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Isolation and Structural Elucidation of New Peptaibols, Bergofungins B, C and D, from *Emericellopsis donezkii* HKI 0059

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Previously we described the isolation and structural elucidation of bergofungin (**1**)[1] by mass spectrometry (FAB; B/E-MIKES (E)-scan; ESI-CID-MS/MS) and NMR-spectroscopic methods. **1** belongs to the peptaibols[2], which are characterized by a high portion of helix-promoting α-aminoisobutyric acid (Aib) in their linear peptide backbone molecules. These secondary metabolites of fungi contain an amino alcohol such as leucinol (Leuol), tryptophanol (Trpol) or phenylalaninol (Pheol) at the carbon terminus but the nitrogen terminus is acetylated.

Similar to other peptaibols such as alamethicins, hypelcins, emerimicins, chrysospermins and ampullosporin the bergofungin A (**1**), too, displays membrane-modifying properties concomitant with various bioactivities such as formation of ion-conducting channels[3,4] or growth inhibition of bacteria and fungi[1,5].

A more detailed study of the producing strain, *Emericellopsis donezkii* HKI 0059, unraveled the presence of three additional homologous bergofungins B (**2**), C (**3**), and D (**4**). In this paper, we report their isolation and structures.

The producer strain was cultivated as surface culture (60 liters) at 26°C in 500 ml Erlenmeyer flasks containing 100 ml malt medium[1]. After 20 days of cultivation at 26°C, the whole mycelium and the culture medium were harvested and separated by filtration. Thereafter, the culture broth and the mycelium were extracted with ethyl acetate. The combined extracts were dried and evaporated. The residue (4 g) was subjected to silica gel chromatography (silica gel 60, 0.063~0.1 mm, Merck), column 600×40 mm, $CHCl_3$-MeOH, 9 : 1 v/v), and 20 ml portions were collected. Fractions containing the bergofungins **2**, **3** and **4**, were detected by ESI-MS. Final purification was achieved by isocratic preparative HPLC (Spherisorb OPS-2, 5 μm, RP_{18}, 250×25 mm Promochem, acetonitrile-H_2O 83 : 17 v/v; 12 ml/minute, UV-detection 210 nm). The structures of **2**, **3** and **4** are shown in Table 1.

In comparison with bergofungin A (**1**) the homologous B (**2**), C (**3**) and D (**4**) are distinguished by different amino acid moieties in positions 8 and 12, respectively. For instance in **2** position 8 is occupied by L-valine but in **3** the Iva in position 12 is replaced by Aib. Compound **4** is discernible from **1**, **2** and **3** by a 14 membered amino acid backbone.

The molecular weights of the new compounds **2**, **3** and **4** were determined by HRFAB-mass spectrometry (double-focusing mass spectrometer AMD 402, Intectra Harpstedt, Germany) (Table 2).

Table 1. Structures of known bergofungin A (**1**), and the new homologues B (**2**), C (**3**) and D (**4**). (Abbreviations: Iva, Pheol, Hyp, AcVal)

		Amino acid No.														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A[1]	Bergofungin (**1**)	Ac-L-Val-	Aib-	Aib-	Aib-L-	Val-	Gly-L-Leu-		Aib-	Aib-L-Hyp-		L-Gln-	L-Iva-	L-Hyp-	Aib-L-Pheol	
B	Bergofungin (**2**)	Ac-L-Val-	Aib-	Aib-	Aib-L-	Val-	Gly-L-Leu-		L-Val-	Aib-L-Hyp-		L-Gln-	L-Iva-	L-Hyp-	Aib-L-Pheol	
C	Bergofungin (**3**)	Ac-L-Val-	Aib-	Aib-	Aib-L-	Val-	Gly-L-Leu-		Aib-	Aib-L-Hyp-		L-Gln-	Aib-	L-Hyp-	Aib-L-Pheol	
D	Bergofungin (**4**)	Ac-L-Val-	Aib-	Aib-	L-Val-		Gly-L-Leu-	Aib-	Aib-	L-Hyp-		L-Gln-	L-Iva-	L-Hyp-	Aib-L-Pheol	

Table 2. Physicochemical properties of bergofungins (**2**), (**3**) and (**4**).

Bergofungin	(**2**)	(**3**)	(**4**)
Appearance	White powder	White powder	White powder
ESI-MS (*m/z*)	1539.5 $[M+H]^+$	1512.5 $[M+H]^+$	1441.4 $[M+H]^+$
HRFAB-MS (*m/z*)	1539.7347 $[M+H]^+$ calcd. 1539.7362	1512.8104 $[M+H]^+$ calcd. 1512.8091	1441.7201 $[M+H]^+$ calcd. 1441.7245
Molecular formula	$C_{74}H_{122}N_{16}O_{19}$	$C_{72}H_{119}N_{16}O_{19}$	$C_{69}H_{114}N_{15}O_{18}$
Melting point	268~270°C	276~278°C	252~254°C
$[\alpha]_D^{25}$ (MeOH, 5 mg/ml)[a]	+ 2.4°	−3.2°	−7.5°
IR (KBr, cm^{-1})[b]	3315, 2960, 1654, 1458, 1280	3310, 2960, 1652, 1460, 1290	3310, 2965, 2935, 1654, 1531, 1458
Retention time on HPLC (min) (Promochem, 250×4.6 mm; Spherisorb 5 ODS-2, 5 μm; 1 ml/min, 210 nm, acetonitrile/H_2O 83 : 17)	10.10	7.24	5.18

[a] Propol polarimeter (Dr. KERNCHEN, Germany).
[b] Shimadzu FT IR-470.

Fig. 1a. FAB mass spectrum of bergofungin B (2).

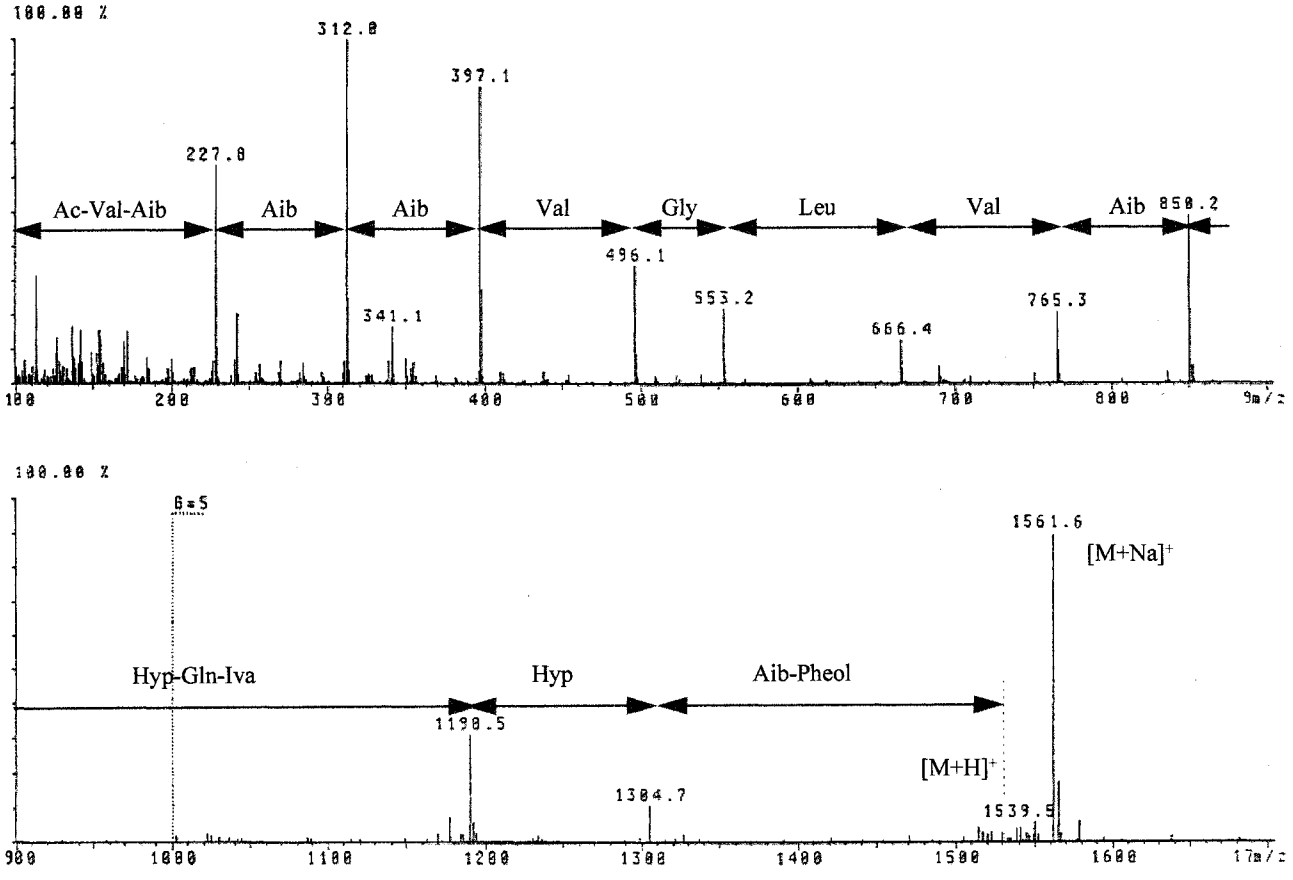


Fig. 1b. FAB mass spectrum of bergofungin C (**3**).

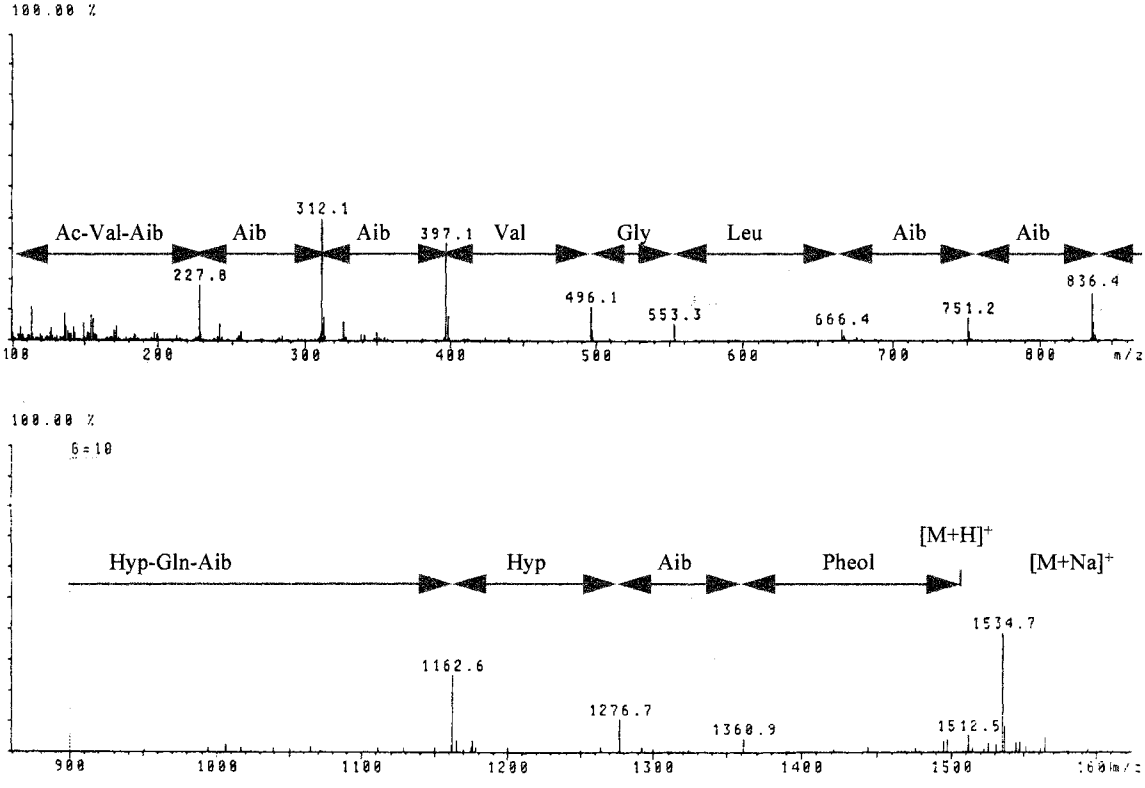
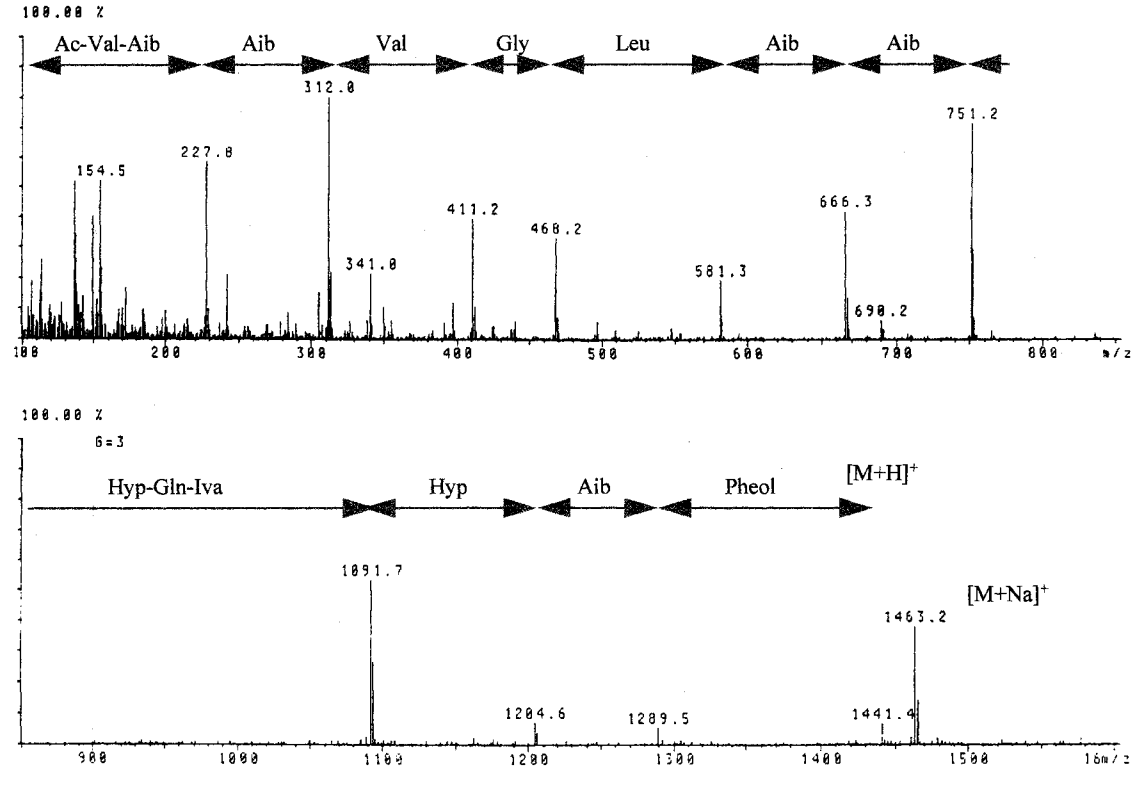


Fig. 1c. FAB mass spectrum of bergofungin D (4).



During FAB-MS a series of diagnostic B-type fragments was observed for the amino acid sequences of **2**, **3** and **4** attributable to the *N*-terminal part of the molecule[6] (see Fig. 1).

ESI-CID-MS/MS of **2** (ammonium acetate as buffer) displayed singly and doubly charged pseudomolecular ions: such as *m/z* 1539 $[M+H]^+$; *m/z* 1561 $[M+Na]^+$ and *m/z* 1577 $[M+K]^+$; *m/z* 792 $[M+H+Na]^{2+}$. The daughter ion scan of *m/z* 1539 $[M+H]^+$ unraveled diagnostic B-type fragments comparable to the results of FAB-MS analysis. Moreover, *m/z*=350; and *m/z*=690 were detected with high intensity resulting from Y-type cleavages (Y_3'' and Y_6'' according to ROEPSTORFF's nomenclature)[6].

According to ROEPSTORFF[6], a Y-type cleavage is characterized by an electrical charge remaining at the *C*-terminal fragments. The subscripted index indicates the number of amino acid residues counted from the *C*-terminus. The number of apostrophes, superscripted at the right side, indicates the number of H-atoms, which transmitted to each fragment ion.

Hydrolysis of **2**, **3** and **4**, derivatization of the amino acids by Marfey's reagent[7] and HPLC-analysis of the derivatives showed the presence of L-valine, L-leucine, α-aminoisobutyric acid (Aib), glycine, trans-4-hydroxy-L-proline, L-glutamine and L-phenylalaninol[1,8].

The compounds **2**, **3** and **4** display antimicrobial activity[9] against *Sporobolomyces salmonicolor* SBUG 549 and *Bacillus subtilis* ATCC 6633 at concentrations $\geq 50\,\mu g/ml$. The following media were used for assay medium: standard I nutrient agar (Serva) and Sabouraud-2%-glucose-agar (Difco). Moreover, bergofungin A (**1**), **2**, **3** and **4** moderately inhibit the activity of prolyl endopeptidase[10] from *Flavobacter* sp. with K_i 0.18 to 0.34 μM.

Acknowledgements

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