NMR STUDIES OF AUREOBASIDINS A AND E

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The ^{1}H and ^{13}C NMR spectra of aureobasidins A and E were analyzed by a variety of 2D NMR techniques. Two isomers of aureobasidin A existed as an equilibrium mixture in deuteriochloroform. The isomerism was associated with *cis-trans* rotation of the amide bond between *N*-methylphenylalanine and proline. Almost all of the aureobasidin E was found in deuteriochloroform as one conformer; the amide bond between β-hydroxy-*N*-methylphenylalanine and proline was in the *cis*-conformation. Experiments with the NOE made identification of the conformation of the amide bonds of aureobasidins A and E possible.

Aureobasidins,[1] new antifungal antibiotics, were isolated from the culture medium of *Aureobasidium pullulans* R106. The structures of aureobasidins A and E (**1** and **2**, Fig. 1) have been reported in the preceding papers.[2,3] Here, we report the total assignment of the ^{1}H and ^{13}C NMR spectra of **1** and **2** in $CDCl_3$, and the conformation of the amide bonds.

The ^{13}C NMR spectrum of **1** in $CDCl_3$ showed about 1.5 times as many signal peaks as the actual number of carbons (Fig. 2). Compound **1** seemed to be a mixture of two isomers (ratio, 57 : 43 in $CDCl_3$) not separated by purification with HPLC under the various conditions tried. The number of carbon signals in ^{13}C NMR of **2** in $CDCl_3$ coincided with the number of carbons of **2** (Fig. 3). The minor signals in Fig. 3 seemed to originate from a conformational isomer, because in the ^{13}C NMR spectrum of **2** measured at 45°C in $CDCl_3$, the minor signal peaks disappeared almost completely. Therefore, the ^{1}H and ^{13}C NMR spectra of **2** in $CDCl_3$ were assigned first.

Assignment of the ^{1}H and ^{13}C NMR Spectra of **2**

Mild alkaline hydrolysis of **1** gave a linear peptide. The assignment of the ^{1}H and ^{13}C NMR spectra of its acetyl and methyl derivative was described in the preceding paper.[2] By comparison with those results, ^{1}H-^{1}H double quantum filtered (DQF) COSY, ^{1}H-^{13}C COSY, and heteronuclear multiple-bond correlation (HMBC) spectra, the assignments of the ^{1}H and ^{13}C signals of **2** were decided. The results are summarized in Table 1. The ^{1}H-^{13}C correlation map of **2** obtained in the HMBC experiment is shown in Fig. 4. The unusual high-field shift of one of the β-protons (0.61 ppm) of the proline ring seemed to be an anisotropic effect of the amido carbonyl in proline.

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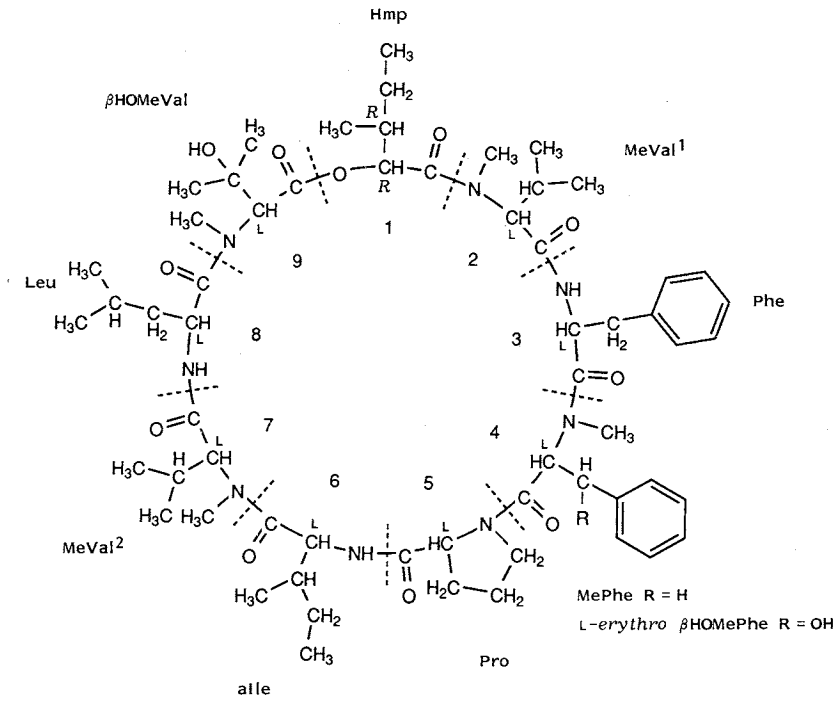
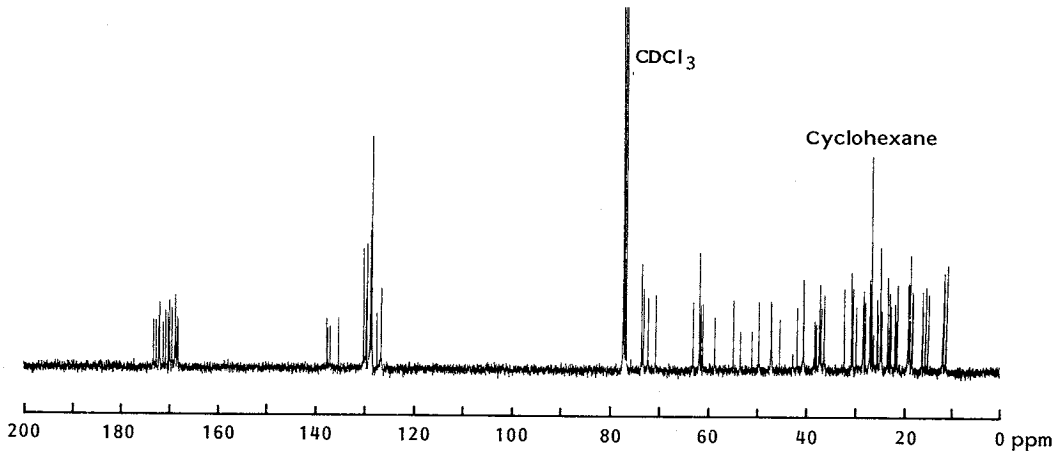
Fig. 1. Structures of aureobasidins A (**1**) and E (**2**).

Hmp: 2-Hydroxy-3-methylpentanoic acid.



Aureobasidin A (**1**) R=H
Aureobasidin E (**2**) R=OH

Fig. 2. ^{13}C NMR spectrum of **1** ($CDCl_3$).



Assignment of the ^1H and ^{13}C NMR Spectra of **1**

The NMR spectrum of **1**, which seemed to exist as an equilibrium mixture of two isomers in $CDCl_3$, was complicated. However, examination of the ^1H and ^{13}C NMR spectra of **1** showed that many of the chemical shift and coupling constant data of **2** were included in the data of **1**. This means that the conformation of one conformer of **1** in $CDCl_3$ is the same as the conformation of **2**. From this

Fig. 3. ^{13}C NMR spectrum of **2** ($CDCl_3$).

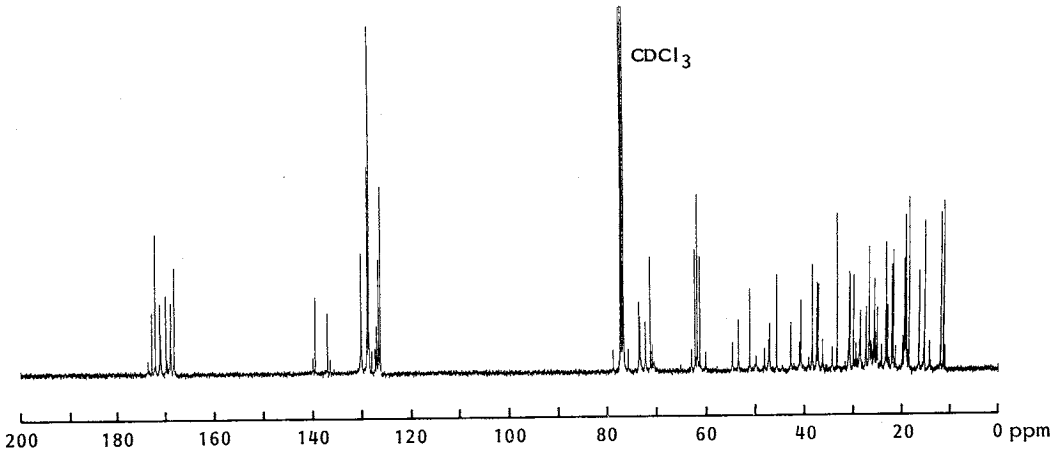


Table 1. ^1H and ^{13}C NMR data for **2** in $CDCl_3$.

Assignment	^1H[a]	^{13}C[b]
Hmp		
CO		168.9 s[c]
2-CH	5.08 s	73.5 d
3-CH	1.66 m[d]	37.3 d
4-CH_2	1.42 m[d], 0.95 m[d]	22.8 t
4-CH_3	0.93 m[d]	16.4 q
5-CH_3	0.76 m[d]	11.3 q
MeVal[1]		
CO		168.0 s
α-CH	4.60 d (11.0)	61.8 d
β-CH	2.10 m[d]	26.6 d
γ-CH_3	0.83 m[d]	19.4 q
γ-CH_3	0.72 d (7.0)	18.4 q
N-CH_3	2.60 s	29.8 q
Phe		
CO		171.9 s
α-CH	5.30 dd (4.0, 10.0)	51.0 d
β-CH_2	3.07 dd (4.0, 14.0), 2.88 dd (10.0, 14.0)	38.3 t
C_γ (ar.)		136.9 s
C_δ (ar.)	7.1 m[d]	130.0 d
C_ε (ar.)	7.1～7.4 m[d]	128.4 d
C_ζ (ar.)	7.3 m	126.5 d
NH	8.13 d (10.0)	
HOMePHe		
CO		168.7 s
α-CH	3.98 d (3.5)	61.2 d
β-CH	5.26 dd (3.5, 9.5)	71.3 d
C_γ (ar.)		139.4 s
C_δ (ar.)	7.1～7.4 m[d]	128.7 d
C_ε (ar.)	7.1～7.4 m[d]	128.4 d
C_ζ (ar.)	7.1～7.4 m[d]	126.1 d
N-CH_3	3.30 s	33.2 q
OH	5.62 d (9.5)	
Pro		
CO		171.0 s
α-CH	3.53 d (7.5)	62.2 d
β-CH_2	2.06 m[d]. 0.61 m	30.6 t
γ-CH_2	1.63 m[d], 1.48 m[d]	21.6 t
δ-CH_2	3.40 m[d], 3.34 m[d]	45.6 t
aIle		
CO		172.6 s
α-CH	4.90 m[d]	53.4 d
β-CH	1.84 m[d]	37.0 d
γ-CH_2	1.32 m[d], 1.11 m	26.6 t
γ-CH_3	1.03 m[d]	15.2 q
δ-CH_3	0.89 m[d]	11.9 q
NH	8.73 d (8.5)	
MeVal[2]		
CO		169.7 s
α-CH	5.21 d (11.0)	61.8 d
β-CH	2.27 m	25.5 d
γ-CH_3	0.91 m[d]	19.1 q
γ-CH_3	0.83 m[d]	19.0 q
N-CH_3	3.17 s	30.7 q
Leu		
CO		171.9 s
α-CH	4.97 m[d]	47.0 d
β-CH_2	1.75 m, 1.32 m[d]	42.7 t
γ-CH	1.52 m[d]	25.0 d
δ-CH_3	0.90 m[d]	23.2 q
δ-CH_3	0.90 m[d]	22.0 q
NH	7.66 d (7.5)	
HOMeVal		
CO		168.2 s
α-CH	3.44 s	72.3 s
β-C		73.3 s
γ-CH_3	1.40 s	28.4 q
γ-CH_3	1.32 s	27.3 q
N-CH_3	3.32 s	40.6 q
OH	4.30 s	

[a] 400 MHz; δ in ppm, *J* in Hz.
[b] 100 MHz; δ in ppm.
[c] Assigned by DEPT experiments.
[d] Overlapping signals.
Hmp: 2-Hydroxy-3-methylpentanoic acid.

Fig. 4. HMBC and NOE experiments of **2**.

⟶ ^{1}H-^{13}C long range coupling detected by HMBC, ⟷ NOE.

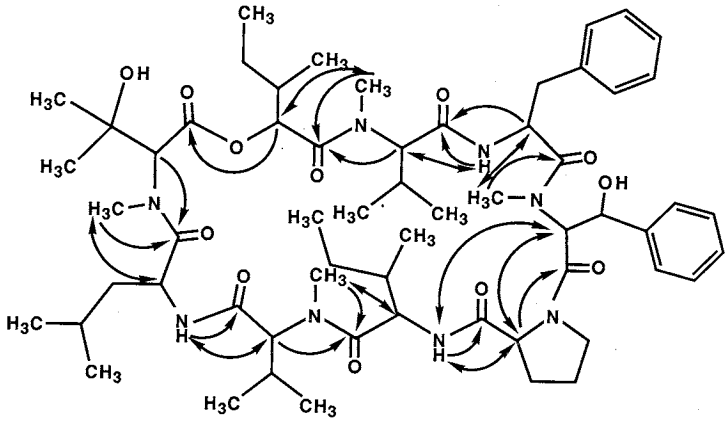


Table 2. ^{1}H and ^{13}C NMR data for conformer A of **1** in $CDCl_3$.

Assignment	^{1}H[a]	^{13}C[b]	Assignment	^{1}H	^{13}C
Hmp			Pro		
CO		168.6 s[c]	CO		171.8 s
2-CH	5.79 s	73.5 d	α-CH	4.80 d (7.5)	63.0 d
3-CH	1.75 m[d]	37.0 d	β-CH_2	2.30 m[d], 1.88 m[d]	28.6
4-CH_2	1.40 m[d], 1.00 m[d]	22.9 t	γ-CH_2	2.06 m[d], 1.85 m[d]	24.7 t
4-CH_3	0.92 m[d]	16.4 q	δ-CH_2	4.22 m[d]. 3.75 m[d]	47.1 t
5-CH_3	0.78 m[d]	11.3 q	aIle		
MeVal[1]			CO		173.1 s
CO		169.3 s	α-CH	4.57 t (9.5)	54.8 d
α-CH	5.01 d (10.5)	61.0 d	β-CH	2.45 m[d]	37.3 d
β-CH	2.02 m[d]	28.1 d	γ-CH_2	1.30 m[d], 1.04 m[d]	25.0 t
γ-CH_3	0.88 m[d]	19.4 q	γ-CH_3	1.18 d (7.5)	15.7 q
γ-CH_3	0.72 m[d]	18.4 q	δ-CH_3	0.91 m[d]	12.0 q
N-CH_3	3.07 s	30.5 q	NH	7.95 d (7.5)	
Phe			MeVal[2]		
CO		170.6 s	CO		169.8 s
α-CH	5.18 m[d]	49.7 d	α-CH	5.29 d (11.5)	61.4 d
β-CH_2	3.20 m[d], 3.05 m[d]	38.3 t	β-CH	2.27 m[d]	25.7 d
C_γ (ar.)		137.5 s	γ-CH_3	0.90 m[d]	18.9 q
C_δ (ar.)	7.2～7.4 m[d]	130.2 d	γ-CH_3	0.85 m[d]	18.9 q
C_ε (ar.)	7.38 m	128.4 d	N-CH_3	3.32 s	30.9 q
C_ζ (ar.)	7.0～7.2 m[d]	126.5 d	Leu		
NH	8.84 d (10.5)		CO		171.8 s
MePhe			α-CH	4.94 m[d]	47.2 d
CO		168.6	β-CH_2	1.79 m[d], 1.32 m[d]	41.9 t
α-CH	3.64 dd (3.0, 12.5)	70.6 d	γ-CH	1.50 m[d]	25.0 d
β-CH	3.25 m[d], 3.15 m[d]	36.4 t	δ-CH_3	0.90 m[d]	21.5 q
C_γ (ar.)		137.7 s	δ-CH_3	0.85 m[d]	23.5 q
C_δ (ar.)	6.49 d (7.5)	128.8 d	NH	7.99 d (7.5)	
C_ε (ar.)	7.0～7.2 m[d]	128.7 d	HOMeVal		
C_ζ (ar.)	7.0～7.2 m[d]	126.6 d	CO		168.0 s
N-CH_3	2.48 s	40.6 q	α-CH	3.40 s	72.2 d
			β-C		73.1 s
			γ-CH_3	1.38 s	28.4 q
			γ-CH_3	1.31 s	27.2 q
			N-CH_3	3.30 s	40.5 q
			OH	4.22 s	

[a] 400 MHz; δ in ppm, J in Hz.
[b] 100 MHz; δ in ppm.
[c] Assigned by DEPT experiments.
[d] Overlapping signals.
Hmp: 2-Hydroxy-3-methylpentanoic acid.

Table 3. ^1H and ^{13}C NMR data for conformer B of **1** in $CDCl_3$.

Assignment	^1H[a]	^{13}C[b]	Assignment	^1H	^{13}C
Hmp			Pro		
CO		168.8 s[c]	CO		171.1 s
2-CH	5.75 s	73.5 d	α-CH	3.75 d (8.0)	61.7 d
3-CH	1.80 m[d]	37.1 d	β-CH_2	2.07 m[d], 0.64 m	30.0 t
4-CH_2	1.40 m[d], 1.00 m[d]	22.8 t	γ-CH_2	1.60 m[d],1.40 m[d]	21.8 t
4-CH_3	0.92 m[d]	16.3 q	δ-CH_2	3.54 m, 3.30 m[d]	45.4 t
5-CH_3	0.78 m[d]	11.3 q	aIle		
MeVal[1]			CO		172.6 s
CO		168.2 s	α-CH	4.97 m[d]	53.4 d
α-CH	4.63 d (11.0)	61.8 d	β-CH	1.67 m[d]	37.4 d
β-CH	2.10 m[d]	26.7 d	γ-CH_2	1.33 m[d], 1.07 m[d]	26.4 t
γ-CH_3	0.78 m[d]	18.5 q	γ-CH_3	0.97 m[d]	15.1 q
γ-CH_3	0.74 m[d]	19.3 q	δ-CH_3	0.87 m[d]	11.9 q
N-CH_3	2.65 s	29.9 q	NH	8.90 (9.0)	
Phe			MeVal[2]		
CO		172.0 s	CO		169.8 s
α-CH	5.22 m[d]	51.0 d	α-CH	5.19 d (11.0)	61.8 d
β-CH_2	3.10 m[d], 2.90 dd (10.0, 14.0)	38.0 t	β-CH	2.28 m[d]	25.5 d
			γ-CH_3	0.90 m[d]	19.2 q
C_γ (ar.)		137.0 s	γ-CH_3	0.85 m[d]	19.1 q
C_δ (ar.)	7.0～7.2 m[d]	129.7 d	N-CH_3	3.17 s	30.7 q
C_ε (ar.)	7.2～7.4 m[d]	128.4 d	Leu		
C_ζ (ar.)	7.2～7.4 m[d]	126.5 d	CO		171.8 s
NH	7.97 d (10.5)		α-CH	4.94 m[d]	47.2 d
MePhe			β-CH_2	1.79 m[d], 1.32 m[d]	41.9 t
CO		170.1 s	γ-CH	1.50 m[d]	25.0 d
α-CH	4.43 dd (6.0, 10.5)	58.6 d	δ-CH_3	0.90 m[d]	22.0 q
β-CH	3.30 m[d], 3.15 m[d]	36.4 t	δ-CH_3	0.88 m[d]	23.1 q
C_γ (ar.)		135.3 d	NH	7.59 d (7.5)	
C_δ (ar.)	7.0～7.2 m[d]	129.5 d	HOMeVal		
C_ε (ar.)	7.2～7.4 m[d]	128.9 d	CO		168.0 s
C_ζ (ar.)	7.2～7.4 m[d]	127.4 d	α-CH	3.40 s	72.2 d
N-CH_3	3.14 s	32.3 q	β-C		73.1 s
			γ-CH_3	1.40 s	28.6 q
			γ-CH_3	1.31 s	27.2 q
			N-CH_3	3.30 s	40.5 q
			OH	4.22 s	

[a] 400 MHz; δ in ppm, *J* in Hz.
[b] 100 MHz; δ in ppm.
[c] Assigned by DEPT experiments.
[d] Overlapping signals.
Hmp: 2-Hydroxy-3-methylpentanoic acid.

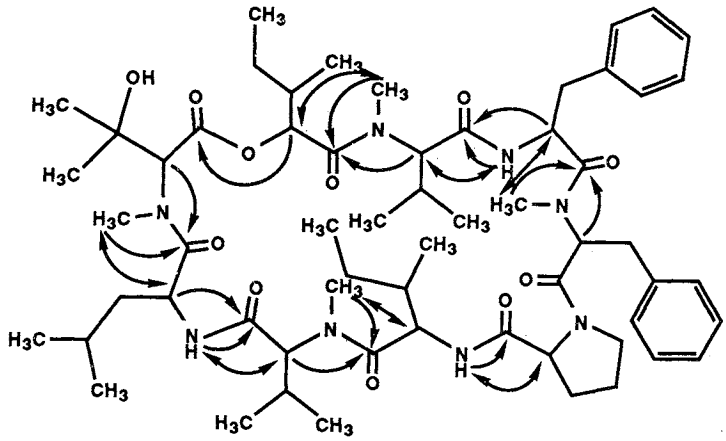
information, the assignment of the ^1H and ^{13}C NMR spectra of **1** was decided by use of the same NMR methods as described for **2**. The ^1H and ^{13}C NMR spectral data of the two conformers, A and B, of **1** are summarized in Tables 2 and 3. The ^1H-^{13}C correlation maps of conformers A and B of **1** obtained in the HMBC experiment are shown in Fig. 5. One of the β-protons (0.64 ppm) in the proline ring of conformer B shifted to a higher field, as in **2**. This indicated that the conformation of conformer B was similar to that of **2**. The reason why the δ-protons (6.49 ppm) of the aromatic ring in *N*-methylphenylalanine (MePhe) in conformer A shift to a higher field seemed to be an anisotropic effect of the amido carbonyl in phenylalanine.

Chemical Shift of γ-Carbon of Proline in **1** and **2**

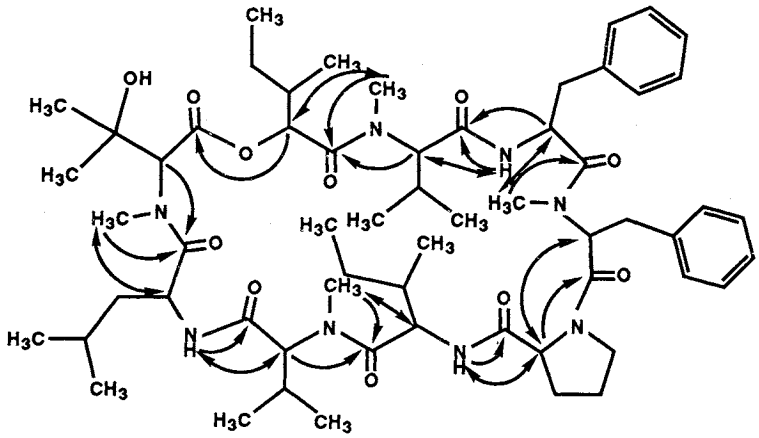
The chemical shift of the γ-carbon of the proline ring in a peptide gives information about the stereochemistry of the amino acid-proline bond. When the amide bond is in the *trans*-conformation, the chemical shift of the γ-carbon of proline is 24～25 ppm. The analogous signal of the *cis*-conformation

Fig. 5. HMBC and NOE experiments of **1**.

⟶ ^{1}H-^{13}C long range coupling detected by HMBC, ⟷ NOE.



Conformer A



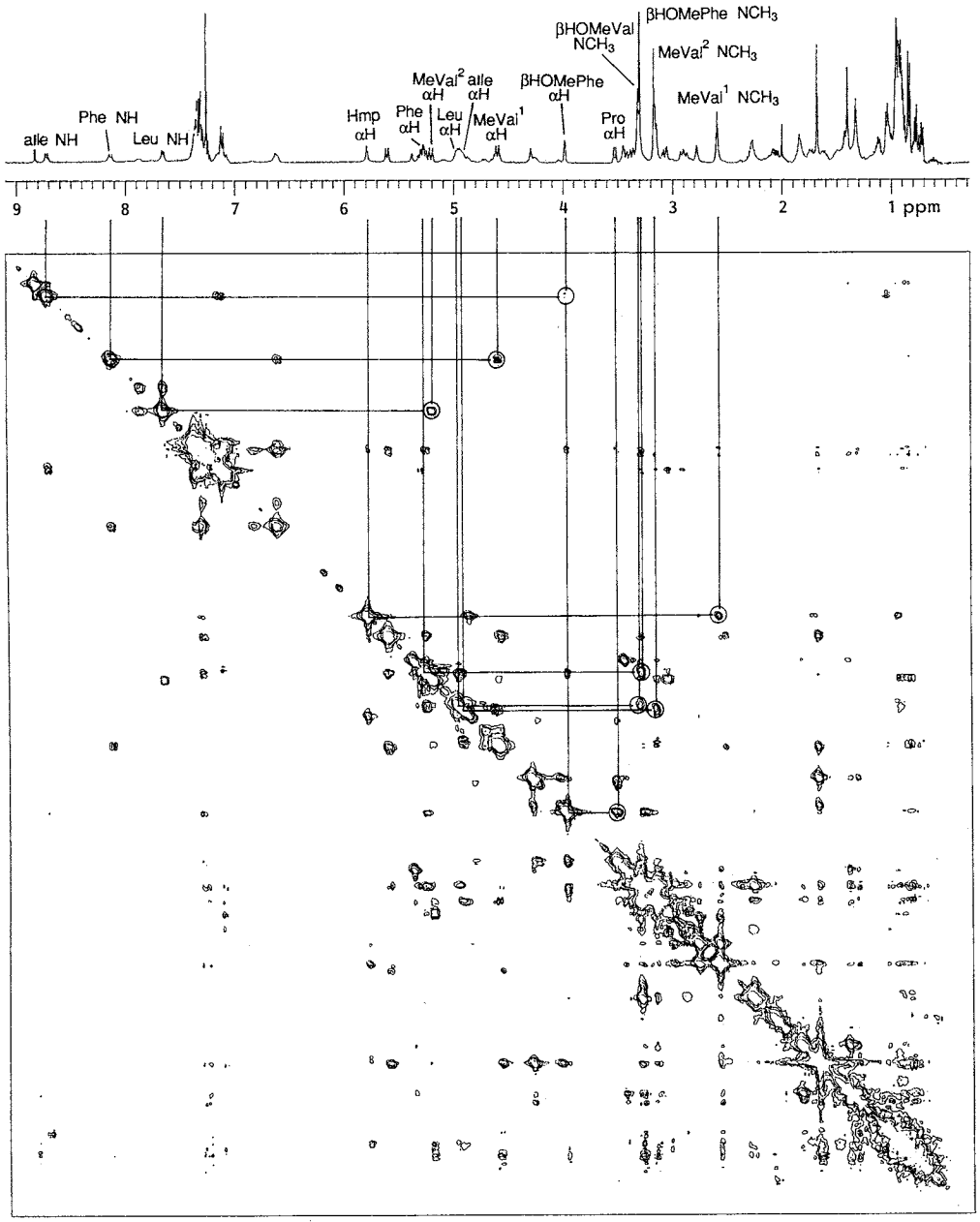
Conformer B

occurs at 22~23 ppm.[4] This principle was applied to analyze the ^{13}C NMR data of **1** and **2**. The amide bond between the β-hydroxy-*N*-methylphenylalanine (βHOMePhe) and the proline of **2** was in the *cis*-conformation because the chemical shift of the γ-carbon in the proline ring was 21.6 ppm. The amide bond between the MePhe and the proline of conformer A in **1** was in the *trans*-conformation because the chemical shift of the γ-carbon in the proline ring was 24.7 ppm; the amide bond of conformer B was in the *cis*-conformation because the chemical shift was 21.8 ppm. These results were consistent with the ^{1}H and ^{13}C NMR spectral data of conformer B being almost the same as those of **2**. Consequently, the isomerism of **1** was associated with *cis-trans* rotation of the amide bond between MePhe and proline.

NOE Experiments of **1** and **2**

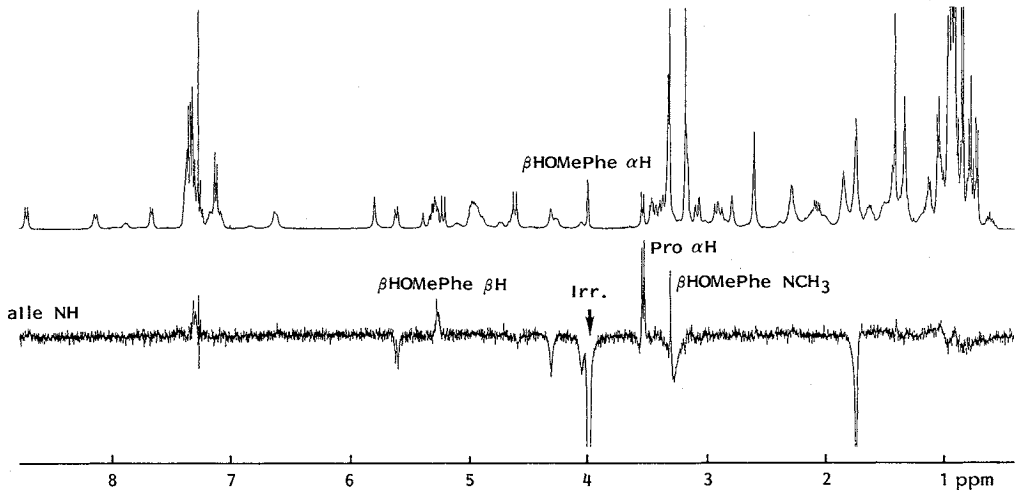
The NOESY and rotating-frame nuclear Overhauser effect spectroscopy (ROESY) spectra of **1** and **2** were measured in $CDCl_3$. The NOESY spectrum of **2** is shown in Fig. 6. The results of **2** showed that

Fig. 6. NOESY spectrum of **2**.



all of the amide bonds except those between βHOMePhe and proline and between proline and alloisoleucine (aIle) were in the *trans*-conformation because NOEs between NH or NCH_3 and the α-protons of the immediately preceding amino acids in the molecule were observed. The amide bond between βHOMePhe and proline was in the *cis*-conformation because NOEs between α-protons of these adjacent amino acid residues were observed. The results of **1** showed that the results of conformer B were similar to those of **2**, and that the amide bond between the MePhe and the proline of conformer B was in the *cis*-conformation.

Fig. 7. NOE difference spectrum of **2**.



The results of the conformer A did not show NOEs between the α-protons of MePhe and proline. Consequently, the conformation of the amide bond was deduced to be *trans*.

The NOE difference spectra were measured to check these results. The NOE difference spectra of **2** is shown in Fig. 7. Irradiation of the α-proton (3.98 ppm) of the βHOMePhe in **2** resulted in NOEs at the α-proton (3.53 ppm) of proline and the NH-proton (8.73 ppm) of aIle. Irradiation of the α-proton of proline in **2** resulted in NOEs at the α-proton of βHOMePhe and the NH-proton of aIle. Irradiation of the α-proton (3.75 ppm) of the proline in conformer B in **1** resulted in NOEs at the α-proton (4.43 ppm) of MePhe and the NH-proton (8.90 ppm) of aIle. Irradiation of the α-proton (4.80 ppm) of the proline in conformer A in **1** did not result in NOEs at the α-proton (3.64 ppm) of MePhe, but gave NOEs at the NH-proton (7.95 ppm) of aIle. (The results of the NOE measurements of **1** and **2** are shown in Figs. 5 and 4.)

From these results, the conformation of all amide bonds of **2** except the amide bond between βHOMePhe and proline was *trans*. The conformation of the amide bond between the βHOMePhe and the proline of **2** was *cis*. The conformation of all amide bonds in conformer A was *trans*. All amide bonds in conformer B had the same conformation as those of **2**.

The reason why **2** exists as a single isomer in $CDCl_3$ seems to be that the hydroxyl group in βHOMePhe forms a hydrogen bond to the amido carbonyl in βHOMePhe, so the *cis*-conformation of the amide bond between the βHOMePhe and the proline is fixed.

Experimental

The NMR spectra were recorded on a Jeol JNM-GX400 spectrometer equipped with a 5-mm $^1H/^{13}C$ dual probe head.

^1H NMR

The 400 MHz ^1H NMR spectra were observed at 399.65 MHz under the following conditions: spectrum width 8,000 Hz, data points 32 K, pulse flip angle 45°, pulse repetition time 4 seconds, and probe temperature 24°C.

^1H-^1H DQF COSY

The DQF COSY spectra resulted from a 256 × 1,024 data matrix, zero-filled in the F1 dimension to

yield a 512×512 data matrix for the 2D spectrum. The spectrum widths were 3,491.6 and 3,385.2 Hz for **1** and **2**, respectively, in both dimensions.

^{13}C and ^{1}H-^{13}C COSY

The ^{13}C NMR and ^{1}H-^{13}C COSY spectra were observed at 100.40 MHz under the following conditions: for the 1D NMR spectrum, spectrum width 23,041.5 Hz, data points 32 K, pulse flip angle 45°, pulse repetition time 1.711 seconds, and a digital resolution of 1.41 Hz; and for the 2D NMR spectrum, spectral widths of 14,005.6 and 12,755.1 Hz for **1** and **2**, respectively, in 2 K data points (^{13}C resonance), 128 FIDs (zero-filled to 256, ^{1}H resonance), and repetition time 1.6 seconds.

HMBC

The HMBC spectra were observed at 399.65 MHz, with spectrum widths of 3,501.4 and 3,385.2 Hz for **1** and **2**, respectively, in F2 (^{1}H resonance), data points 1 K, 20 KHz in F1 (^{13}C resonance), 256 FIDs (zero-filled to 512), and $\Delta 1$ and $\Delta 2$ durations of 3.7 and 60.0 mseconds, respectively.

NOESY

The NOESY spectra were measured at 399.65 MHz under the following conditions: spectrum width in both dimensions of 3,745.3 and 3,531.1 Hz, 256 FIDs (zero-filled to 512) containing 1 K data in the F2 dimension, and mixing times 800 and 500 mseconds for **1** and **2**, respectively.

ROESY

ROESY spectra of **1** and **2** were obtained with the pulse sequence $90°-t_1-(\tau_P-\tau_I-)_n-t_2$ by use of a mixing sequence $(\tau_P \cdot \tau_I)_n$ with $\tau_P = 30° = 12.0$ μseconds, $\tau_I = 8.5$ μseconds, and $n = 2{,}032$. Total mixing time was 250 mseconds, and 256 t_1-values with 32 scans each were recorded: 1 K data points in the F2 dimension, spectral width in both dimensions 5,000 Hz, zero-filling to 1 K in the F1 dimension.

NOE Difference Spectrum

NOE difference spectra of **1** and **2** were taken with a preirradiation of 5 seconds at the frequency of the selective proton signal. A set of one pulse sequence alternately acquired with the irradiation RF power of off and on. The resulting FIDs were subtracted and then accumulated for 128 times. The FID obtained thus was Fourier-transformed. The sample was heated at 40°C for about 5 minutes before the NOE difference spectra were taken.

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