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Site-Specific Ring Opening of Depsipeptide Aureobasidin A in Hydrogen Fluoride

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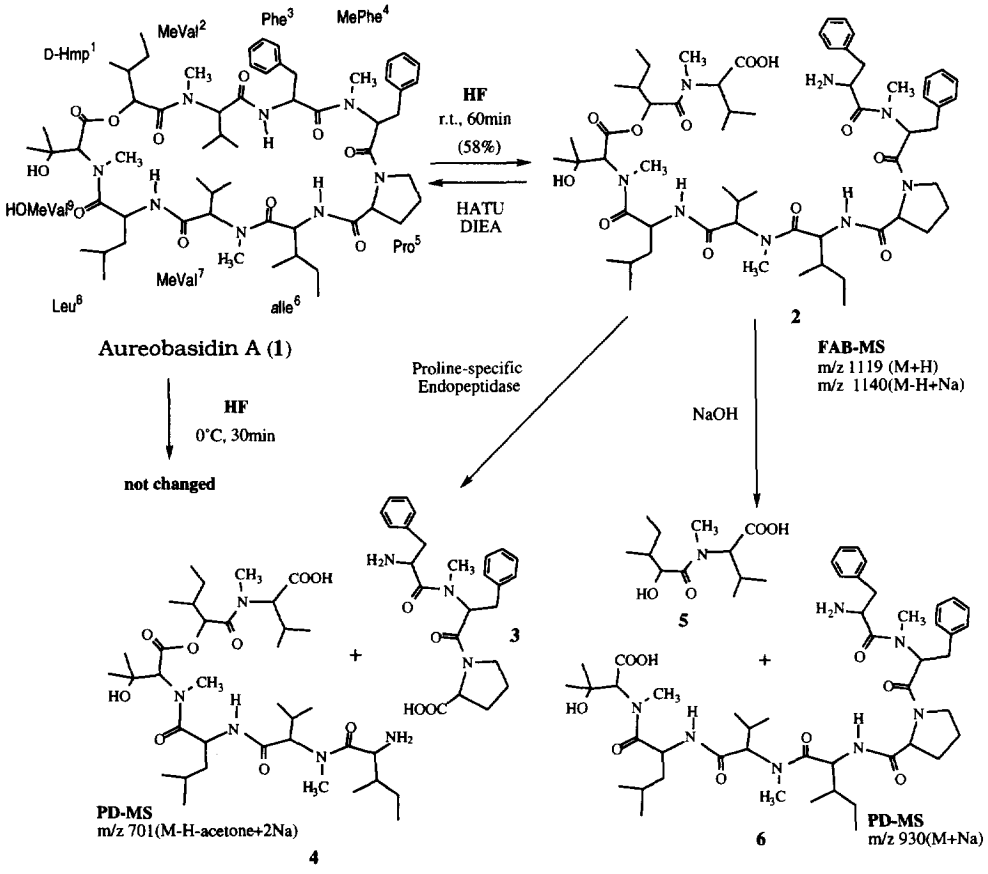
Abstract: A site-specific ring opening reaction for the antifungal depsipeptide aureobasidin A occurred on treatment with HF at room temperature for 1 hour. The open-chain peptide thus obtained was recyclized to afford the original aureobasidin A without any modification.

Anhydrous hydrogen fluoride has been widely used as a powerful deprotecting reagent, especially for the final deprotection in solid phase peptide synthesis.[1] Usually, the deprotection procedure with HF is carried out at 0°C for 30 min. We evaluated the stability, under these conditions, of aureobasidins isolated from *Aureobasidium pullulans* R106 as antifungal cyclic depsipeptides,[2] whose major component is aureobasidin A (AbA).

AbA was treated with HF at 0°C for 30 min and the reaction mixture was analyzed by silica gel TLC. Although AbA was found to be virtually unchanged on this treatment, an unknown ninhydrin-positive product was detected in a minute amount by TLC (developed with $CHCl_3$-MeOH-AcOH 95:5:3) at Rf 0.2. In order to confirm and isolate this byproduct, whose structure was first supposed to be the *N,O*-acyl migration product at that time, AbA was exposed to HF under severer conditions (rt, 1 h) to give the expected product as a major spot on TLC. The product was purified by preparative TLC and then reverse-phase HPLC (RP-HPLC) in a moderate yield of 58%. It was identified unexpectedly as a linear nonapeptide having a Phe residue in its *N*-terminus from the results of fast-atom bombardment mass spectroscopy (FAB-MS) (m/z 1119 M+H, 1140 M+Na) and *N*-terminal analysis by the dansyl method[3] (Scheme). Mild alkaline hydrolysis of the peptide with 1N NaOH at room temperature for 30 min[4] as well as enzymic digestion with proline-specific endopeptidase at 37°C for 2 h[5] also supported the structure of the nonapeptide having an *N*-terminus of a Phe residue. Thus, the whole structure of the peptide was concluded to be H-Phe-MePhe-Pro-*a*Ile-MeVal-Leu-HOMeVal-D-Hmp-MeVal-OH (2).[6] Why the linkage between MeVal and Phe in this peptide was selectively cleaved under above conditions is not clear.[7] There is no structural information on aureobasidin A in HF to elucidate of this problem because the conformational analysis can not be easily carried out in HF by NMR. However, anyhow a constrained environment surrounding the Phe residue may cause this unusual cleavage.

This open peptide was then recyclized with *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) and diisopropylethylamine (DIEA) in DMF at a concentration of 10^{-3}M[8] to afford a cyclic peptide in a yield of 12%. The cyclic peptide thus obtained was identical with the original AbA (RP-HPLC, ^{1}H-NMR, and plasma-desorption mass spectroscopy (PD-MS)). Consequently, the linear nonapeptide obtained by HF treatment at room temperature for 1 h from AbA, had not suffered any other change in the molecule and may serve as a valuable intermediate to construct modified analogs of the mother molecule.

This successful opening and recyclization of the cyclic depsipeptide AbA offers a very useful method for further synthesis of aureobasidin analogs which are essential to the study of structure-activity relationships. This novel and specific cleavage reaction of the cyclic peptide in HF should also prompt mechanism investigations as well as further applications to other peptides.



References and Notes

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3. Two samples, one of which was obtained by acid hydrolysis (6 N HCl, 110°C) of the dansylated peptide and the other by dansylation of the acid hydrolyzate of this peptide, were analyzed by RP-HPLC. Both peaks detected as dansyl amino acid were identical with that of the authentic dansyl Phe.
4. The reaction mixture of the alkaline hydrolysis was analyzed by PD-MS and a peak derived from the hydrolyzed heptapeptide H-Phe-MePhe-Pro-*a*Ile-MeVal-Leu-HOMeVal-OH (**6**) was detected as the Na adduct (M+2Na, 937).
5. The enzymic reaction mixture was also analyzed by PD-MS and a peak derived from the digested hexapeptide H-*a*Ile-MeVal-Leu-HOMeVal-D-Hmp-MeVal-OH (**4**) was detected as the Na adduct of the retro-aldol reaction product of **4** (M-acetone+Na, 701).
6. It should be emphasized that the open-chain peptide thus obtained retained its antifungal activity against *Candida albicans* TIMM0136 (MIC, 50 μg/ml) probably with the same mechanism as that of the original cyclic aureobasidin A molecule (MIC, 0.05 μg/ml).
7. Although a similar cleavage reaction at the peptide linkage of the amino side of the Phe residue with CF_3CO_2H-EtSH-anisole was recently reported by Goodman and his coworkers (Spencer, J. R., Delaet, N. G. J., Toy-Palmer, A., Antonenko, V. V., Goodman, M., *J. Org. Chem.* **1993**, *58*, 1635), aureobasidin A could not be cleaved under such acidolytic conditions.
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