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# STRUCTURES OF β-AMINO ACIDS IN ANTIBIOTICS ITURIN A

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Summary: An antibiotic iturin A was separated into several components (A-1∿8) using HPLC and the structures of the β-amino acids of each component were established based on NMR and mass spectral analysis.

During our screening search for antibiotics against phytopathogenic fungi, we isolated a peptidal substance produced by a Bacillus species and tentatively termed 10W. Physicochemical studies on 10W revealed that it was identical with iturin A[1], which is a mixture of homologous cyclic peptide composed of each one mole of a lipophilic β-amino acid, glutamine, tyrosine, proline and serine, and three moles of asparagine. The structures of two main components have been proposed as I-a and b by Peypoux et al[2-5].

However, results of our preliminary studies on the antibiotic were not coincident with the proposed structures. Therefore, we attempted to carry out a complete characterization of the structures of each component of iturin A.

An antibiotic 10W was a mixture of homologous peptides and separated into eight components using HPLC on ODS (Nucleosil $5C_{18}$) with acetonitrile-10mM ammonium acetate (2:3) as in Fig.1-a, while iturin A gave seven peaks in the chromatogram as in Fig.1-b and each of them corresponded to 10W-2∿8. Though the contents of each component were slightly different between these two antibiotics, two abundant components of iturin A were evidently identical with 10W-2 and 4. Therefore, hereafter 10W-1∿8 are called iturin A-1∿8, respectively.

The mass spectra of each component of iturin A obtained with FAB ionization system showed homologous M+H ion peaks at m/z 1029 for iturin A-1, 1043 for A-2, 1057 for A-3, A-4 and A-5, 1071 for A-6 and A-7, and 1085 for A-8. The mass spectra of the N-acetyl methyl ester of the lipophilic amino acids of iturin A-2 and A-4 isolated from the hydrolyzate by solvent extraction were identical with the published data of the same derivatives of $C_{14}$- and $C_{15}$-β-amino acids of iturin A, respectively[3].

After being partially hydrolyzed, iturin A-2 afforded a mixture of oligopeptides, among which were identified Glu-Pro, Tyr-Asp, Ser-$C_{14}$-β-amino acid, Glu-Pro-Asp and Ser-$C_{14}$-β-amino acid-(Asp, Tyr).

In the $^{13}C$-NMR spectra methyl carbons of aliphatic long chain compounds

usually resonate nearly at δ 14 ppm for normal-type compounds, 23 ppm for iso-type, and 11 and 19 ppm for anteiso-type compounds[6]. The $^{13}$C-NMR spectrum of N-acetyl methyl ester of $C_{14}$-β-amino acid of iturin A-2 showed only one aliphatic methyl signal at δ 14.1 ppm, indicating that this β-amino acid is not of iso-type but of normal structure. The other signals[7] were well coincident with those of long chain aliphatic compounds[6]. Thus the structure of $C_{14}$-β-amino acid of iturin A-2 should be 3-amino-tetradecanoic acid.

The above mentioned chemical shifts of the derivative of the isolated β-amino acid were very similar to those in the whole peptide, suggesting that those did not shift due to any steric effects in the peptide.

The $^{13}$C-NMR spectrum of iturin A-4 showed two equivalent methyls at δ 23.0 ppm, indicating that the $C_{15}$-β-amino acid of iturin A-4 is of iso-type and 3-amino-13-methyltetradecanoic acid. Chemical shifts in $^{13}$C-NMR spectra of terminal parts of $C_{14}$- and $C_{15}$-β-amino acids in iturin A-2, A-3, A-4 and A-5 are summarized in Table 1, confirming that the structure of the β-amino acid in iturin A-3 is 3-amino-12-methyltetradecanoic acid and that the $C_{15}$-β-amino acid in A-5 is 3-amino-pentadecanoic acid.

$^{1}$H-NMR spectra of the above four components thus determined were clearly distinguishable from each others in their methyl regions; a 3H distorted triplet for a normal-type compound, a 6H doublet for an iso-type and a 6H multiplet due to triplet plus doublet for an anteiso-type compound.

$^{1}$H-NMR spectra of the other minor homologs, iturin A-1, A-6 and A-7 and the mass spectra of the β-amino acids are shown in Table 1. All the structures of β-amino acids in iturin A components were established as in II-1∿II-7 for iturin A-1∿7, respectively. Iturin A-8 was deduced to have an anteiso-type $C_{17}$-β-amino acid from the mass and $^{1}$H-NMR spectra, but it could be obtained too little to be fully characterized by $^{13}$C-NMR spectrum.

The data disclosed above and the reported ones on iturin A[2] suggest the structures of iturin A-1∿7 to be II-1∿7.

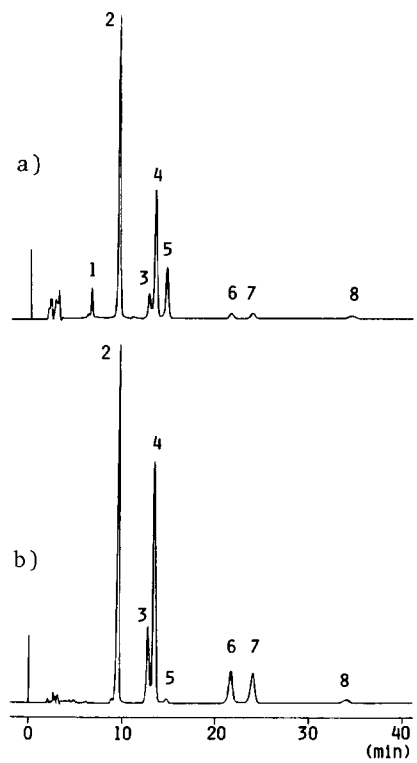
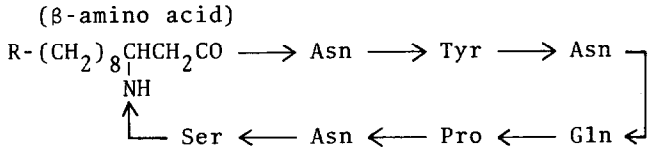



Fig.1. Chromatograms of the antibiotics on HPLC (ODS).
a) 10W, b) iturin A.




R: I-a) $(CH_3)_2CH-$

b) $CH_3CH_2\underset{\displaystyle CH_3}{\underset{|}{C}}H-$

II -1) $CH_3CH_2-$

2) $CH_3CH_2CH_2-$

3) $CH_3CH_2\underset{\displaystyle CH_3}{\underset{|}{C}}H-$

4) $(CH_3)_2CHCH_2-$

5) $CH_3CH_2CH_2CH_2-$

6) $(CH_3)_2CHCH_2CH_2-$

7) $CH_3CH_2CH_2CH_2CH_2-$

| | MS[a] | $^{13}$C-NMR, δ(ppm)[b] | | | | | $^{1}$H-NMR | MS of β- |
|---|---|---|---|---|---|---|---|---|
| | $(M+H)^+$ | C-15 | C-14 | C-13 | C-12 | $CH_3$[c] | δ($CH_3$) | amino acid ($M^+$)[d] |
| A-1 | 1029 | | | | | | 0.9 (3H,t) | 285 |
| A-2 | 1043 | | 14.5 | 23.7 | 33.1 | | 0.9 (3H,t) | 299 |
| A-3 | 1057 | | 11.8 | 30.0 | 35.7 | 19.7 | 0.9 (6H,m) | 313 |
| A-4 | 1057 | | 23.0 | 29.1 | 40.0 | 23.0 | 0.9 (6H,d) | 313 |
| A-5 | 1057 | 14.4 | 23.7 | 33.1 | 30.0 | | 0.9 (3H,t) | 313 |
| A-6 | 1071 | | | | | | 0.9 (6H,d) | 327 |
| A-7 | 1071 | | | | | | 0.9 (3H,t) | 327 |
| A-8 | 1085 | | | | | | 0.9 (6H,m) | 341 |

Table 1. Mass and NMR Spectra of Iturin A-1 ∿ A-8 and Mass Spectra of Their β-Amino Acid Derivatives.

a) measured with FAB ionization system.

b) carbons of β-amino acid residue.

c) branch methyl

d) obtained as N-acetyl methyl ester.

In recent years, Peypoux, Delcambe and their co-workers have studied the antibiotics belonging to iturin group, such as iturin C[8], mycosubtilin[9], bacillomycin L[10] and bacillomycin D[11]. All these antibiotics also have $C_{14}$∿$C_{17}$-β-amino acids. The structures of these β-amino acids should be reinvestigated because these have been determined in comparison with those of iturin A.

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6) "$^{13}$C-NMR Spectroscopy" by E. Breitmaier and W. Voelter, 2nd ed., p. 131, Verlag Chemie, Weinheim, 1978.
7) Chemical shifts of the derivative were as follows ($CDCl_3$): 14.1 (C-14), 22.7 (C-13), 31.9 (C-12), 30.0 (C-6∿C-11), 26.2 (C-5), 34.1 (C-4), 46.2 (C-3), 38.2 (C-2), 172.5, 169.7 (C-1, acetyl CO), 51.6 ($OCH_3$), 23.4 (acetyl $CH_3$).
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