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Structure of argifin, a new chitinase inhibitor produced by *Gliocladium* sp.

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Abstract

A new chitinase inhibitor, named argifin, was isolated from the cultured broth of a fungal strain *Gliocladium* sp. FTD-0668. Argifin was a water-soluble cyclic pentapeptide, and its structure was elucidated as cyclo(N^{ω}-(*N*-methylcarbamoyl)-L-arginyl-*N*-methyl-L-phenylalanyl-β-L-aspartyl-β-L-aspartyl-D-alanyl). The IC_{50} value of argifin against blowfly (*Lucilia cuprina*) chitinase was 3.7 μM.

Keywords: enzyme inhibitor; fungi; natural product; peptide.

Chitinase hydrolyzes chitin into oligomers of *N*-acetylglucosamine, and it has been expected to be a specific target for insecticides.[1] An inhibitor of chitinase may suppress moulting of insects and prevent their maturation to the adult reproductive stage. However, only allosamidin[2] and a few other but far less active inhibitors have been reported. In the course of screening for chitinase inhibitors, we have found a new cyclic pentapeptide named argifin (**1**) from the cultured broth of *Gliocladium* sp. FTD-0668. Here we report the structure elucidation of **1** (Fig. 1).

The mycelia obtained from the cultured broth (8 liters) were extracted with methanol, and the extract was concentrated in vacuo. It was chromatographed successively by using Diaion® SK1B cation exchange, Diaion® HP20, Dowex 1 anion exchange, ODS, and Sephadex™ G-10 columns, and 20.0 mg of a white powder of **1** was obtained ($[\alpha]_D^{25}$ −82.8°, *c* 0.1, H_2O). It showed positive reaction to Rydon–Smith reagent while its reaction to ninhydrin reagent was negative. The IR spectrum suggested the presence of amide, carboxyl, and guanidine (1720, 1645, 1600, 1550, 1500 cm^{-1}). HR-FAB-MS of **1** revealed the molecular formula as $C_{29}H_{41}N_9O_{10}$ (found ($M-H^-$), 674.2930; calcd for $C_{29}H_{40}N_9O_{10}$, 674.2898).

As **1** was suggested to be a peptide from the IR and NMR[3] spectra, amino acids analysis was carried out for the acid hydrolysate. The chiral HPLC revealed the presence of 1 mol of L-arginine, *N*-methyl-

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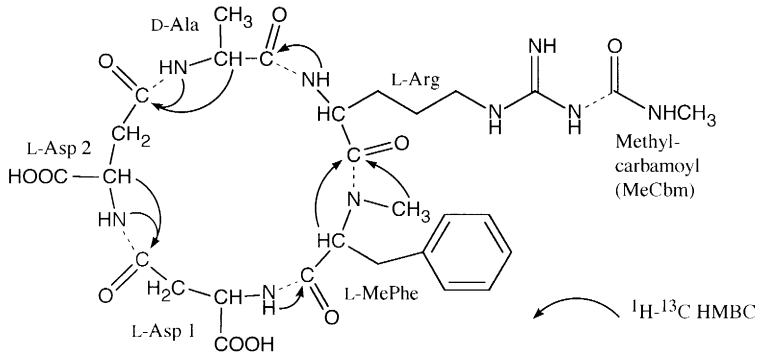

Fig. 1. Structure of **1** elucidated by ^{1}H–^{13}C HMBC

L-phenylalanine, and D-alanine, and 2 mol of L-aspartic acid (column, Sumichiral OA-5000; mobile phase, 1 mM $CuSO_4$ for Arg and Ala, 2 mM $CuSO_4$:MeOH (85:15) for MePhe and Asp). Except for five amino acids, an *N*-methylcarbamoyl residue (-$CONHCH_3$) was revealed by the ^{1}H–^{1}H COSY and ^{1}H–^{13}C HMBC. Thus, all atoms except three guadinino hydrogens in Arg and two carboxyl hydrogens in Asp 1 and Asp 2 were assigned, and both Asp residues were suggested to be aspartic acids, not asparagines. The α-carbonyl carbons of Asp 1 and Asp 2 shifted more than 3 ppm highfield in acidic solution, but their β-carbonyl carbons shifted by only 0.7–1.0 ppm. Therefore, the former carbons were suggested to be carboxylic acids.

The index of hydrogen deficiency of **1** was 14, which indicated that **1** is a cyclic peptide. The linkages of the five amino acids were elucidated by ^{1}H–^{13}C HMBC as shown in Fig. 1. The remaining carbamoyl should be linked to the guanidino nitrogen of Arg, and thus the structure of **1** was elucidated as cyclo(N^{ω}-(*N*-methylcarbamoyl)-L-arginyl-*N*-methyl-L-phenylalanyl-β-L-aspartyl-β-L-aspartyl-D-alanyl). It is interesting that one proton signal of Arg β-CH_2 was observed in extremely highfield (δ −0.43). The proton may be positioned on the benzene ring of MePhe and is shielded.

Argifin (**1**) inhibited chitinase from *Lucilia cuprina* (blowfly) in a dose-dependent manner with IC_{50} values of 3.7 μM at 37°C and 0.10 μM at 20°C. It is the first non-sugar chitinase inhibitor showing submicromolar range inhibition. It was also the first chitinase inhibitor produced by fungi. When 20 μg of **1** was injected into cockroach larvae, **1** showed 73% mortality while the control mortality was only 12%.

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3. NMR experiments were performed on a Varian Unity 400 spectrometer using D_2O or H_2O–D_2O (4:1, for amino protons) as solvents. ^{1}H NMR (400 MHz) δ 8.42 (1H, d, J=5, Asp 1 NH), 8.34 (1H, d, J=7, Arg α-NH), 8.10 (1H, d, J=6, Ala NH), 7.75 (1H, d, J=9, Asp 2 NH), 7.36 (2H, dd, J=7, 7, MePhe ε_1, ε_2), 7.29 (1H, dd, J=7, 7, MePhe ζ), 7.23 (2H, d, J=7, MePhe δ_1, δ_2), 6.99 (1H, br.s, MeCbm NH), 5.10 (1H, dd, J=11, 3, MePhe α), 4.49 (1H, dd, J=14, 3, Asp 2 α), 4.36 (1H, dd, J=12, 3, Asp 1 α), 4.34 (1H, dd, J=14, 2, Arg α), 4.18 (1H, q, J=7, Ala α), 3.14 (1H, dd, J=14, 3, MePhe β_1), 3.06 (1H, m, MePhe

β_2), 3.01 (2H, m, Arg δ), 2.95 (1H, dd, J=17, 3, Asp 1 β_1), 2.87 (3H, s, MePhe NMe), 2.79 (1H, dd, J=17, 12, Asp 1 β_2), 2.76 (3H, s, MeCbm Me), 2.70 (1H, dd, J=14, 3, Asp 2 β_1), 2.41 (1H, dd, J=14, 14, Asp 2 β_2), 1.43 (1H, m, Arg γ_1), 1.32 (3H, d, J=7, Ala β), 1.21 (1H, m, Arg γ_2), 1.08 (1H, m, Arg β_1), −0.43 (1H, m, Arg β_2); ^{13}C NMR (100 MHz) δ 178.3 (s, Asp 1 C=O), 177.2 (s, Asp 2 C=O), 175.2 (s, Ala C=O), 174.2 (s, Arg C=O), 172.2 (s, Asp 2 γ), 170.6 (s, MePhe C=O), 170.5 (s, Asp 1 γ), 155.1 (s, MeCbm C=O), 153.5 (s, Arg ζ), 137.6 (s, MePhe γ), 129.6 (d×2, MePhe δ_1, δ_2), 129.0 (d×2, MePhe ε_1, ε_2), 127.1 (d, MePhe ζ), 62.4 (d, MePhe α), 52.7 (d, Asp 1 α), 52.1 (d, Asp 2 α), 49.5 (d, Ala α), 48.6 (d, Arg α), 40.5 (t, Arg δ), 38.7 (t, Asp 2 β), 36.0 (t, Asp 1 β), 33.3 (t, MePhe β), 29.6 (q, MePhe NMe), 26.3 (t, Arg β), 26.0 (q, MeCbm Me), 23.9 (t, Arg γ), 16.6 (q, Ala β); ^{15}N NMR (40 MHz) δ 133.6 (Ala NH), 128.5 (Asp 1 NH), 127.8 (Asp 2 NH), 125.7 (Arg α-NH), 122.5 (MePhe NMe), 86.3 (MeCbm NH).