Conformational Preference of β-Aryldehydroalanine. Solid-State Conformation of Tripeptide Possessing a (*Z*)-β-(1-Pyrenyl)dehydroalanine Residue in the Second Position

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In this paper we attempted to reveal how a large β-substituent (pyrenyl group) of dehydroresidue affects the structural and conformational preferences of (*Z*)-β-substituted α,α-dehydroalanine. An X-ray crystallographic analysis was carried out for Boc–L-Ala–Δ^{Z}Pyr–L-Val–OMe (Boc = *t*-butyloxycarbonyl; OMe = methoxy) possessing a novel (*Z*)-β-(1-pyrenyl)dehydroalanine (Δ^{Z}Pyr) residue. Single crystals were obtained by slow evaporation from an ethanol solution in the monoclinic space group $P2_1$. The bond lengths and bond angles of the Δ^{Z}Pyr residue were essentially similar to other β-substituted dehydroalanines. The tripeptide took a type II β-turn conformation involving an intramolecular hydrogen bond between CO(Boc) and NH(Val), like small peptides possessing a (*Z*)-β-(1-naphthyl)dehydroalanine or a (*Z*)-β-phenyldehydroalanine residue. The similarity between Δ^{Z}Pyr and other dehydroalanines in structural property and conformational preference should be responsible for the non-planarity of the Δ^{Z}Pyr residue to give an extremely high energy in the planar orientation of the pyrenyl group relative to the $C^{\alpha}=C^{\beta}\text{-}C^{\gamma}$ plane; the non-planarity should weaken the π-conjugation effect of the pyrenyl group on the structural property and conformational preference of the Δ^{Z}Pyr residue. The pyrenyl rings were packed in a partially overlapped face-to-face orientation with the shortest center-to-center distance of 5.96 Å. The CD spectrum of a KBr disk prepared from the crystal showed strong excitonic interactions between pyrenyl groups, reflecting a regular arrangement of the pyrenyl groups in the crystal structure.

Recently, unusual amino acids (e.g., α,α-disubstituted residues, α,β-dehydroresidues, and β-amino acid residues) have been used as an effective tool for constructing a specific and compact conformation. These amino acids differ from protein amino acids in chemical structure around the C^{α} carbon, i.e., in conformational preference, thus providing specific conformational constrains on the main and side chains. As a result, a peptide possessing an unusual residue can be expected to show well-defined and predictable conformations, leading to specific molecular shapes or specific arrangements of the functional groups.

α,β-Dehydroamino acid (unsaturated) residues, being naturally present in many bioactive peptides and in certain proteins,[1] have been found to influence the main-chain conformation as well as the side-chain conformation characteristically. The inherent conformational preference is largely governed by the structural feature of the $C^{\alpha}=C^{\beta}$ double bond (Chart 1).

β-Substituted α,β-dehydroalanines give a smaller degree of side-chain freedom than such naturally occurring saturated residues as Phe, Asp, Glu, and Lys, because the variable side-chain torsion angle is regarded as being single due to prohibited rotation about the $C^{\alpha}=C^{\beta}$ double bond. Thus, β-substituted α,β-dehydroalanines can be expected to be a unique residue for spatially and regularly arranging a functional β-substituent along its peptide backbone.

Extensive work has been reported on the conformational preference of dehydroresidues corresponding to conventional saturated amino acids: i.e., (*Z*)-β-phenyl-α,β-dehydroalanine (Δ^{Z}Phe),[2] (*Z*)-dehydroleucine (Δ^{Z}Leu),[3] dehydrovaline (ΔVal),[4] dehydroalanine (ΔAla),[5] and so on. The conformations induced by dehydroresidue depend on the size of the β-substituent: e.g., β-turn or 3_{10}-helix for the Δ^{Z}Phe residue,[2] β-turn for Δ^{Z}Leu,[3] extended conformation or γ-turn for ΔAla,[5] and extended conformation for 2-amino-2-butenoic acid.[6] On the other hand, little is known about the conformational preference in dehydroresidues having a larger β-substituent. Understanding the effect of the β-substituent on the conformational preference in dehydroresidue is important when designing a novel dehydropeptide for arranging a wide variety of functional β-substituents.

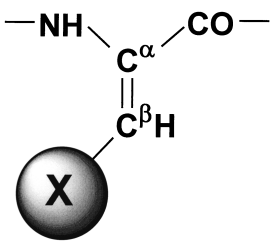

Chart 1. (Z)-α,β-Dehydroresidue.

We have studied how the β-aryl group (size and π-conjuga-

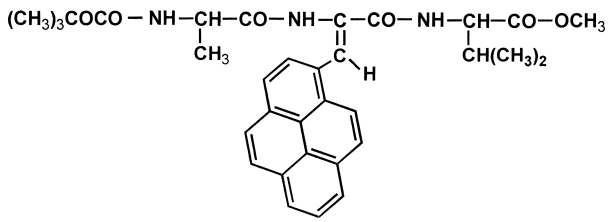

Chart 2. Boc–L-Ala–Δ^{Z}Pyr–L-Val–OMe **1**
(Boc = *t*-butyloxycarbonyl; OMe = methoxy)

tion) of α,β-dehydroalanine affects the structural property and conformational preference.[7,8] In the present study, an X-ray crystallographic analysis was performed for tripeptide **1** (Chart 2) possessing a novel (*Z*)-β-(1-pyrenyl)dehydroalanine (Δ^{Z}-Pyr), as an extension of our previous work.

Tripeptide containing a Δ^{Z}Phe or (*Z*)-β-(1-naphthyl)dehydroalanine (Δ^{Z}Nap)[7] residue at the second position has been found in the β-turn conformation both in the solid state and in solution. Peptide **1** in solution was also found to form a β-turn conformation.[8] However, the detailed conformation of peptide **1** as well as the structural parameter of the Δ^{Z}Pyr residue are still not clear. The present work aims to provide both structural and conformational data of a novel Δ^{Z}Pyr residue having a more bulky and π-extended pyrenyl β-substituent and, particularly, to reveal whether peptide **1** retains the conformational preference characteristic of other dehydroresidues.

Experimental

Sample Preparation of Peptide 1. The synthesis and characterization of peptide **1** followed Ref. 8. The Δ^{Z}Pyr residue was introduced by the ring-opening Boc–Ala–Δ^{Z}Pyr azlactone with an amino group of Val–OMe. Single crystals were obtained by gradually evaporating a solution of peptide **1** in absolute ethanol.

X-ray Structure Determination. A yellow, dry, single crystal (0.20 × 0.30 × 0.30 mm^3) was used for collecting three-dimensional X-ray data on a RIGAKU AFC7R diffractometer. A phase determination was carried out by a direct method (SHELXS),[9] and expanded using Fourier techniques.[10] All nonhydrogen atoms were refined anisotropically, and hydrogen atoms were refined isotropically. The final cycle of a full-matrix least-squares refinement on F^2 was based on 1888 observed reflections ($I > 3.00\sigma(I)$) and 528 variable parameters, and converged with $R = 3.6\%$ and $R_w = 4.3\%$. In the final stage, a refinement was carried out with anisotropic temperature factors for all nonhydrogen atoms. The crystallographic details are summarized in Table 1.
The complete data including the final positional parameters, equivalent thermal factors, anisotropic temperature factors, bond lengths, bond angles, and van der Waals contacts for peptide **1** are deposited as Document No. 74027 at the Office of the Editor of Bull. Chem. Soc. Jpn. Crystallographic data have been deposited at the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK and copies can be obtained on request, free of charge, by quoting the publication citation and the deposition number 156302.

CD Measurement. CD and absorption spectra were recorded using JASCO J-500 and JASCO V-550 spectrometers, respectively. The sample preparation for a solid-state CD measurement followed Ref. 11. A crystal of peptide **1** (ca. 0.5 mg) and dry KBr (110 mg) were finely ground, and the powder was pressed at 350 kg cm^{-2} for 10 min to prepare a clear disk of 2 cm diameter. The disk, held by a disk holder, was placed normal to a light beam. In the CD measurement, the sample disk was rotated manually in 90° steps, and then measured (at rotation angles of 0°, 90°, 180°, and 270°) to check for the absence of artifacts, such as light scattering. The spectrum of a 100% KBr disk was used as a blank. The ordinate of the CD spectrum in KBr was expressed in terms of the ellipticity (θ in mdeg) when the corresponding absorption spectrum was normalized to unity for the maximum absorbance (A_{max}) at around 360 nm, assignable to the Δ^{Z}Pyr residue. For a comparison, CD and absorption spectra of peptide **1** in pure chloroform were recorded with a peptide concentration of 0.4 mM using a quartz cell of 1 mm optical length (1 M = 1 mol dm^{-3}).

Table 1. Crystallographic Details for Boc–Ala–Δ^{Z}Pyr–Val–OMe

Empirical formula	$C_{33}H_{37}N_3O_6$
Molecular weight	571.67
Crystal dimensions	0.20 × 0.30 × 0.30 mm^3
Crystal system/space group	monoclinic/$P2_1$
Cell parameters	
a = 5.957(1) Å	
b = 27.498(2) Å	
c = 9.557(1) Å	
β = 102.61(1)°	
V = 1527.8(4) Å^3	
Z 2	
Density calculated (g cm^{-3})	1.243
Radiation used	Cu$K\alpha$ (λ = 1.54178 Å)
$F(000)$	608.00
Temperature	296 K
Scan type	ω-2θ
$2\theta_{max}$ (up to which data were collected)	120.1°
Observed reflections ($I >$ 3.00$\sigma(I)$)	1888
Variables	528
Refinement method	Full-matrix least squares on F^2
Final agreement factors for observed data	R = 3.6% R_w = 4.3%

Conformational Energy Calculation. An empirical conformational energy calculation was carried out using the structural and energy parameters based on the ECEPP system.[12] The ECEPP parameters of the Δ^{Z}Pyr residue were determined in this study, although a tentative calculation was made previously.[8] The structural parameters were assessed from the present structural data of peptide **1**, and the partial charges were determined from the CNDO/2 (ON) molecular orbital calculation.[13] The torsional barrier about the C$^{\beta}$–C$^{\gamma}$ bond of the Δ^{Z}Phe residue (6.4 kcal mol^{-1})[14] was used for the Δ^{Z}Pyr residue. The program PEPCON,[12,15] written by M. Sisido for a conformational energy calculation and graphics of a given peptide, was modified to be applicable to β-aryldehyroalanine-containing peptides.[16]

Results and Discussion

Structural Property of the Δ^{Z}Pyr Residue. Most of the bond lengths and bond angles were normal, except for those of the Δ^{Z}Pyr residue. In the Δ^{Z}Pyr residue (Table 2), the C2$^{\alpha}$=C2$^{\beta}$ distance of 1.327(5) Å corresponds to the standard double-bond distance of 1.337 Å.[17] The C2$^{\alpha}$–C2′ and N2–C2$^{\alpha}$ distances of 1.493(5) Å and 1.421(5) Å, respectively, were

Table 2. Selected Bond Lengths and Bond Angles of the ΔZPyr and Other Dehydroresidues[a)]

	Δ^ZPyr	Δ^ZNap[b)]	Δ^ZPhe[c)]	Δ^ZLeu[c)]
$C2^\alpha$=$C2^\beta$	1.327(5)	1.353(9)	1.329	1.323
$C2^\alpha$–C2′	1.493(5)	1.473(8)	1.504	1.491
N2–$C2^\alpha$	1.421(5)	1.411(7)	1.425	1.421
C2′=O2	1.224(4)	1.230(7)	1.237	1.258
C1′–N2–$C2^\alpha$	120.9(3)	119.7(5)	121.4	123.2
N2–$C2^\alpha$–C2′	117.7(3)	120.6(5)	116.5	116.9
N2–$C2^\alpha$=$C2^\beta$	123.4(4)	121.3(5)	124.5	122.9
C2′–$C2^\alpha$=$C2^\beta$	118.2(4)	117.4(5)	118.4	119.9
$C2^\alpha$=$C2^\beta$–$C2^\gamma$	127.2(4)	125.0(6)	130.8	127.3

a) The atomic abbreviation is defined as follows: –C1′–N2–$C2^\alpha$(=$C2^\beta$–$C2^\gamma$)–(C2′O2)–. b) From Ref. 7. c) From Ref. 18.

markedly shorter than the corresponding values of saturated peptides:[18] e.g., 1.53 and 1.45 Å for Phe residue. The C2′=O2 distance of 1.224(4) Å was somewhat longer than the corresponding distance of the Phe residue (1.203 Å)[18] as a saturated residue. The shortness of the N2-$C2^\alpha$ and $C2^\alpha$-C2′ bonds, and the slightly longer value of the C2′=O2 double bond should be interpreted by the presence of $C2^\alpha$=$C2^\beta$ double bond that leads to an extended delocalization of the π-electron system. This tendency has also been found in other dehydroresidues.[19]

To understand the effect of the β-substituent on the structural property of dehydroresidues, the data of other β-substituted dehydroresidues are listed in Table 2.[7,19] The bond lengths of the Δ^ZPyr residue were close to those of the other residues, although the C2′=O2 distance was slightly shorter. The bond angles of the Δ^ZPyr residue were 117.7(3)° for N2–$C2^\alpha$–C2′, 123.4(4)° for N2–$C2^\alpha$=$C2^\beta$, and 127.2(4)° for $C2^\alpha$=$C2^\beta$–$C2^\gamma$, being somewhat deviated from 120°, expected for an sp^2 hybridized carbon atom. It has been pointed out that the closing of the N2–$C2^\alpha$–C2′ bond angle and the opening of N2–$C2^\alpha$=$C2^\beta$ and $C2^\alpha$=$C2^\beta$-$C2^\gamma$ are responsible for steric constraints induced by the β-substituent of dehydroresidues.[19b] As shown in Table 2, the three bond angles of the Δ^ZPyr were similar to those of the other β-substituted dehydroalanines.

To summarize, no marked difference between the Δ^ZPyr and other dehydroresidue was seen in the structural properties, essentially, indicating that the largest size and π system of the pyrenyl group do not affect the structural parameters characteristic of the β-substituted dehydroalanines.

Conformation of Peptide 1. An ORTEP view of peptide **1** is shown in Fig. 1, and the torsion angles are summarized in Table 3. The backbone took a type II β-turn structure characterized by $\phi_1 = -51.0(5)°$, $\psi_1 = 137.7(4)°$, $\phi_2 = 54.3(5)°$, and $\psi_2 = 32.0(5)°$. Herein, an intramolecular hydrogen bond was observed between CO of the Boc group and NH of the Val residue. The type II β-turn is somewhat deviated from the standard one ($\phi_i = -60°$, $\psi_i = 120°$, $\phi_{i+1} = 80°$, and $\psi_{i+1} = 0°$).[20] A distorted β-turn conformation was also observed for an analogous Δ^ZNap-containing tripeptide, Boc–Ala–Δ^ZNap–Val–OMe (**2**),[7] as shown in Table 4. The solution conformation of peptide **1** was investigated using ^{1}H NMR spectroscopy.[8] The solvent dependence on the NH chemical shifts revealed that the Val NH participates in intramolecular hydrogen bonding. In addition, a strong nuclear Overhauser effect (11.3%) was observed for the Ala C^αH–Δ^ZPyr NH proton pairs, indicating that the protons are very close to each other (< 3 Å). These NMR data correspond to the conformation in the present

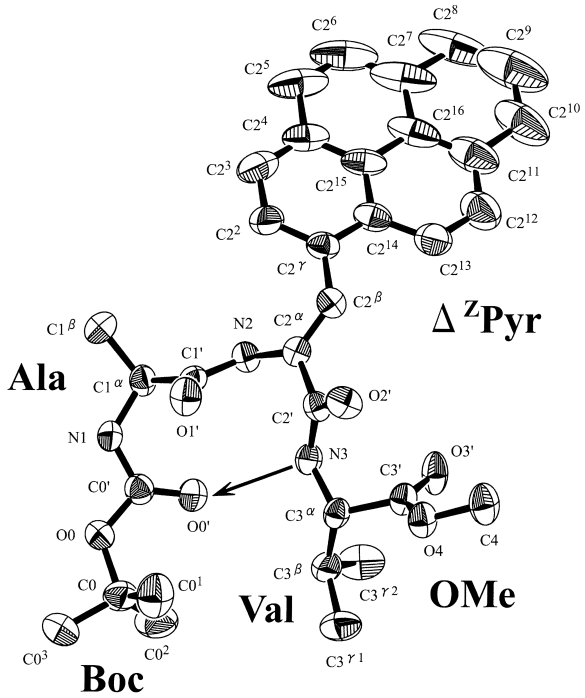

Fig. 1. Conformation of peptide **1** forming a type II β-turn. The arrow represents an intramolecular hydrogen bond between NH(Val) and CO(Boc). The $C2^8$, $C2^9$, $C2^{10}$ atoms of the pyrenyl groups were somewhat disordered.

Table 3. Selected Torsion Angles (°)

θ_0^1	$C0^1$–C0–O0–C0′	62.5(6)	θ_0^2	$C0^2$–C0–O0–C0′	−62.7(6)
θ_0^3	$C0^3$–C0–O0–C0′	179.2(4)	θ_0	C0–O0–C0′–N1	−168.1(4)
ω_0	O0–C0′–N1–$C1^\alpha$	174.7(4)	υ_0	O0′–C0′–N1–$C1^\alpha$	−7.3(7)
ϕ_1	C0′–N1–$C1^\alpha$–C1′	−51.0(5)	ψ_1	N1–$C1^\alpha$–C1′–N2	137.7(4)
ω_1	$C1^\alpha$–C1′–N2–$C2^\alpha$	178.3(4)	υ_1	O1′–C1′–N2–$C2^\alpha$	−0.9(6)
ϕ_2	C1′–N2–$C2^\alpha$-C2′	54.3(5)	ψ_2	N2–$C2^\alpha$–C2′–N3	32.0(5)
ω_2	$C2^\alpha$–C2′–N3–$C3^\alpha$	169.3(3)	υ_2	O2′–C2′–N3–$C3^\alpha$	−5.1(6)
χ_2^1	N2–$C2^\alpha$–$C2^\beta$–$C2^\gamma$	3.5(7)	$\chi_2^{1'}$	C2′–$C2^\alpha$–$C2^\beta$–$C2^\gamma$	174.0(4)
χ_2^2	$C2^\alpha$–$C2^\beta$–$C2^\gamma$–$C2^2$	42.5(7)	$\chi_2^{2'}$	$C2^\alpha$–$C2^\beta$–$C2^\gamma$–$C2^{14}$	−137.4(5)
ϕ_3	C2′–N3–$C3^\alpha$–C3′	−50.4(5)	$\psi_{3(T)}$	N3–$C3^\alpha$–C3′–O4	136.4(4)
$\theta_{3(T)}$	$C3^\alpha$–C3′–O4–C4	−175.1(4)	$\upsilon_{3(T)}$	O3′–C3′–O4–C4	6.3(7)
χ_3^1	N3–$C3^\alpha$–$C3^\beta$–$C2^{\gamma 1}$	−165.2(4)	$\chi_3^{1'}$	C3′–$C3^\alpha$–$C3^\beta$–$C3^{\gamma 2}$	68.9(5)

Table 4. Conformational Parameters of Di- and Tripeptides Possessing a Dehydroresidue at the Second Position in the Solid States[a)]

Peptide	ϕ_1	ψ_1	ϕ_2	ψ_2	ϕ_3	ψ_3	Conformation	Ref.
Boc–Ala–Δ^ZPyr–Val–OMe	−51.0	137.7	54.3	32.0	−50.4	136.4	Type II β-turn	Present Work
Boc–Ala–Δ^ZNap–Val–OMe	−54.9	141.6	57.7	28.0	−54.5	140.0	Type II β-turn	7
Boc–Ala–Δ^ZPhe–NHPr	−63.9	135.4	75.8	7.8			Type II β-turn	2b
Boc–Gly–Δ^ZPhe–NHMe	−57.2	141.2	71.5	7.2			Type II β-turn	2a
Boc–Pro–Δ^ZPhe–Gly–OH	−48	137.0	65	15	−166		Type II β-turn	2c
Boc–Phe–Δ^ZPhe–Val–OMe	−45.4	−45.6	−46.5	−38.0	51.7	43.0	Type III β-turn	2d
Boc–Pro–Δ^ZLeu–NHMe	−51	133	74	8			Type II β-turn	3a
Boc–Phe–Δ^ZLeu–Val–OMe	−44.5	134.5	54.5	31.1	51.9	139.0	Type II β-turn	3b

a) ϕ_i and ψ_i correspond to ith residue from N-terminus.

study: i.e., the intramolecular hydrogen bond of NH(Val) with CO(Boc) and the Ala $C^{\alpha}H$–Δ^ZPyr NH distance of 2.2 Å. Thus, the β-turn conformation in the solid state was retained in solution.

The urethane group was frequently found in a planar conformation ($\theta_0 = 180^\circ$ and $\omega = 180^\circ$).[21] In the present case, the Boc group also gave a *trans-trans* conformation characterized by $\theta_0 = -168.1(4)^\circ$ and $\omega_0 = 174.7(4)^\circ$, essentially, although θ_0 is slightly deviated from 180°. The conformation is required for the presence of the O0′ oxygen atom of the Boc group participating in the 4 → 1 hydrogen bond.[21] The methyl carbon atoms of the Boc group were staggered with respect to the O0–C0′ bond [$\theta_0^1 = 62.5(6)^\circ$, $\theta_0^2 = -62.7(6)^\circ$, and $\theta_0^3 = 179.2(4)^\circ$]. Like other dehydropeptides,[18] the two amide bonds around the Δ^ZPyr residue took *trans* conformations, characterized by $\omega_1 = 178.3(4)^\circ$ and $\omega_2 = 169.3(3)^\circ$, although ω_2 is slightly deviated from 180°. The side chain of the Val residue showed the (*t*, *g*) conformation [$\chi_3^1 = -165.2(4)^\circ$ and $\chi_3^{1'} = 68.9(5)^\circ$] corresponding to the least staggering of a methyl group between the NH and CO groups.[22] The conformation was most commonly observed,[23] being very similar to those in Boc–Phe–Δ^ZPhe–Val–OMe[2d] and Boc–Phe–Δ^ZLeu–Val–OMe.[3b]

The side-chain conformation of the Δ^ZPyr residue was characterized by $\chi_2^1 = 3.5(7)^\circ$ and $\chi_2^{1'} = 174.0(4)^\circ$, meaning that the stereochemistry about the $C2^{\alpha}$=$C2^{\beta}$ double bond is essentially planar. On the other hand, the orientation of the pyrenyl group, characterized by $\chi_2^2 = 42.5(7)^\circ$ and $\chi_2^{2'} = -137.4(5)^\circ$, was found to be nonplanar relative to the $C2^{\alpha}$=$C2^{\beta}$–$C2^{\gamma}$ plane. The nonplanar orientation should weaken the π-extended effect of the pyrenyl group on the structural and conformational preferences of the Δ^ZPyr residue.

Side- and Main-Chain Conformations of β-Aryldehydroalanines. Figure 2a shows the effect of the χ_2^2 angle of the Δ^ZPyr residue on the conformational energy of Ac–Ala–Δ^Z-Pyr–NHMe (Ac = acetyl, NHMe = *N*-methylamide), in which all torsion angles, expect for the χ_2^2 angle, were set to the experimental values given in Table 4. There were mainly two stable orientations for the pyrenyl group; one in $\chi_2^{2'} = 46^\circ$ (type A) and the other in $\chi_2^2 = 246^\circ$ (type B). The type A orientation was very close to the experimental value [$\chi_2^2 = 42.5(7)^\circ$]. The planar orientation [$\chi_2^2 = 0(360)^\circ$ or 180°], giving an extremely high energy, should be energetically prohibited; an intense steric repulsion works between the peptide bond and the pyrenyl ring in the Δ^ZPyr residue, as shown in Chart 3.

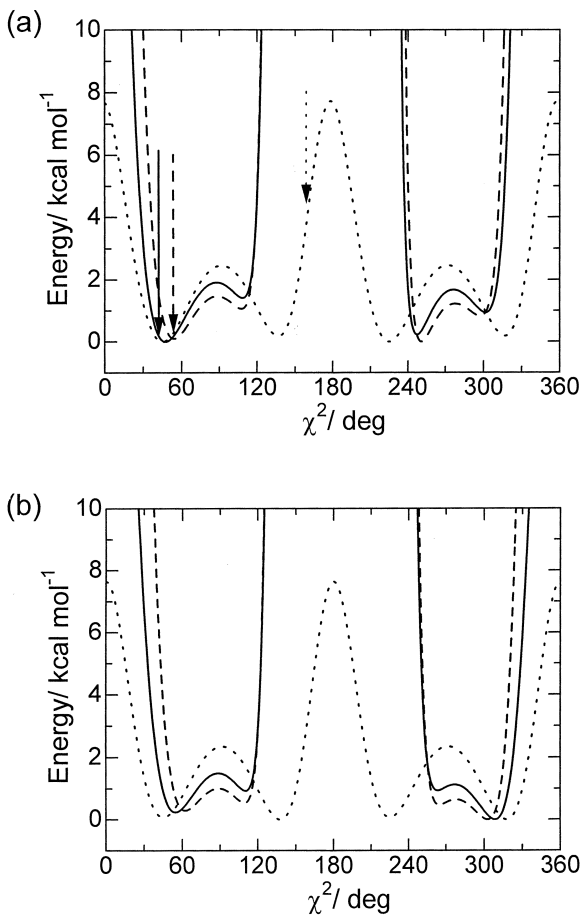

Fig. 2. Dependence of the χ^2 angle of β-aryldehydroalanines on conformational energy of (a) Ac–Ala–X–NHMe and of (b) Ac–X–NHMe: X = Δ^ZPyr (solid line), Δ^ZNap (broken line), and Δ^ZPhe (dotted line). All torsion angles expect for the χ_2^2 angle were set to the experimental values shown in Table 4. The arrows represent the experimental value of peptide **1** (solid arrow), Boc–Ala–Δ^ZNap–Val–OMe (broken arrow),[7] and Boc–Ala–Δ^ZPhe–NHPr (dotted arrow).[2b]

Figure 2a also shows the χ_2^2 dependence on the conformational energy of Ac–Ala–Δ^ZNap–NHMe and of Ac–Ala–Δ^Z-Phe–NHMe; the main chains were set to the X-ray data of Boc–Ala–Δ^ZNap–Val–OMe[7] and Boc–Ala–Δ^ZPhe–NHPr

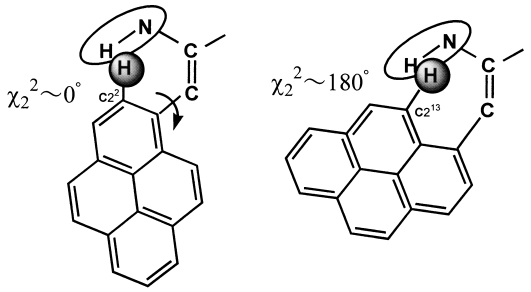

Chart 3.

(NHPr = *N*-2-propyl amide),[2b] respectively. The side-chain freedom of Δ^ZPyr residue was almost the same as that of the Δ^ZNap residue, giving two stable orientations,[7] but much smaller than that of the Δ^ZPhe residue. Obviously, β-substituents larger than the phenyl group tend to give a more restricted orientation of the side chain. The χ_2^2 angle observed for the Δ^ZPyr and Δ^ZNap residues agreed well with one of the stable orientations predicted, while the experimental χ_2^2 angle of the Δ^ZPhe residue was considerably deviated from the stable regions predicted. This might also be ascribed to the side-chain orientation inherent in the Δ^ZPyr and Δ^ZNap residues, having such a large β-aryl group; the orientation should be largely determined by the specific steric repulsion between the peptide bond and the aryl group, as shown in Chart 3. Actually, a similar χ_2^2 dependence on conformational energy was obtained for Ac–Δ^ZPyr–NHMe and Ac–Δ^ZNap–NHMe, in which the preceding Ala residue in Fig. 2a was deleted, as shown in Fig. 2b. The remarkable steric repulsion between the β-substituent and the peptide bond should lead to the conformational preference of the β-substituted dehydroalanines.

A main-chain energy contour map was calculated for Ac–Δ^ZPyr–NHMe in order to know the conformational preference of the Δ^ZPyr residue. As shown in Fig. 3a, the Δ^ZPyr residue favors two stable conformations: one is ($\phi \sim -120^\circ$, $\psi \sim 20^\circ$), corresponding to the ($i + 2$) residue of a type II β-turn; the other is ($\phi \sim -44^\circ$, $\psi \sim -34^\circ$), corresponding to a 3_{10}-helix.[24] Thus, the Δ^ZPyr energy map agrees well with the present experimental results of the type II β-turn for peptide **1**. As shown in Figs. 3b and 3c, a similar energy contour map was obtained for Ac–Δ^ZNap–NHMe[7] and Ac–Δ^ZPhe–NHMe, which essentially resembled the Ac–Δ^ZPhe–NHMe reported by Ajo et al.[14] Therefore, β-aryldehydroalanine tends to show almost the same conformational preference, irrespective of the aromatic size of the β-substituent.

To obtain more information, Table 4 gives the solid-state conformation of di- and tripeptides containing the β-substituted dehydroresidues. The –Y–Δ^ZPhe– and –Y–Δ^ZLeu– segments also induce a type II β-turn. However, it has been pointed out that the torsion angle of the peptide backbone (ϕ, ψ) for the β-turn is significantly influenced by the side-chain size of the *N*-terminal residue (Y).[19a] The β-turn observed for Y = Gly with no side chain and Ala with the smallest side chain was closing to an ideal type II β-turn. The segment of Y = Phe with a bulky side chain induces a distorted type II β-turn, which is responsible for a more prominent steric hindrance between the phenyl group of the Phe and β-substituent of the dehydroresidue (Δ^ZPhe or Δ^ZLeu). The backbone of peptide **1** favors a distorted type II β-turn, although the Y position of peptide **1** is the Ala residue with the smallest side chain. This tendency was also observed for the –Ala–Δ^ZNap– segment.[7]

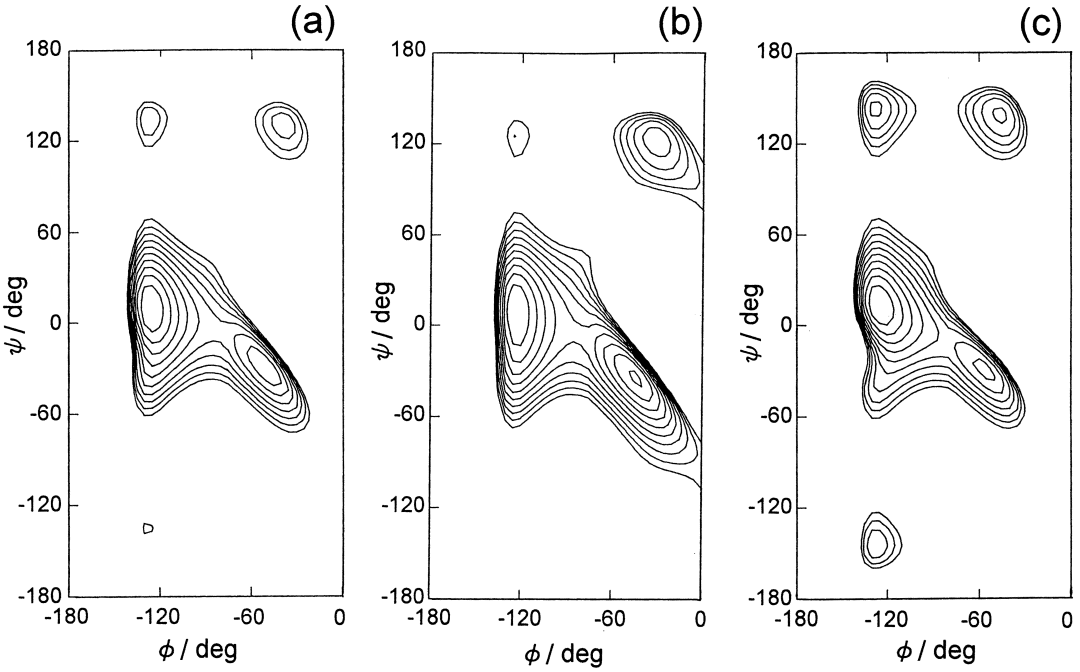

Fig. 3. Main-chain energy contour maps of β-aryldehydroalanines: (a) Ac–Δ^ZPyr–NHMe, (b) Ac–Δ^ZNap–NHMe,[7] and (c) Ac–Δ^Z-Phe–NHMe. The contours are drawn in 0.5 kcal mol^{-1} increments from the minimum point. Only the region of $-180^\circ \leq \phi \leq 0^\circ$ is shown because of the molecular symmetry. In each (ϕ, ψ), the χ^2 angle was taken as the value giving the minimal conformational energy.

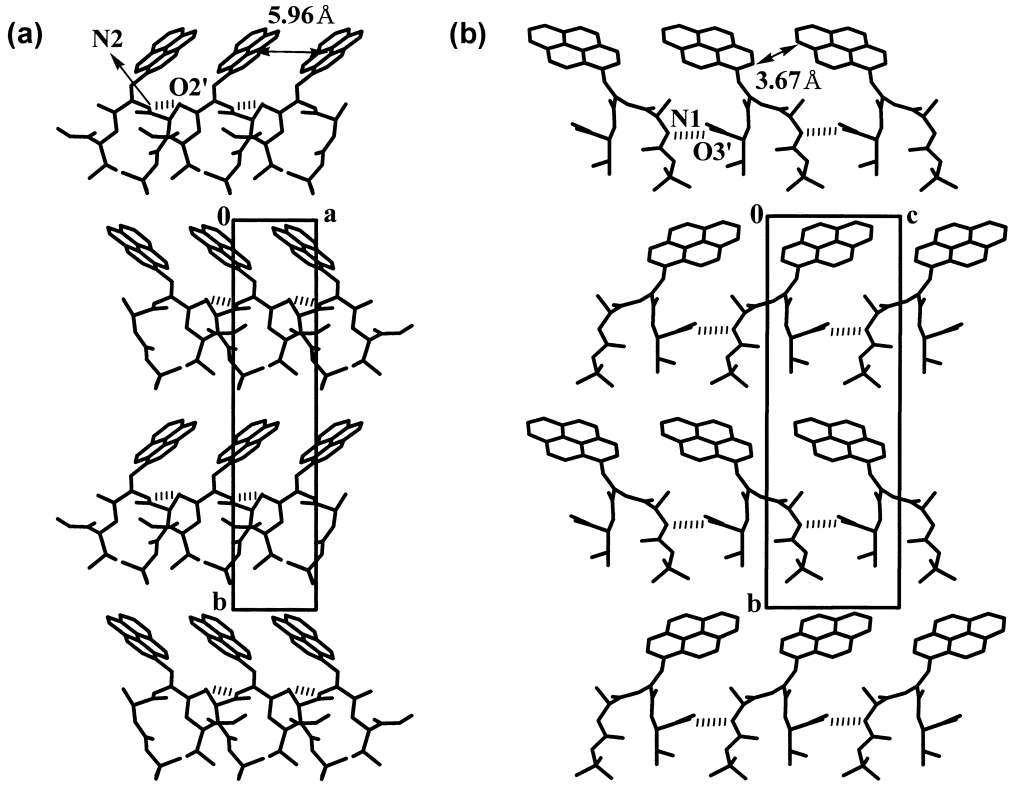

Fig. 4. Crystal packing of peptide **1**: view normal to (a) *ab* and (b) *bc* planes. The dotted lines represent two kinds of intermolecular hydrogen bonds as shown in Table 5.

The preference for a distorted type II β-turn should be characterized by the Δ^ZPyr or Δ^ZNap residue having a large bulky β-substituent, which interacts with the peptide chain to give a remarkable steric repulsion for deviating from an ideal type II β-turn.

Crystal Structure. The crystal structure of peptide **1** is shown in Fig. 4. The hydrogen-bonding and van der Waals parameters are given in Table 5. Here, two hydrogen bonds are present for N2$\cdots$O2′ = 2.856(3) Å and N1$\cdots$O3′ = 2.981(3) Å. The molecular packing is stabilized by the intermolecular hydrogen bonds and van der Waals interactions in Table 5. The pyrenyl rings are arranged in a partially overlapped face-to-face orientation: the shortest center-to-center distance is 5.96 Å between symmetries (x, y, z) and ($x + 1$, y, z), and the edge-to-edge distance is 3.67 Å for C2^2 (x, y, $z - 1$) and C2^{10} (x, y, z). Here, photo-functional pyrenyl groups can be arranged in a specific manner using a crystal structure of novel dehydropeptides. An overview of the crystal structure for peptide **1** is similar to that for peptide **2**,[7] which carries naphthyl groups with a center-to-center distance of 5.97 Å.

Figure 5 shows CD and absorption spectra of peptide **1** in the solid state and in chloroform. Peptide **1** in chloroform showed two intense absorption maxima at around 360 and 280 nm ($\varepsilon_{362} = 2.6 \times 10^4$; $\varepsilon_{287} = 2.1 \times 10^4$; $\varepsilon_{280} = 2.2 \times 10^4$), which are assignable to the Δ^ZPyr residue. For the two bands, marked vibronic patterns characteristic of pyrenyl groups al-

Table 5. Hydrogen-Bond Parameters and Intermolecular van der Waals Contact Distances (Å)

Hydrogen bond	Distance (Å)		Angle (°)	Symmetry[a)]
	N$\cdots$O′	H$\cdots$O′	N–H$\cdots$O′	
N3–H$\cdots$O0′	3.182(4)	2.50(2)	147(2)	x, y, z
N1–H$\cdots$O3′	2.981(3)	2.16(3)	176(3)	$x + 1$, y, $z + 1$
N2–H$\cdots$O2′	2.856(3)	1.90(3)	164(3)	$x + 1$, y, z

van der Waals contact	Distance (Å)[b)]	Symmetry[c)]
O4$\cdots$C2$^\gamma$	3.293(5)	$x - 1$, y, z
O3′$\cdots$C4	3.377(5)	$x + 1$, y, z
O1′$\cdots$C1$^\beta$	3.359(7)	$x - 1$, y, z
O2′$\cdots$C2^2	3.181(6)	$x - 1$, y, z

a) The symmetry operations are applied to O0′ (intramolecular), O2′, and O3′ atoms. b) The van der Waals contacts out to 3.40 Å are listed. c) The symmetry operations are applied to the B atom for A$\cdots$B pairs.

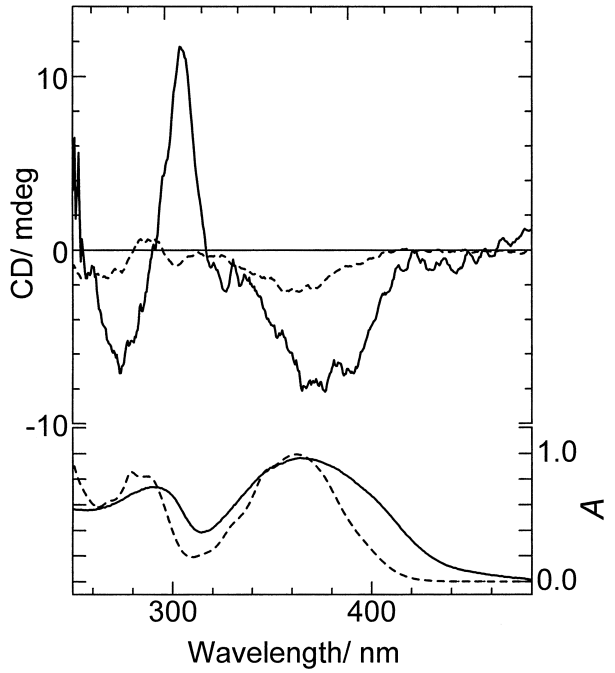

Fig. 5. CD (top) and absorption (bottom) spectra of peptide **1** in the solid state (solid line) and in chloroform (broken line). For both samples, the maximum absorbance around 360 nm was normalized to unity.

most disappeared, suggesting that the presence of the $C^{\alpha}=C^{\beta}$ double bond influences the excited state (more exactly, the Frank–Condon state) of the pyrenyl group significantly. Peptide **1** in the solid state also gave two bands, but the patterns are broadened remarkably. This broadening should arise from the presence of ground-state interactions between the pyrenyl groups that are arranged in a specific manner in the solid state. The ground-state interactions were observed more prominently in the corresponding CD spectrum. Interestingly, peptide **1** in the solid state showed an intense negative signal around 370 nm, and strong exciton couplets with a positive peak at longer wavelengths around 280 nm, while only weak signals were observed in chloroform. The solid-state CD spectrum did not change with every 90° rotation, essentially, not being based on artifacts, such as light scattering. The remarkable CD signals reflect the regular arrangement of pyrenyl groups in the crystal structure (in Fig. 4), since a single peptide **1** forms the same β-turn conformation in the solid state and in chloroform. Therefore, the relative distance and orientation of the pyrenyl groups in the crystal structure can lead to strong excitonic interactions between the pyrenyl groups.

Conclusions

We have attempted here to reveal how the large β-substituent (pyrenyl group) of dehydroresidue affects the structural and conformational preferences of (*Z*)-β-substituted α,β-dehydroalanine. The solid-state conformation was presented for a novel dehydrotripeptide possessing one Δ^{Z}Pyr residue in the second position. The bond lengths and bond angles essentially did not change in Δ^{Z}Pyr and the other dehydroresidues. Like small peptides possessing a Δ^{Z}Nap or Δ^{Z}Phe residue, peptide **1** took a type II β-turn conformation involving an intramolecular hydrogen bond between (*i*)th CO and (i + 3)th NH. Consequently, the conformational preference of the Δ^{Z}Pyr residue should resemble those of the Δ^{Z}Nap and Δ^{Z}Phe residues, being responsible for the non-planarity of the Δ^{Z}Pyr residue to give an extremely high energy in the planar orientation of the pyrenyl group relative to the $C^{\alpha}=C^{\beta}$–C^{γ} plane. Here, the non-planarity should weaken the π-conjugation effect of the pyrenyl group on structural and conformational preferences of the Δ^{Z}-Pyr residue.

The present findings indicate that bulky β-substituent, such as the pyrenyl group, can be arranged regularly along a peptide backbone while retaining a conformational preference characteristic of a conventional dehydroresidue. Thus, β-substituted dehydroalanine will be a unique tool for spatially and regularly arranging β-substituent(s) along the peptide backbone, and for constructing a variety of functional and bioactive peptides.

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