STRUCTURES OF AUREOBASIDINS B TO R

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Structures of 17 minor forms of aureobasidins (Abs), Abs B~R, were elucidated by mass fragmentation and amino acid analysis. The fragmentation patterns by FAB-MS spectroscopy of Abs A~E seemed to follow predictable rules, so we used the rules to elucidate the 13 other Abs. All Abs consisted of eight amino acids and one hydroxy acid.

A new antifungal antibiotic, aureobasidin (Ab) A (Fgi. 1),[1] has been isolated from the fermentation broth of *Aureobasidium pullulans* R106. This strain also produces many antifungal antibiotics with structures that might be related to Ab A. We isolated 17 of these antibiotics and named them Abs B~R.[1] The structures of Abs B~E were elucidated by chemical degradation. Acid hydrolysis of Abs B~E gave amino acids and hydroxy acids. These amino acids were analyzed by HPLC or NMR. The structures of the hydroxy acids were confirmed by comparison with synthetic compounds. Alkaline hydrolysis of Abs B~E gave linear chain peptides. The fragmentation patterns of their derivatives by FAB-MS were used to deduce the amino acid sequences, from which the structures of Abs B~E were identified. We found that the fragmentation patterns by FAB-MS of Abs A~E seemed to follow predictable rules, so we applied the rules to elucidate the structure of the other Abs. The structures of Abs A~R are shown in Fig. 1.

Structure of Ab B

Ab B had a molecular ion peak at *m/z* 1,087 (M+H) by FAB-MS; this peak was 14 daltons smaller than that of Ab A. The acid hydrolysate (6N HCl, 110°C, 22 hours) of Ab B contained 0.6 mol of β-hydroxy-*N*-methylvaline (βHOMeVal), 2 mol of *N*-methylvaline (MeVal), 1 mol each of proline, alloisoleucine (aIle), *N*-methylphenylalanine (MePhe), leucine, and phenylalanine, and 0.3 mol of methylamine. This result was obtained by amino acid autoanalysis and HPLC analysis with a strong cation-exchange resin column (detection by fluorescence; color development with *o*-phthalaldehyde-NaClO).[2] The hydroxy acid was purified from the hydrolysate by Dowex 50W. By NMR, its structure was found to be 2-hydroxyisovaleric acid (Hiv). Hiv from Ab B coincided with the *R* form of the synthetic Hiv[3] on chiral HPLC (Chiralpak WH, Daicel Chemical Industries, Ltd.). Thus, Ab B had the same amino acids as Ab A, and Hiv instead of the 2-hydroxy-3-methylpentanoic acid (Hmp) of Ab A.

Alkaline hydrolysis (0.25N NaOH in aqueous MeOH, room temperature, 15 hours) of Ab B gave **B1** and **B2**. By FAB-MS, the MW of **B1** was 1,046 and that of **B2** was 1,068. Compound **B1** was methylated to give **B1a**. The acid hydrolysate of **B1a** contained sarcosine (Sar) instead of the βHOMeVal and methylamine found in the acid hydrolysate of Ab B.

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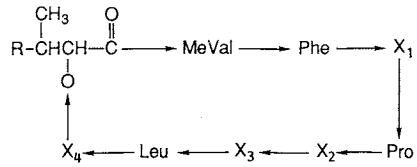
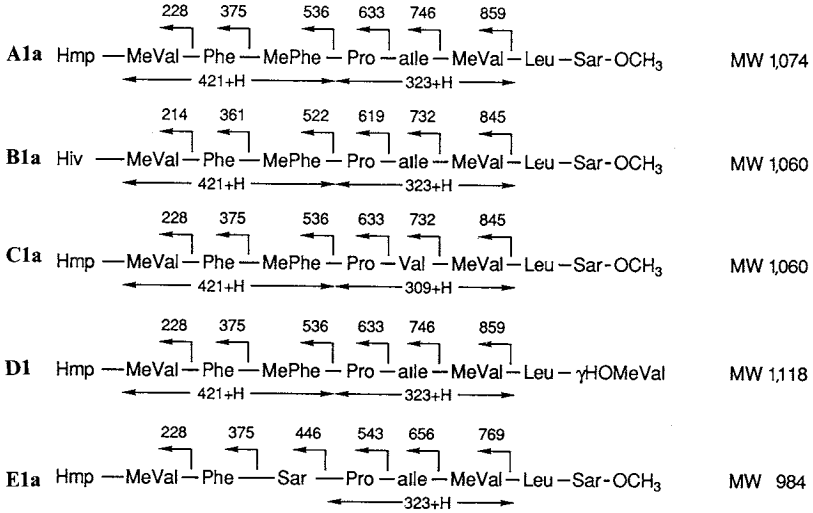
Fig. 1. Structures of aureobasidins (Abs) A~R.



Compound	R	X_1	X_2	X_3	X_4
Ab A	C_2H_5	MePhe	aIle	MeVal	βHOMeVal
Ab B	CH_3	MePhe	aIle	MeVal	βHOMeVal
Ab C	C_2H_5	MePhe	Val	MeVal	βHOMeVal
Ab D	C_2H_5	MePhe	aIle	MeVal	γHOMeVal
Ab E	C_2H_5	βHOMePhe	aIle	MeVal	βHOMeVal
Ab F	C_2H_5	MePhe	aIle	Val	βHOMeVal
Ab G	C_2H_5	MePhe	aIle	MeVal	MeVal
Ab H	C_2H_5	MePhe	aIle	MeVal	Val
Ab I	C_2H_5	MePhe	Leu	MeVal	βHOMeVal
Ab J	C_2H_5	MePhe	aIle	MeVal	*N*,βMeAsp
Ab K	CH_3	MePhe	aIle	MeVal	MeVal
Ab L	C_2H_5	MePhe	Val	MeVal	MeVal
Ab M	C_2H_5	Phe	aIle	MeVal	MeVal
Ab N	C_2H_5	MePhe	aIle	MeVal	$DH_{3,4}$MeVal
Ab O	C_2H_5	MePhe	aIle	MeVal	βHOMePhe
Ab P	C_2H_5	MePhe	aIle	Val	MeVal
Ab Q	C_2H_5	MePhe	aIle	MeVal	MePhe
Ab R	C_2H_5	βHOMePhe	aIle	MeVal	MeVal

Fig. 2. Mass fragmentation of **A1a**, **B1a**, **C1a**, **D1**, and **E1a**.

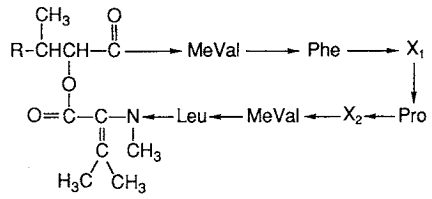


The structure of **B1a** (Fig. 2) was elucidated by comparison of its fragment ion peaks in FAB-MS with the peaks of **A1a**, the methyl ester of **A1**, which was a hydrolysate of Ab A cleaved to form a linear chain.[2] The fragment ion peaks of **B1a** were observed at *m/z* 214, 361, 522, 619, 732, and 845, each peak of which was 14 daltons smaller than the corresponding peak of **A1a**. Fragment ion peaks at *m/z* 422 and

324 were detected in both **A1a** and **B1a**; they seemed to be the tripeptides MeVal-Phe-MePhe (+H) and Pro-aIle-MeVal (+H), respectively. Therefore, the *N*-terminal component might be 14 daltons smaller than Hmp. Hiv was this size. So, the structure of **B1a** was deduced to be that shown in Fig. 2. aIle, MeVal, and leucine were not distinguishable by mass spectra. The sequence containing these three amino acids was arranged by reference to the structure of **A1a**.

As mentioned above, **B1** corresponded to **A1** having Sar at the *C*-terminal, and the Sar of **A1** was derived from βHOMeVal of Ab A. Ab B was found also to have βHOMeVal by HPLC analysis, so the Sar of **B1** was the decomposed product of βHOMeVal in Ab B. Therefore, Ab B was as shown in Fig. 1.

Fig. 3. Structures of **A2**, **B2**, **C2**, **E2**, and **E3**.



Compound	R	X_1	X_2
A2	C_2H_5	MePhe	aIle
B2	CH_3	MePhe	aIle
C2	C_2H_5	MePhe	Val
E2	C_2H_5	Sar	aIle
E3	C_2H_5	βHOMePhe	aIle

The structure of **B2** was deduced by the amino acid composition and the MW. We found that the Hmp of **A2**,[2] in which βHOMeVal was changed to 2,3-didehydro-*N*-methylvaline ($DH_{2,3}$MeVal) by alkaline treatment of Ab A, was changed in **B2** to Hiv (Fig. 3).

Structure of Ab C

Ab C showed a molecular ion peak by FAB-MS at *m/z* 1,087 (M+H); this peak was the same as that of Ab B. The acid hydrolysate of Ab C contained 0.6 mol of βHOMeVal, 2 mol of MeVal, 1 mol each of proline, valine, MePhe, leucine, and phenylalanine, and 0.3 mol of methylamine. The hydroxy acid was found to be 2*R*,3*R*-Hmp by chiral HPLC. Thus, the difference between Ab C and A was valine and aIle.

Ab C was hydrolyzed in alkaline solution to give **C1** and **C2**, which had the MW of 1,046 and 1,068, respectively, by FAB-MS. Compound **C1** was methylated to give **C1a**. The acid hydrolysate of **C1a** contained Sar instead of the βHOMeVal and methylamine found in the acid hydrolysate of Ab C. The structure of **C1a** was elucidated by FAB-MS fragmentation. The fragment ion peaks of **C1a** were observed at *m/z* 228, 375, 536, 633, 732, and 845. The peaks at *m/z* 228 to 633 were in exactly the same pattern as **A1a**, but the peaks at *m/z* 732 and 845 were 14 daltons smaller than those of **A1a**. The fragment ion peak at *m/z* 422, which we assigned as MeVal-Phe-MePhe (+H) in **A1a**, was also detected in **C1a**. However, in **C1a**, the peak at *m/z* 324 that arose from Pro-aIle-MeVal (+H) in **A1a** was not detected, and another peak appeared at *m/z* 310. Therefore, the aIle of **A1a** seemed to be a valine in **C1a** (Fig. 2). In this way, the structure of Ab C was found to be as shown in Fig. 1.

It was deduced that the aIle of **A2** was changed to valine in **C2** (Fig. 3).

Structure of Ab D

Ab D had a molecular ion peak at *m/z* 1,101 (M+H) by FAB-MS; the peak was the same as that of Ab A. The acid hydrolysate of Ab D contained an undefined compound, 2 mol of MeVal, and 1 mol each of proline, aIle, leucine, MePhe, and phenylalanine. The undefined compound was purified by Dowex 50W and it was found to be γ-hydroxy-*N*-methylvaline (γHOMeVal)[4] by NMR and FAB-MS. The hydroxy acid component was purified and found by chiral HPLC to be 2*R*,3*R*-Hmp.

Alkaline hydrolysis of Ab D gave only compound **D1**, which had the MW of 1,118 by FAB-MS.

Treatment of **D1** with diazomethane gave **D1a**, which was 18 daltons smaller than **D1**. The fragmentation patterns of **D1** (Fig. 2) and **D1a** were very similar. Both spectra had ion peaks at *m/z* 228, 375, 536, 633, 746, and 859, which were the same as the peaks of **A1a**. Fragment ion peaks at *m/z* 422 and 324, which we assigned as MeVal-Phe-MePhe (+H) and Pro-aIle-MeVal (+H), respectively, in **A1a**, were both detected in both **D1** and **D1a**. So, the Sar methyl ester of **A1a** seemed to be replaced by γHOMeVal in **D1**. βHOMeVal was decomposed to Sar by alkaline hydrolysis, but γHOMeVal was not. The ^{1}H NMR spectrum of **D1a** showed the absence of a methoxy proton. The IR spectrum (1790 cm^{-1}) of **D1a** showed the presence of γ-lactone. Therefore, the *C*-terminal of **D1** was γHOMeVal, and it was cyclized by elimination of MeOH from the methyl ester derived with diazomethane treatment to form the γ-lactone. Thus, the structure of Ab D was that shown in Fig. 1.

Structure of Ab E

Ab E had a molecular ion peak at *m/z* 1,117 (M+H) by FAB-MS; this peak was 16 daltons larger than that of Ab A. Amino acid autoanalysis of the acid hydrolysate of Ab E showed that the amino acid composition was 1 mol each of proline, aIle, leucine, and phenylalanine. The hydrolysate was analyzed by HPLC, and 0.5 mol of βHOMeVal, 2 mol of MeVal, and 1 mol of methylamine were detected. The acid hydrolysate was chromatographed on a Dowex 50W column to isolate Hmp, βHOMeVal, MeVal, proline, aIle, leucine, phenylalanine, and an undefined compound. The undefined compound was purified by reversed-phase HPLC and gave two compounds at about the weight ratio of 7 (**E5**) and 3 (**E6**). They were identified as isomers of β-hydroxy-*N*-methylphenylalanine (βHOMePhe)[5] by NMR and FAB-MS. From CD analysis, **E5** showed $[\theta]_{220}+7{,}714°$ and **E6** showed $[\theta]_{220}+7{,}803°$ in 0.5 N HCl, and they both seemed to be in the L form.[6] Compounds **E5** and **E6** were compared with DL-*threo*- and DL-*erythro*-βHOMePhe synthesized from DL-*threo*-β- and DL-*erythro*-β-hydroxyphenylalanine,[7] respectively. The ^{1}H NMR spectra of **E5** and **E6** coincided with those of DL-*threo*- and DL-erythro-βHOMePhe, respectively. Thus, the configurations of βHOMePhe of **E5** and **E6** were identified as L-*threo* (2*S*,3*R*) and L-*erythro* (2*S*,3*S*), respectively. ^{13}C NMR data of Ab E[8] suggested that Ab E was a mixture of conformational isomers, but not a mixture of diastereoisomers. Ab E was oxidized with Jones reagent, followed by reduction by sodium borohydride, to give Ab E and Ab E isomer. Ab E isomer had the MW of 1,116 by FAB-MS, which value was the same as that of Ab E. The fragmentation patterns of Ab E and Ab E isomer were very similar. The analysis of chiral HPLC[2] of the acid hydrolysate of Ab E isomer gave about the same results as those of Ab E. From these results, Ab E isomer seemed to be the stereosiomer of the β-position of βHOMePhe in Ab E. Feeding experiments with DL-*threo*-β- and DL-*erythro*-β-hydroxyphenylalanine in the culture medium of *Aureobasidium pullulans* R106 were done. The experiment with DL-*threo*-β-hydroxyphenylalanine gave Ab E and Ab E isomer, but that with DL-*erythro*-β-hydroxyphenylalanine gave only Ab E. These results will be reported in detail elsewhere. The ratio of L-*threo*- and L-*erythro*-βHOMePhe in the acid hydrolysate of Ab E isomer was 24:1. Therefore, the configurations of βHOMePhe in Ab E and Ab E isomer were assigned to be L-*erythro* (2*S*,3*S*) and L-*threo* (2*S*,3*R*), respectively. All other amino acids were found by chiral HPLC to be in the L form.[2] The hydroxy acid of Ab E was identified as 2*R*,3*R*-Hmp by chiral HPLC. Thus, the MePhe of Ab A was the βHOMePhe in Ab E.

Ab E was hydrolyzed in alkaline solution to give **E1**, **E2**, and **E3**. Compound **E1** was treated with diazomethane to give **E1a**. By FAB-MS, the MW of **E1** was 970, that of **E1a** was 984, that of **E2** was 992, and that of **E3** was 1,098. The acid hydrolysate of **E1a** contained 2 mol each of MeVal and Sar, and

1 mol each of proline, aIle, leucine, and phenylalanine. Fragment ion peaks of **E1a** (Fig. 2) were observed at *m/z* 228, 375, 446, 543, 656, 769, and 882. So, the MePhe of **A1a** might change to Sar in this compound. βHOMePhe and βHOMeVal of Ab E were decomposed to Sar in **E1a**, and the structure of Ab E was therefore as shown in Fig. 1. The structures of **E2** and **E3** were deduced by the amino acid compositions and mass fragmentations; the MePhe of **A2** was changed in **E2** and **E3** to Sar and βHOMePhe, respectively (Fig. 3).

Structures of Abs F~R

The fragmentation of Abs A~E observed by FAB-MS is depicted in Fig. 4. The fragment ion peaks at *m/z* 210, 324, 391, 485, 518, 665, and 1,101 of Ab A and the peaks at *m/z* 324, 393, 575, 1,011, and 1,117 of Ab E were assigned by linked-scan spectra (data not shown) in FAB-MS. On the basis of the assignments and comparisons of the fragmentation patterns for Abs A~E, Fig. 4 was made. Many fragments were common to all five compounds, and they were made use of in the elucidation of the structures of Abs F~R.

The molecular formulas of Abs B~R were found by HRFAB-MS (Table 1), and their amino acids were identified by amino acid autoanalysis and HPLC analysis of their acid hydrolysates (Table 2). From these results and by the comparison of their FAB-MS fragment ion peaks (Fig. 5) with the fragmentation patterns of Abs A~E (Fig. 4), the structures of Abs F~R were elucidated.

The acid hydrolysate of Ab F contained 1 mol each of βHOMeVal, MeVal, proline, valine, aIle,

Fig. 4. Mass fragmentation of aureobasidins (Abs) A~E.

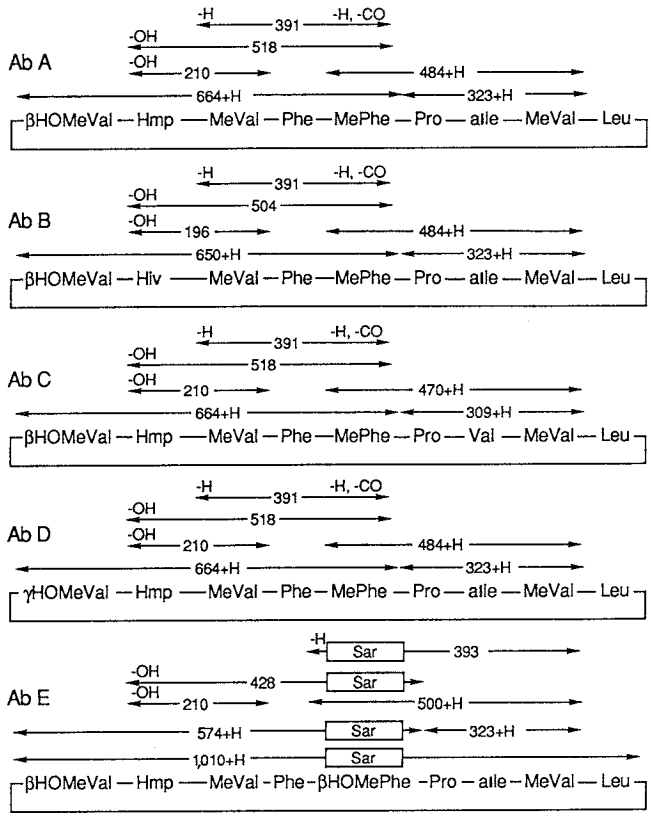


Table 1. HRFAB-MS data of aureobasidins (Abs) B~R.

Compound	Found m/z $(M+H)^+$	Calcd (M+H)	Molecular formula	Compound	Found m/z $(M+H)^+$	Calcd (M+H)	Molecular formula
Ab B	1,087.679	1,087.681	$C_{59}H_{90}N_8O_{11}$	Ab K	1,071.685	1,071.686	$C_{59}H_{90}N_8O_{10}$
Ab C	1,087.680	1,087.681	$C_{59}H_{90}N_8O_{11}$	Ab L	1,071.687	1,071.686	$C_{59}H_{90}N_8O_{10}$
Ab D	1,101.700	1,101.696	$C_{60}H_{92}N_8O_{11}$	Ab M	1,071.682	1,071.686	$C_{59}H_{90}N_8O_{10}$
Ab E	1,117.693	1,117.691	$C_{60}H_{92}N_8O_{12}$	Ab N	1,083.682	1,083.686	$C_{60}H_{90}N_8O_{10}$
Ab F	1,087.681	1,087.681	$C_{59}H_{90}N_8O_{11}$	Ab O	1,149.698	1,149.696	$C_{64}H_{92}N_8O_{11}$
Ab G	1,085.698	1,085.701	$C_{60}H_{92}N_8O_{10}$	Ab P	1,071.685	1,071.686	$C_{59}H_{90}N_8O_{10}$
Ab H	1,071.686	1,071.686	$C_{59}H_{90}N_8O_{10}$	Ab Q	1,133.701	1,133.701	$C_{64}H_{92}N_8O_{10}$
Ab I	1,101.697	1,101.696	$C_{60}H_{92}N_8O_{11}$	Ab R	1,101.697	1,101.696	$C_{60}H_{92}N_8O_{11}$
Ab J	1,115.681	1,115.676	$C_{60}H_{90}N_8O_{12}$				

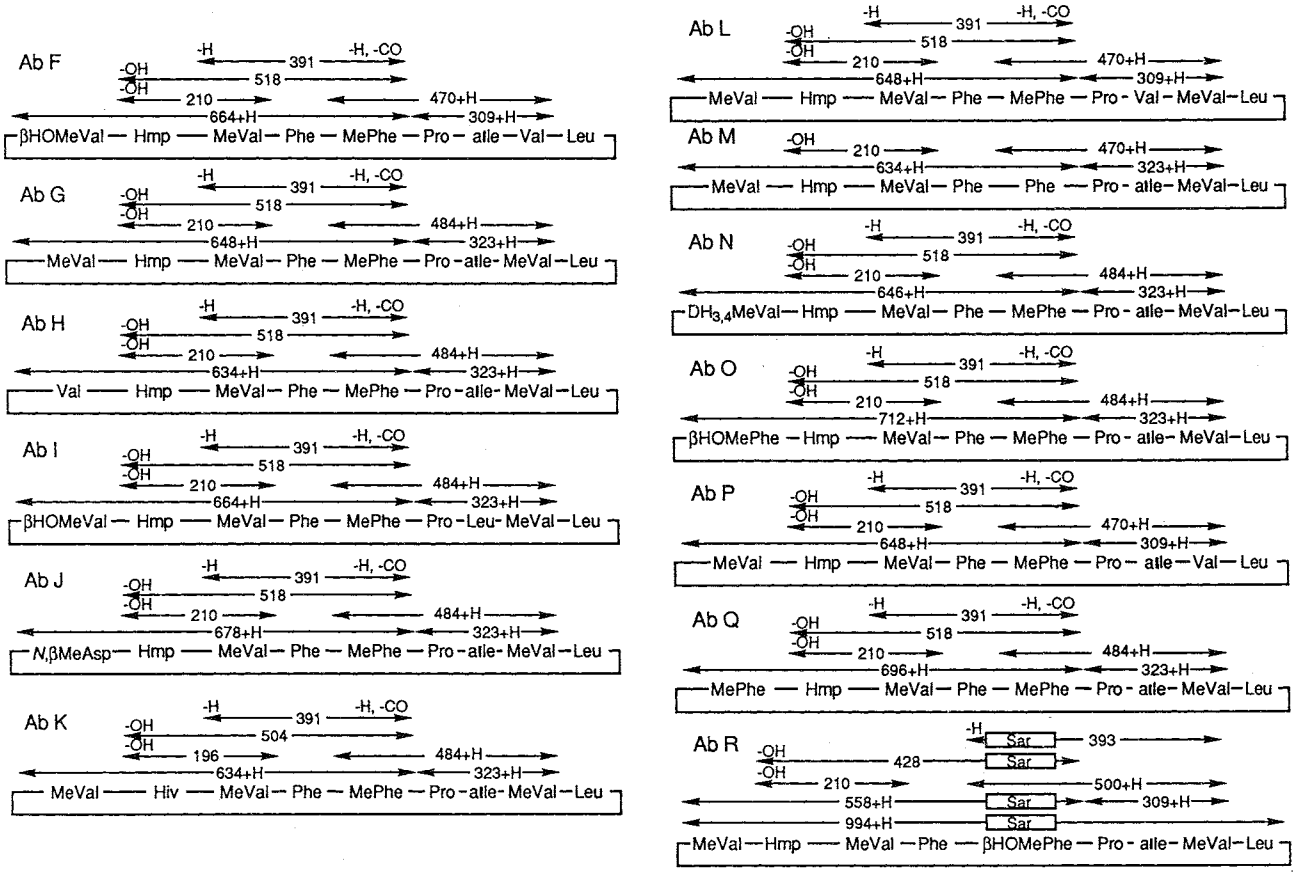
Table 2. Amino acid composition of aureobasidins (Abs) B~R and their derivatives.

Compound	Amino acids
Ab B	βHOMeVal (0.6), MeVal (2), proline (1), alloisoleucine (1), MePhe (1), leucine (1), phenylalanine (1), methylamine (0.3).
B1, B1a	MeVal (2), Sar (1), proline (1), alloisoleucine (1), MePhe (1), leucine (1), phenylalanine (1).
B2	MeVal (2), proline (1), alloisoleucine (1), MePhe (1), leucine (1), phenylalanine (1), methylamine (1).
Ab C	βHOMeVal (0.6), MeVal (2), proline (1), valine (1), MePhe (1), leucine (1), phenylalanine (1), methylamine (0.3)
C1, C1a	MeVal (2), Sar (1), proline (1), valine (1), MePhe (1), leucine (1), phenylalanine (1).
C2	MeVal (2), proline (1), valine (1), MePhe (1), leucine (1), phenylalanine (1), methylamine (1).
Ab D	γHOMeVal (1), MeVal (2), proline (1), alloisoleucine (1), leucine (1), MePhe (1), phenylalanine (1).
D1	γHOMeVal (1), MeVal (2), proline (1), alloisoleucine (1), leucine (1), MePhe (1), phenylalanine (1).
D1a	γHOMeVal (1), MeVal (2), proline (1), alloisoleucine (1), leucine (1), MePhe (1), phenylalanine (1).
Ab E	βHOMeVal (0.5), MeVal (2), proline (1), alloisoleucine (1), leucine (1), phenylalanine (1), methylamine (1).
E1, E1a	MeVal (2), Sar (2), proline (1), alloisoleucine (1), leucine (1), phenylalanine (1).
E2	MeVal (2), Sar (1), proline (1), alloisoleucine (1), leucine (1), phenylalanine (1), methylamine (1).
E3	MeVal (2), proline (1), alloisoleucine (1), leucine (1), phenylalanine (1), methylamine (2).
Ab F	βHOMeVal (1), MeVal (1), proline (1), valine (1), alloisoleucine (1), MePhe (1), leucine (1), phenylalanine (1).
Ab G	MeVal (3), proline (1), alloisoleucine (1), MePhe (1), leucine (1), phenylalanine (1).
Ab H	MeVal (2), proline (1), valine (1), alloisoleucine (1), MePhe (1), leucine (1), phenylalanine (1).
Ab I	βHOMeVal (0.6), MeVal (2), proline (1), MePhe (1), leucine (2), phenylalanine (1), methylamine (0.3).
Ab J	*N*,βMeAsp (1), MeVal (2), proline (1), alloisoleucine (1), MePhe (1), leucine (1), phenylalanine (1).
Ab K	MeVal (3), proline (1), alloisoleucine (1), MePhe (1), leucine (1), phenylalanine (1).
Ab L	MeVal (3), proline (1), valine (1), MePhe (1), leucine (1), phenylalanine (1).
Ab M	MeVal (3), proline (1), alloisoleucine (1), leucine (1), phenylalanine (2).
Ab N	MeVal (2), proline (1), alloisoleucine (1), MePhe (1), leucine (1), phenylalanine (1), methylamine (0.3).
Ab O	MeVal (2), proline (1), alloisoleucine (1), MePhe (1), leucine (1), phenylalanine (1), methylamine (1).
Ab P	MeVal (2), proline (1), valine (1), alloisoleucine (1), MePhe (1), leucine (1), phenylalanine (1).
Ab Q	MeVal (2), proline (1), alloisoleucine (1), MePhe (2), leucine (1), phenylalanine (1).
Ab R	MeVal (3), proline (1), alloisoleucine (1), leucine (1), phenylalanine (1), methylamine (1).

Abbreviations: βHOMeVal, β-hydroxy-*N*-methylvaline; MeVal, *N*-methylvaline; MePhe, *N*-methylphenylalanine; Sar, sarcosine; γHOMeVal, γ-hydroxy-*N*-methylvaline; *N*,βMeAsp, *N*,β-dimethylaspartic acid.

MePhe, leucine, and phenylalanine. So, one or the other of the two MeVal of Ab A seemed to be replaced by valine in Ab F. The fragmentation pattern of Ab F showed peaks at m/z 310 and 471 instead of the peaks at m/z 324 and 485 in Ab A (Figs. 4 and 5). Both compounds had a peak at m/z 391. Therefore, aIle or its adjacent MeVal in Ab A was changed to an amino acid 14 daltons smaller. From its MW and

Fig. 5. Mass fragmentation of aureobasidins (Abs) F~R.



the amino acid composition of Ab F, valine was present instead of one of the MeVal of Ab A. Thus, Ab F was as shown in Fig. 1.

The structures of Abs G, H, I, L, M, and O~R were decided by the same method as used for the structure of Ab F, and these were as shown in Fig. 1.

One undefined component was detected in the acid hydrolysate of Ab J and one in that of Ab N. The MW of the component (in depsipeptide form) in Ab J was 143, deduced from the MW of Ab J and the composition of the other amino acids. So, this component could be *N*,*β*-dimethylaspartic acid (*N*,*β*MeAsp) or *N*-methylglutamic acid. Jones oxidation of Ab D gave Ab J, and we concluded that the undefined component of Ab J was *N*,*β*MeAsp, a newly reported amino acid. The structure of Ab J was elucidated to be as shown in Fig. 1. The undefined component of Ab N had the MW of 111 (in depsipeptide form). Alkaline treatment (0.25 N NaOH in aqueous MeOH, room temperature, 24 hours) of Ab N gave a compound identical with **A2** by HPLC and FAB-MS. Therefore, we decided that the undefined component was 3,4-didehydro-*N*-methylvaline ($DH_{3,4}$MeVal), a newly reported amino acid, and that the structure of Ab N was as shown in Fig. 1. The acid hydrolysate of Ab K was analyzed by chiral HPLC; *R*-Hiv, which was the same as in Ab B, was detected. In the structure of Ab K, the Hmp of Ab G was Hiv.

Experimental

FAB-MS and HRFAB-MS were obtained on a Jeol JMS-DX302 spectrometer. NMR spectra were recorded on a Jeol JNM-FX200 spectrometer, IR spectra on a Hitachi 270-30 spectrophotometer, and CD curves on a Jasco J-600 spectropolarimeter. Amino acid autoanalysis was done with a Jeol JCL-300 amino acid autoanalyzer. HPLC amino acid analysis was carried out as described in the preceding paper.[2)]

Mild Alkaline Hydrolysis of Ab B—Isolation **B1** and **B2**

To a solution of Ab B (25 mg) in MeOH (6 ml) was added 1 N NaOH (2 ml), and the mixture was stirred for 15 hours at room temperature. The reaction mixture was neutralized with 1 N HCl and concentrated under reduced pressure. The concentrate was acidified with 1 N HCl and extracted with EtOAc. The extract was washed with H_2O and concentrated under reduced pressure. The residue was chromatographed on a Capcell Pak C_{18} column (Shiseido Co., Ltd.) with 70% CH_3CN, giving **B1** (9 mg) and **B2** (3 mg) as colorless powders.

B1: ^{1}H NMR ($CDCl_3$) δ 2.45 (3H, s, NCH_3), 2.91 (3H, s, NCH_3), 3.21 (3H, s, NCH_3), 3.23 (3H, s, NCH_3). FAB-MS *m/z* 1,047 (M+H), 1,069 (M+Na), 1,085 (M+K). IR (KBr) cm^{-1} 3470, 3340, 2970, 1730, 1640, 1460, 1095, 710.

B2: FAB-MS *m/z* 1,069 (M+H), 1,091 (M+Na). IR (KBr) cm^{-1} 3480, 2980, 1740, 1640, 1540, 1225, 710.

B1-Methyl Ester (**B1a**)

In a solution of **B1** (9 mg) in a 1:1 mixture of MeOH and ether (4 ml), diazomethane was bubbled until the solution turned yellow. After the reaction mixture was concentrated under reduced pressure, the residue was chromatographed on a Capcell Pack C_{18} column with 60% CH_3CN, giving **B1a** (6 mg) as a colorless powder.

^{1}H NMR ($CDCl_3$) δ 2.47 (3H, s, NCH_3), 3.04 (3H, s, NCH_3), 3.12 (3H, s, NCH_3), 3.17 (3H, s, NCH_3), 3.71 (3H, s, OCH_3). FAB-MS *m/z* 1,061 (M+H), 1,083 (M+Na). IR (KBr) cm^{-1} 3480, 3330, 2970, 1755, 1630, 1210, 1090, 705.

Acid Hydrolysis of Ab B—Isolation of Hiv

Ab B (117 mg) was hydrolyzed with 6 N HCl (5 ml) at 110°C for 22 hours in a sealed tube. The reaction mixture was concentrated under reduced pressure. The residue was chromatographed on a Dowex 50W (60 ml) column equilibrated with 0.1 M pyridine-formic acid buffer (pH 3.1). Appropriate fractions were

combined and concentrated under reduced pressure. The residue was desalted on a Dowex 50W (H^+) column with H_2O, giving Hiv (6 mg) as a colorless oil.

^1H NMR ($CDCl_3$) δ 0.93 (3H, d, J=6.8 Hz), 1.06 (3H, d, J=7.1 Hz), 2.0~2.3 (1H, m), 4.14 (1H, d, J=3.4 Hz). ^{13}C NMR ($CDCl_3$) δ 15.9 (q), 18.7 (q), 32.0 (d), 74.8 (d), 178.4 (s).

Synthesis of Hiv

The Hiv corresponding to L- and D-valine were prepared from optically pure parent amino acids by the procedure described elsewhere.[3]

^1H NMR ($CDCl_3$) δ 0.92 (3H, d, J=6.8 Hz), 1.06 (3H, d, J=7.1 Hz), 2.0~2.3 (1H, m), 4.15 (1H, d, J=3.4 Hz).

Mild Alkaline Hydrolysis of Ab C—Isolation **C1** and **C2**

To a solution of Ab C (10 mg) in MeOH (3 ml) was added 1 N NaOH (1 ml), and the mixture was stirred for 15 hours at room temperature. The reaction mixture was neutralized with 1 N HCl and concentrated under reduced pressure. The concentrate was acidified with 1 N HCl and extracted with EtOAc. The extract was washed with H_2O and concentrated under reduced pressure. The residue was chromatographed on a Capcell Pack C_{18} column with 70% CH_3CN, giving **C1** (5 mg) and **C2** (2 mg) as colorless powders.

C1: ^1H NMR ($CDCl_3$) δ 2.47 (3H, s, NCH_3), 2.98 (3H, s, NCH_3), 3.23 (6H, s, $NCH_3 \times 2$). FAB-MS m/z 1,047 (M+H), 1,069 (M+Na), 1,085 (M+K). IR (KBr) cm^{-1} 3430, 3325, 2970, 1730, 1630, 1455, 1095, 705.

C2: FAB-MS m/z 1,069 (M+H), 1,091 (M+Na).

C1-Methyl Ester (**C1a**)

In a solution of **C1** (4 mg) in a 1:1 mixture of MeOH and ether (4 ml), diazomethane was bubbled until the solution turned yellow. After the reaction mixture was concentrated under reduced pressure, the residue was chromatographed on a Capcell Pak C_{18} column with 60% CH_3CN, giving **C1a** (3 mg) as a colorless powder.

^1H NMR ($CDCl_3$) δ 2.47 (3H, s, NCH_3), 3.04 (3H, s, NCH_3), 3.18 (6H, s, $NCH_3 \times 2$), 3.70 (3H, s, OCH_3). FAB-MS m/z 1,061 (M+H), 1,083 (M+Na). IR (KBr) cm^{-1} 3450, 3330, 2980, 1755, 1640, 1215, 1090, 705.

Mild Alkaline Hydrolysis of Ab D—Isolation of **D1**

To a solution of Ab D (48 mg) in MeOH (6 ml) was added 1 N NaOH (2 ml), and the mixture was stirred for 19 hours at room temperature. The reaction mixture was neutralized with 1 N HCl and concentrated under reduced pressure. The concentrate was acidified with 1 N HCl and extracted with EtOAc. The extract was washed with H_2O and concentrated under reduced pressure. The residue was chromatographed on a Capcell Pak C_{18} column with 85% CH_3CN, giving **D1** (23 mg) as a colorless powder.

^1H NMR ($CDCl_3$) δ 2.38 (3H, s, NCH_3), 3.02 (3H, s, NCH_3), 3.19 (3H, s, NCH_3), 3.23 (3H, s, NCH_3). FAB-MS m/z 1,119 (M+H), 1,141 (M+Na), 1,157 (M+K). IR (KBr) cm^{-1} 3460, 3330, 2980, 1735, 1635, 1460, 1095, 705.

Diazomethane Treatment of **D1** (**D1a**)

In a solution of **D1** (23 mg) in a 1:1 mixture of MeOH and ether (4 ml), diazomethane was bubbled until the solution turned yellow. After the reaction mixture was concentrated under reduced pressure, the residue was chromatographed on a Capcell Pak C_{18} column with 80% CH_3CN, giving **D1a** (12 mg) as a colorless powder.

^1H NMR ($CDCl_3$) δ 2.46 (3H, s, NCH_3), 3.01 (3H, s, NCH_3), 3.08 (3H, s, NCH_3), 3.19 (3H, s, NCH_3). FAB-MS m/z 1,101 (M+H), 1,123 (M+Na). IR (KBr) cm^{-1} 3460, 3330, 2980, 1790, 1640, 1460, 1085, 1010, 705.

Acid Hydrolysis of Ab D—Isolation of γHOMeVal

Ab D (130 mg) was hydrolyzed with 6 N HCl (5 ml) at 110°C for 24 hours in a sealed tube. The reaction

mixture was concentrated under reduced pressure. The residue was chromatographed on a Dowex 50W (60 ml) column equilibrated with 0.1 M pyridine-formic acid buffer (pH 3.1). The column was developed with the same buffer solution followed by a 0.2-M pyridine-formic acid buffer (pH 3.1). γHOMeVal was eluted with 0.2 M pyridine-formic acid buffer (pH 3.1). The amino acid was desalted on a Dowex 50W (H^+) column with 1 N NH_4OH, giving γHOMeVal (6 mg) as a colorless powder.

^1H NMR (D_2O) δ 1.01 (3H, d, J=7.3 Hz), 2.2~2.4 (1H, m), 2.73 (3H, s, NCH_3), 3.5~3.8 (3H, m). ^{13}C NMR (D_2O) δ 14.8 (q), 36.4 (q), 38.8 (d), 69.8 (t), 70.5 (d), 175.6 (s). FAB-MS m/z 148 (M+H).

Acid Hydrolysis of Ab E — Isolation of L-*threo*-(**E5**) and L-*erythro*-βHOMePhe (**E6**)

Ab E (109 mg) was hydrolyzed with 6 N HCl (5 ml) at 110°C for 24 hours in a sealed tube. The reaction mixture was concentrated under reduced pressure. The residue was chromatographed on a Dowex 50W (60 ml) column equilibrated with 0.1 M pyridine-formic acid buffer (pH 3.1). Appropriate fractions were combined and concentrated under reduced pressure. The residue was chromatographed on a Capcell Pak C_{18} column with H_2O, giving **E5** (4.1 mg) and **E6** (1.8 mg) as colorless powders.

L-*threo*-βHOMePhe (**E5**): ^1H NMR (D_2O) δ 2.67 (3H, s, NCH_3), 3.78 (1H, d, J=7.2 Hz), 5.06 (1H, d, J=7.2 Hz), 7.46 (5H, m). FAB-MS m/z 196 (M+H). CD $[\theta]_{220}$ +7,714° (c 0.003, 0.5 N HCl).

L-*erythro*-βHOMePhe (**E6**): ^1H NMR (D_2O) δ 2.74 (3H, s, NCH_3), 3.93 (1H, d, J=4.0 Hz), 5.35 (1H, d, J=4.0 Hz), 7.3~7.5 (5H, m). FAB-MS m/z 196 (M+H). CD $[\theta]_{220}$ +7,803° (c 0.0025, 0.5 N HCl).

Synthesis of DL-*threo*-β- and DL-*erythro*-β-Hydroxyphenylalanine

DL-*threo*-β- and DL-*erythro*-β-hydroxyphenylalanine were synthesized by a method described previously.[7]

DL-*threo*-β-Hydroxyphenylalanine: ^1H NMR (D_2O) δ 3.93 (1H, d, J=4.4 Hz), 5.32 (1H, d, J=4.4 Hz), 7.48 (5H, m).

DL-*erythro*-β-Hydroxyphenylalanine: ^1H NMR (D_2O) δ 4.10 (1H, d, J=4.0 Hz), 5.37 (1H, d, J=4.0 Hz), 7.43 (5H, m).

Synthesis of DL-*threo*- and DL-*erythro*-βHOMePhe

To a solution of DL-*threo*-β-hydroxyphenylalanine (181 mg) in 0.1 N NaOH (10 ml) was added formalin (0.2 ml), and the mixture was stirred for 2 hours at room temperature. To this mixture, $NaBH_4$ (70 mg) was added with cooling in an ice-bath and the mixture was stirred for 4 hours at room temperature. The reaction mixture was acidified with 1 N HCl and concentrated under reduced pressure. The residue was chromatographed on a Nucleosil $5C_{18}$ column (Macherey Nagel Co.) with H_2O, giving DL-*threo*-βHOMePhe (41 mg) as a colorless powder. DL-*erythro*-βHOMePhe was also obtained from DL-*erythro*-β-hydroxyphenylalanine by the above-mentioned synthetic method.

DL-*threo*-βHOMePhe: ^1H NMR (D_2O) δ 2.67 (3H, s, NCH_3), 3.78 (1H, d, J=7.2 Hz), 5.06 (1H, d, J=7.2 Hz), 7.46 (5H, m).

DL-*erythro*-βHOMePhe: ^1H NMR (D_2O) δ 2.73 (3H, s, NCH_3), 3.92 (1H, d, J=4.0 Hz), 5.33 (1H, d, J=4.0 Hz), 7.3~7.5 (5H, m).

Synthesis of Ab E Isomer

To a solution of Ab E (81 mg) in acetone (5 ml) was added Jones reagent (0.2 ml) with cooling in an ice-bath, and the mixture was stirred for 0.5 hour at room temperature. To this mixture, PrOH was added with cooling in an ice-bath. The reaction mixture was poured into H_2O and extracted with EtOAc. The extract was washed with saturated $NaHCO_3$ solution and H_2O, and concentrated under reduced pressure. The residue was 63 mg as a colorless powder.

FAB-MS m/z 1,115 (M+H), 1,137 (M+Na).

To a solution of the residue (63 mg) in MeOH (5 ml), $NaBH_4$ (50 mg) was added with cooling in an ice-bath and the mixture was stirred for 1 hour at room temperature. The reaction mixture was acidified with 1 N HCl and concentrated under reduced pressure. The residue was chromatographed on a Capcell Pak C_{18} column with 70% CH_3CN, giving a mixture of Ab E and Ab E isomer. The mixture was chromatographed on a Nucleosil silica 100-5 column with hexane, PrOH, and CH_3CN (90:10:5) giving Ab E (11 mg) and Ab E isomer (16 mg) as colorless powders.

Ab E isomer: ^{1}H NMR ($CDCl_3$) δ 2.46 (3H, s, NCH_3), 3.02 (3H, s, NCH_3), 3.24 (3H, s, NCH_3), 3.25 (3H, s, NCH_3). FAB-MS *m/z* 1,117 (M+H), 1,139 (M+Na). IR (KBr) cm^{-1} 3435, 2965, 1740, 1640, 1410, 1085, 705.

Mild Alkaline Hydrolysis of Ab E—Isolation of **E1**, **E2**, and **E3**

To a solution of Ab E (108 mg) in MeOH (12 ml) was added 1 N NaOH (4 ml), and the mixture was stirred for 19 hours at room temperature. The reaction mixture was neutralized with 1 N HCl and concentrated under reduced pressure. The concentrate was acidified with 1 N HCl and extracted with EtOAc. The extract was washed with H_2O and concentrated under reduced pressure. The residue was chromatographed on a Capcell Pak C_{18} column with 70% CH_3CN, giving **E1** (49 mg), **E2** (7 mg), and **E3** (12 mg) as colorless powders.

E1: ^{1}H NMR ($CDCl_3$) δ 2.67 (3H, s, NCH_3), 3.25 (6H, s, $NCH_3 \times 2$), 3.33 (3H, s, NCH_3). FAB-MS *m/z* 993 (M+Na), 1,009 (M+K). IR (KBr) cm^{-1} 3430, 3320, 2970, 1635, 1095, 705.

E2: FAB-MS *m/z* 993 (M+H), 1,015 (M+Na). IR (KBr) cm^{-1} 3450, 3330, 2970, 1730, 1635, 1530, 1220, 1090, 700.

E3: FAB-MS *m/z* 1,099 (M+H), 1,121 (M+Na). IR (KBr) cm^{-1} 3450, 3350, 2980, 1740, 1640, 1415, 1225, 1095, 710.

E1-Methyl Ester (**E1a**)

In a solution of **E1** (33 mg) in a 1 : 1 mixture of MeOH and ether (10 ml), diazomethane was bubbled until the solution turned yellow. After the reaction mixture was concentrated under reduced pressure, the residue was chromatographed on a Capcell Pak C_{18} column with 70% CH_3CN, giving **E1a** (12 mg) as a colorless powder.

^{1}H NMR ($CDCl_3$) δ 2.75 (3H, s, NCH_3), 3.30 (3H, s, NCH_3), 3.33 (3H, s, NCH_3), 3.39 (3H, s, NCH_3), 3.71 (3H, s, OCH_3). FAB-MS *m/z* 985 (M+H), 1,007 (M+Na). IR (KBr) cm^{-1} 3470, 3320, 2980, 1755, 1640, 1460, 1215, 1095, 705.

Jones Oxidation of Ab D—Synthesis of Ab J

To a solution of Ab D (96 mg) in acetone (5 ml) was added Jones reagent (0.5 ml) with cooling in an ice-bath, and the mixture was stirred for 1 hour at room temperature. To this mixture, PrOH was added with cooling in an ice-bath. The reaction mixture was neutralized with barium hydroxide solution and was filtered. The filtrate was concentrated under reduced pressure. The residue was chromatographed on a Capcell Pak C_{18} column with 85% CH_3CN containing 0.1% TFA, giving Ab J (32 mg) as colorless powder.

FAB-MS *m/z* 1,115 (M+H), 1,137 (M+Na). IR (KBr) cm^{-1} 3460, 3320, 2980, 1735, 1635, 1420, 1085, 705.

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