, 0.76 g (50%); mp 301-302°C (dec); $[\alpha]_D^{20}$ -108° (*c* 1, $CHCl_3$); R_f^2 (TLC with $CHCl_3$:MeOH = 9:1) 0.78. Found: C, 64.69; H, 8.05; N, 11.16%; M^+, 500. Calcd for $C_{27}H_{40}O_5N_4$: C, 64.77; H, 8.05; N, 11.19%; M, 500.3. Similar cyclization reaction (1 mmol) and following purifications using Dowex 1 (OH^-), Dowex 50 (H^+), and Sephadex LH-20 column chromatographies yielded 1L (48 mg, 10%); mp 296-297°C (dec); $[\alpha]_D^{20}$ -103° (*c* 1, Me_2SO); R_f^2 0.55. Found: C, 64.63; H, 8.00; N, 11.13%; M^+, 500.

^{1}H-NMR spectra were measured on a JNM PS100 spectrometer and ^{13}C-NMR on a Hitachi H-90 instrument. Temperature dependences were studied in a range from 30°C to 60°C. D-H exchange experiments were carried out in a mixture of $CDCl_3$-CD_3OD (1:1) in the presence of catalytic amount of trifluoroacetic acid. Differential N-methylation experiment was performed according to the procedure of Kawai and Nagai.[2] Table 1 shows the chemical shifts, coupling constants, temperature coefficients of NH protons in 1D, and NH-C$^\alpha$H dihedral angles estimated from observed J values.[3] In D-H exchange, NH protons of Tyr(Me) and Leu were totally exchanged within 25 min after the addition of trifluoroacetic acid, however, NH of Ile partially remained after 50 min. For side chain orientation,

Table 1. Amide proton resonances and related data of 1D[a]

Residue	δ (ppm)	J (Hz)	Temperature coefficient[b]	NH-C$^\alpha$H dihedral angle
D-Tyr(Me)	7.08	10.5	0.0035	160-180
L-Ile	7.35	9.5	0.0087	150-160, 0
L-Leu	6.48	10.5	0.0039	160-180

a) Solvent, $CDCl_3$; internal reference, tetramethylsilane; at 29°C.

b) Part per million per degree, mean values in four points.

the coupling constants of α and β protons in Tyr(Me) are estimated as $J_{\alpha\beta_1}$ = 9.3 Hz, $J_{\alpha\beta_2}$ = 5.7 Hz, and $J_{\beta_1\beta_2}$ = 13.2 Hz. Populations of each conformer were calculated to be n_1 or n_2 = 0.61, n_2 or n_1 = 0.28, and n_3 = 0.11.[4] The values of $J_{\alpha\beta}$ of Ile (9.5 Hz) indicates αH and βH locate mainly at *trans* positions. The $J_{\alpha\beta}$ value of Leu was 6.4 Hz: the side chain of Leu seems to rotate freely.

^{13}C-NMR spectrum of 1D in $CDCl_3$ was unambiguously assigned except for β carbons of Tyr(Me), Ile, and Leu. Difference in the chemical shifts between β and γ carbons in Pro (9.21 ppm) shows Ile-Pro bond to be in *cis* conformation.[5]

Different from 1D, ^{1}H-NMR spectrum of 1L was recorded in $(CD_3)_2SO$ solution because of low solubility of 1L in $CDCl_3$. Signals of $C^{\alpha}H$ protons of 1L appeared in 3.8-4.4 ppm region as unresolved and overlapped multiplets, suggesting the presence of a mixture of conformers. Further analysis was not tried for 1L.

Since Ile-Pro bond was shown to be *cis*, *cis-trans-cis-trans* backbone conformations were considered at first because several cyclic tetrapeptides, *e.g.* *cyclo*(-L-Ala-L-Ala-Sar-Sar-),[6] have been shown to possess these conformations.[6,7] However, large J values for Ile and Leu were incompatible to these conformations as far as the dihedral angles reported in X-ray studies were conserved.[6,7] Structures containing consecutive *cis* bonds should be strained severely and dihedral angles for these structures also were incompatible with the observed J values. On the other hand, a *trans-trans-cis-trans* backbone conformation was compatible with the observed results such as chemical shifts, coupling constants, D-H exchange, and differential N-methylation. A large temperature coefficient of isoleucine NH indicated exposure to the solvent, this being compatible with the fact that this NH was the most sensitive to differential N-methylation.[2] Slow exchange of the exposed NH of Ile was explained in terms of shielding of the carbonyl in Tyr(Me) with the neighboring side chains of Tyr(Me) and Ile, and this shielding should cause retardation of the acid-catalyzed D-H exchange. A similar effect was recently reported by Kopple and Go.[8] Small temperature coefficients of NH protons of Leu and Tyr(Me) could be explained with the shielding by side chains of Leu and Pro and by a non-linear hydrogen bond between Pro-CO and NH-Tyr(Me), respectively. The presence of this hydrogen bond was proposed by Pullman and Pullman for a leucine residue with similar dihedral angles.[9] Such a weak hydrogen bond may be easily exchanged by CD_3OD.

In conclusion, we propose a *trans-trans-cis-trans* backbone conformation with

the dihedral angles shown in Table 2 as a model of 1D (Fig. 1). This result should be useful for elucidation of conformation of Cyl-2.

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Table 2. Dihedral angles postulated for 1D[a)]

Angle[b)]	Tyr(Me)	Ile	Pro	Leu
ϕ	110°	-140°	-80°	-110°
ψ	-70°	120°	-20°	70°
ω	170°	10°	-170°	-160°
χ_1	60° or 180°	-60°		

a) Notations for the angles according to IUPAC-IUB Commission, *Biochemistry*, 9, 3471 (1970), are used. b) The values of ψ and ω could not be derived experimentally and were estimated from CPK model building.

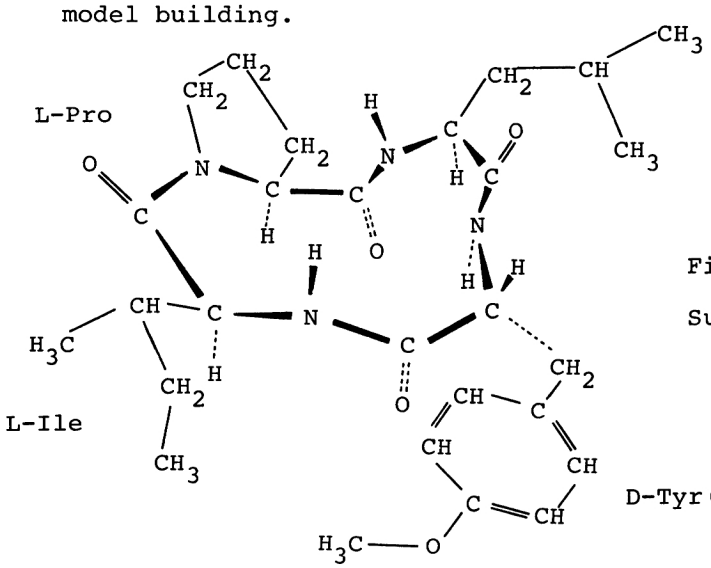

Fig. 1.
Supposed conformation of 1D.

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