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Peptides in Higher Plants. I.* The Conformation of Frangulanine

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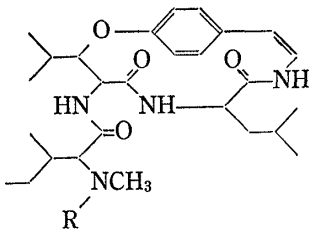
Frangulanine is a peptide alkaloid, with a 14 membered macrocyclic ring, from *Hovenia dulcis* THUNB. The X-ray analysis of frangulanine derivative and the synthetic study revealed the conformation of frangulanine as shown in Fig. 1 and Fig. 2.

The tetrapeptide, Ile–Hyleu–Leu–Tyr, a hypothetical intermediate of frangulanine biosynthesis, was also synthesized.

Previously we reported the isolation of frangulanine (I) and hovenine-A (II) from *Hovenia dulcis* (Rhamnaceae)[2] and X-ray analysis of frangulanine derivative.[3] Present paper deals with the conformation of frangulanine by discussing the result of X-ray analysis and synthetic study.

The tripeptide, methyl carbobenzoxy-L-isoleucyl-L-β-hydroxyleucyl-L-leucinate, was synthesized to clarify the conformation of frangulanine by comparison of their nuclear magnetic resonance (NMR) spectra. The tripeptide was also used to synthesize isoleucyl-β-hydroxyleucyl-leucyl-tyrosine, the hypothetical intermediate of frangulanine biosynthesis.[4]



I : R=CH_3
II : R=H

Chart 1

Discussion of X-Ray Analysis

The projection of the molecular structure of tri-N-methyl-frangulanine methiodide (III) viewed along the C-axis is already reported.[3] Another projection viewed along the b-axis is shown in Fig. 1.

Fig. 1 reveals the conformation of tri-N-methylfrangulanine methiodide. When the ϕ and ψ values are plotted on the Ramachandran ϕ—ψ chart, they fall within the upper right part of the allowed region for β-pleated sheets structure. The benzene ring and the neighbouring double bond are twisted 73 degrees as suggested by ultra-violet (UV) absorption study.[4]

The projection of the structure viewed along the a-axis after rotated for 40 degrees around the b-axis is shown in Fig. 2 (In Fig. 2 the three amide N-methyl groups are displaced by protons.)

If frangulanine has the same conformation as the frangulanine derivative, the protons H_4 and H_5 on the macrocyclic ring should be in the shielding zone of the aromatic ring, while the protons H_1, H_2 and H_7 in the deshielding zone of the aromatic ring. However the protons H_3 and H_6 are not affected by the aromatic ring.

To confirm the conformation of frangulanine, the NMR spectra of some amino acid derivatives and non-macrocyclic peptides are studied.

* Dedicated to the memory of Prof. Eiji Ochiai.

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2) M. Takai, Y. Ogihara, and S. Shibata, *Phytochemistry*, **12**, 2985 (1973).

3) M. Takai, K. Kawai, Y. Ogihara, Y. Iitaka, and S. Shibata, *J.C.S. Chem. Commum.*, **1974**, 653.

4) E.W. Warnhoff, "Fortscritte der Chemie organiscer Naturstoffe," Bd. Springer, Wien, 28, s. 192.

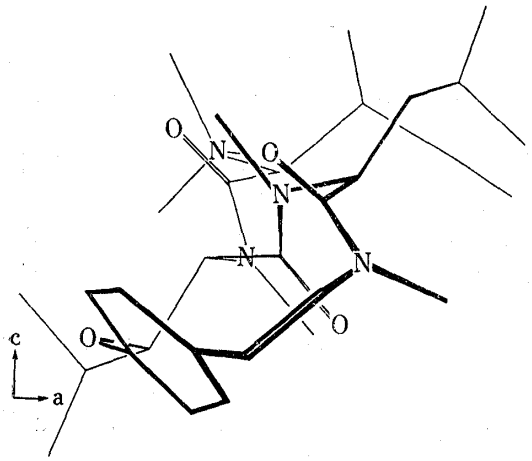

Fig. 1. The Projection of the Molecular Structure of Tri-N-methylfrangngulanine Methiodide viewed along b-Axis

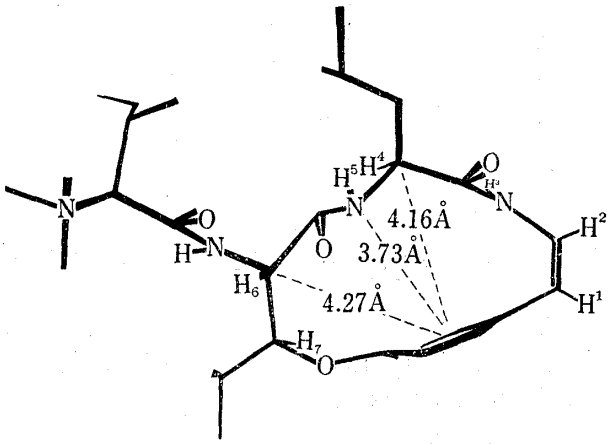

Fig. 2. The Projection of the Molecular Structure of Tri-N-methylfrangulanine Methiodide viewed along a-Axis after rotated for 40 degrees around b-Axis

Syntheses of Amino Acid Derivatives and Peptides

Esterification of L-leucine with methanol and thionyl chloride gave methyl L-leucinate hydrochloride (IV).

threo-β-Hydroxyleucine was prepared from isobutyraldehyde and glycine copper complex by Akabori's method.[5] Resolution of the D,L-*threo*-β-hydroxyleucine was achieved *via* the brucine salt of the phthaloyl derivative to afford pure L-*threo*-β-hydroxyleucine monohydrate.[6]

Carbobenzoxy-D,L- and L-*threo*-β-hydroxyleucine (Va, -b), which were prepared in aqueous alkaline solution from carbobenzoxy chloride and the corresponding amino acid,[7] were coupled with IV by dicyclohexylcarbodiimide (DCC) and N-hydroxy-succinimide (SuOH) to yield methyl carbobenzoxy-D,L-*thero*-β-hydroxyleucyl-L-leucinate (VIa) and methyl carbobenzoxy-L-threo -β-hydroxyleucyl-L-leucinate (VIb) (Chart 2).

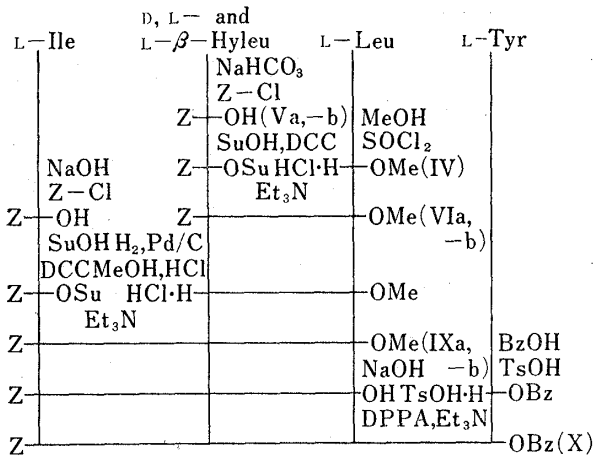

Chart 2

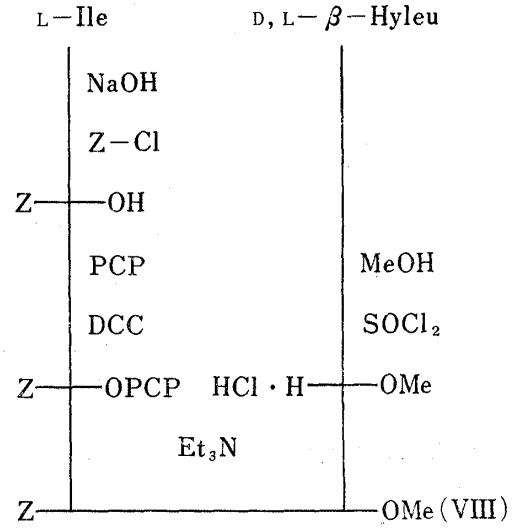

Chart 3

threo-D,L-β-Hydroxyleucine was esterified by methanol and thionyl chloride to give methyl *threo*-D,L-β-hydroxyleucinate hydrochloride (VII), which was coupled with pentachlorophenyl

5) Y. Ikutani, T. Okuda, and S. Akabori, *Bull. Chem. Soc. Japan*, **33**, 582 (1960).
6) S. Dalby, G.W. Kenner, and R.C. Sheppard, *J. Chem. Soc.*, **1960**, 968.
7) J. Marchand, M. Pais, and F.-X.Jarreau, *Bull. Soc. Chim. France*, **1971**, 3742.

carbobenzoxy-L-isoleucinate (Z-Ile-OPCP)[8] to afford methyl carbobenzoxy-L-isoleucyl-D,L-*threo*-β-hydroxyleucinate (VIII) (Chart 3). The diastereoisomeric compounds of VIII were separated using preparative thin-layer chromatography (TLC). These compounds are very useful for the following NMR study.

Hydrogenolysis of the compounds VIa and VIb in methanol containing hydrogen chloride furnished the corresponding amine hydrochlorides, which were converted to methyl carbobenzoxy-L-isoleucyl-D,L-*threo*-β-hydroxyleucyl-L-leucinate (IXa) and methyl carbobenzoxy-L-isoleucyl-L-*threo*-β-hydroxyleucyl-L-leucinate (IXb) by the addition of carbobenzoxy-L-isoleucine N-hydroxy-succinimide ester[9] and triethylamine.

The compound IXa was saponified and then coupled with benzyl L-tyrosinate[10] by diphenylphosphoryl azide (DPPA)[11] to yield diastereoisomeric benzyl carbobenzoxy-L-isoleucyl-D,L-*threo*-β-hydroxyleucyl-L-leucyl-L-tyrosinate (X), of which free tetrapeptide is a hypothetical intermediate of frangulanine that could be used as a material for biosynthetic study (Chart 2).

Table I. The Chemical Shift (δ) and Coupling Constant Values (Hz) in NMR Spectra ($CDCl_3$, 100 MHz) of Each α-Protons of Amino Acid Components and β-Proton of β-Hydroxyleucine of Compounds I, Va, VIb, VIII and IXb

	Ile		β-Hyleu			Leu	
	NH	α-H	NH (H_8)	β-H (H_7)	α-H (H_6)	NH (H_5)	α-H (H_4)
Z-Hyleu (Va)			6.11	3.71	4.50		
			d 10	bd 9	bd 9[a]		
Z-Hyleu-OMe (VIb)			5.89	3.7[b]	4.34	6.94	4.56
			d 9		dd 2, 9	d 9	t-like
Z-Ile-Hyleu-OMe (VIII)	5.46	4.07	6.78	3.7[b]	4.81		
	d 10	dd 8, 10	d 10		dd 2, 10		
Z-Ile-Hyleu-Leu-OMe (IXb)	5.70	4.10	7.13	3.7[b]	4.58	7.13	4.50
	d 9	dd 7, 9	d 9		dd 2, 8	d 9	dd 6, 9
Frangulanine (I)		2.60		4.94	4.45	5.92	4.0
				dd 2, 8	dd 8, 10	d 9	m

a) bs after D_2O exchange
b) overlapping with -OMe

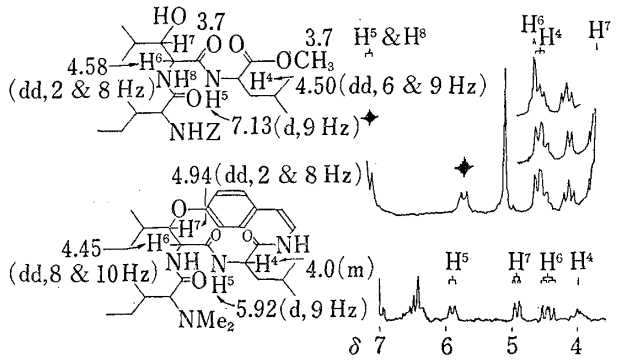

Fig. 3. NMR Spectra of Frangulanine and Compound IXb

NMR Study

The chemical shift values in NMR spectra of each α-protons of amino acid components and β-proton of β-hydroxyleucine are listed in Table I (The compound X is insoluble in $CDCl_3$).

The NMR spectra of frangulanine and the compound IXb are shown in Fig. 3.

When carbobenzoxy group is converted to peptide bond, the α-proton is shifted to lower field *ca.* 0.2 ppm, while the conversion of carboxyl group or carbomethoxy group to peptide bond causes the shift of α-proton to upper field *ca.* 0.2 ppm. Thus, in the case of non-macrocyclic tetrapeptide X, the signal of α-proton (H_4) of leucine will appear at δ *ca.* 4.3.

8) J. Kovacs, M.Q. Ceprini, C.A. Dupraz, and G.N. Schmit, *J. Org. Chem.*, **32**, 3696 (1967).
9) American Cyanamid Co., Fr. Patent 1406785 (1965) [*C.A.*, **63**, 13413*g* (1965)].
10) N. Izumiya, *Nippon Kagaku Zasshi*, **78**, 130 (1957).
11) T. Shioiri, K. Ninimiya, and S. Yamada, *J. Am. Chem. Soc.*, **94**, 6203 (1972).

The protons H_4, H_5, H_6, and H_7 of frangulanine are shielded by aromatic ring for 0.3, 1.2, 0.1, and −1.2 ppm respectively. This agreed with the X-ray analysis, namely, frangulanine has the same conformation as tri-N-methylfrangulanine methiodide.

Experimental[12)]

Tri-N-methylfrangulanine Methiodide (III)——A mixture of sodium hydride (750 mg) in 10 ml of dimethyl sulfoxide (DMSO) was warmed at 65—70° for 45 min. To this solution frangulanine (50 mg) in 5 ml of DMSO was added. After stirring for 10 min at room temperature, methyl iodide (3 ml) was added to the solution and the stirring was continued further 30 min.

After the addition of water, the mixture was extracted with $CHCl_3$. Evaporation of the solvent gave fine crystals. Recrystallization from methanol–ether yielded colourless prisms, mp 242—244° (45 mg).

NMR ($CDCl_3$) δ 2.98, 3.09 and 3.39 (3H, s, $-CONCH_3-$), 3.66 (9H, s, $-\overset{+}{N}(CH_3)_3$).

Z-D,L-Hyleu-L-Leu-OMe (VIa) and Z-L-Hyleu-L-Leu-OMe (VIb)——Equimolar DCC was added to the solution of Z-Hyleu and equimolar SuOH in EtOAc at 0°. After stirring at 0° for overnight, the precipitated N,N-dicyclohexylurea (DCU) was filtered. The filtrate was evaporated and then dissolved in dimethylformamide (DMF). To this solution 10% excess amount of the compound IV and triethylamine were added at 0°.

After stirring at room temperature overnight, the reaction mixture was diluted with EtOAc and then washed with 1N HCl, water, 5% $NaHCO_3$ and water. EtOAc layer was dried over Na_2SO_4 and evaporated to give 2.7 g of oily compound VIa in 66% yield and 540 mg of oily compound VIb in 88% yield.

Z-L-Ile-D,L-Hyleu-OMe (VIII)——Pentachlorophenyl carbobenzoxy-L-isoleucinate (10.2 g) and methyl D,L-*threo*-β-hydroxyleucinate hydrochloride (3.9 g) were dissolved in EtOAc (150 ml). Triethylamine was added to the solution until the pH became to 9. After 2 days, more methyl D,L-*threo*-β-hydroxyleucinate hydrochloride (0.4 g) was added.

In the next day, the reaction mixture was diluted with EtOAc (500 ml), washed with 0.1N NaOH, water, 1N HCl and dried over Na_2SO_4. After evaporation, the residue was recrystallized from EtOAc and petroleum ether to give colourless needles, 5.02 g, 62%.

The diastereoisomeric compounds of VIII were separated by preparative TLC (Kiesel gel, GF_{254}, developing solvent was $CHCl_3$: MeOH=20: 1.)

VIII (small *Rf* value), mp 104—105°. *Anal.* Calcd. for $C_{21}H_{32}O_6N_2$: C, 61.74; H, 7.90; N, 6.86. Found: C, 61.93; H, 7.98; N, 6.89.

VIII (large *Rf* value), mp 146—147°. *Anal.* Found: C, 60.99; H, 7.83; N, 6.70.

Z-L-Ile-D,L-Hyleu-L-Leu-OMe (IXa) and Z-L-Ile-L-Hyleu-L-Leu-OMe (IXb)——The compound VI was hydrogenated in 20% HCl–MeOH containing 10% palladium-on-charcol catalyst. The catalyst was removed by filtration, and evaporation of the solvent gave an oily H-Hyleu-Leu-OMe·HCl. This oily material was dissolved in DMF and added with Z-Ile-OSu and Et_3N at 0°.

After stirring at room temperature overnight, the reaction mixture was diluted with EtOAc and washed with 1N HCl, water, 5% $NaHCO_3$, water and dried over Na_2SO_4. After the evaporation of the solvent, recrystallization of oily residue from EtOAc–pet.ether gave 1.8 g of colourless needles IVa in 50% yield, mp 139—143°. *Anal.* Calcd. for $C_{27}H_{43}O_7N_3$. C, 62.16; H, 8.31; N, 8.06. Found: C, 61.65; H, 8.21; N, 8.16. and 340 mg of colourless needles IXb in 50% yield, mp 159—161°. *Anal.* Found: C, 61.03; H, 8.37; N, 8.32.

Z-L-Ile-D,L-Hyleu-L-Leu-Tyr-OBz (X)——The compound IXa (677 mg) was treated with a solution of sodium hydroxide (1N, 1.5 ml) in methanol (10 ml) for 2 hr, water was added and then the bulk of the methanol was removed by evaporation. After washing with ether, the aqueous phase was acidified with 1N-HCl solution and extracted with EtOAc. The EtOAc layer was washed with water and dried over Na_2SO_4. Evaporation of the solvent gave an oily Z-L-Ile-D,L-Hyleu-L-Leu-OH (490 mg, 74%).

This oily material was dissolved in DMF. To this solution H-Tyr-OBz·TsOH (532 mg) and DPPA (264 mg) in DMF were added at 0°, followed by the addition of Et_3N (0.3 ml). The mixture was stirred at 0° for several hous and at room temperature overnight. The reaction mixture was diluted with EtOAc and washed with 1N HCl, water, 5% $NaHCO_3$ and water and dried over Na_2SO_4 and evaporated. The residue was chromatographed on silica gel to give colourless powders, 360 mg, 51%, mp 218—220°. *Anal.* Calcd. for $C_{42}H_{56}O_9N_4+2H_2O$.: C, 63.29; H, 7.58; N, 7.03. Found: C, 63.37; H, 7.48; N, 7.17.

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12) All melting points are uncorrected.