REVIEW

New Sequences, Constituents, and Producers of Peptaibiotics: An Updated Review

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To date, 18 genera of imperfect and ascomycetous fungi have been recognized to produce *ca.* 700 individual sequences of peptaibiotics. These are linear polypeptide antibiotics which *i*) have a molecular weight between 500 and 2,200 Dalton, thus containing 5–21 residues; *ii*) show a high content of α-aminoisobutyric acid; *iii*) are characterized by the presence of other nonproteinogenic amino acids and/or lipoamino acids; *iv*) possess an acylated N-terminus, and *v*) have a C-terminal residue that, in most of them, consists of a free or acetylated amide-bonded 1,2-amino alcohol, but might also be an amine, amide, free amino acid, 2,5-dioxopiperazine, or sugar alcohol. From April 2003 until present, *ca.* 300 new individual sequences of peptaibiotics have been published in the literature, but most of them have not yet been included in databases. To summarize these new sequences and novel constituents, as well as to introduce fungal species hitherto unknown as producers of peptaibiotics, the relevant literature is reviewed. Furthermore, ecophysiological and taxonomic aspects of the producing fungi are discussed.

1. Introduction. – Peptaibiotics constitute a constantly growing family of peptide antibiotics of fungal origin. The term 'peptaibiotics' was introduced in [1] and reconsidered in [2]. Peptaibiotics are defined as linear peptide antibiotics which *i*) have a molecular weight between 500 and 2,200 Dalton, thus containing 5–21 residues; *ii*) show a high content of α-aminoisobutyric acid (Aib); *iii*) are characterized by the presence of other nonproteinogenic amino acids and/or lipoamino acids; *iv*) possess an acylated N-terminus, and *v*) have a C-terminal residue that, in most of them, consists of a free or acetylated amide-bonded 1,2-amino alcohol, but might also be an amine, amide, free amino acid, 2,5-dioxopiperazine, or sugar alcohol. Since the majority of Aib-containing peptides carries a C-terminal residue representing a 1,2-amino alcohol, this subgroup is referred to as **peptaibols**. Very lipophilic peptaibols, the N-terminus of which is acylated by octanoic, decanoic, or *cis*-dec-4-enoic acid, are named **lipopeptaibols** [2][3]. In the third subfamily of **lipoaminopeptides** (also reported as **aminolipopeptides**), the N-terminus is substituted by unbranched, α- or γ-methyl-branched, saturated, or unsaturated C_4–C_{15} fatty acids. An L-proline-, *trans*-4-hydroxy-L-proline, or *cis*-4-methyl-L-proline residue is found in position 1 of the peptide chain, and, in most cases, it is followed by a lipoamino acid residue in position 2. To our

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present knowledge, this compound, 2-amino-6-hydroxy-4-methyl-8-oxo-decanoic acid (AHMOD), has only been recorded from this subfamily. A fourth subfamily comprises all other peptaibiotics that cannot be classified in any of the other three preceding subfamilies. A review introducing nine subfamilies according to structural homologies of peptaibiotics has been published [4].

Usually, peptaibiotics are classified according to their main chain length, as long-chain (17–21 residues), medium-chain (11–16), and short-chain (5–10) sequences. Although not yet reported in literature, the detection of very short chain (<5) peptaibiotics that may represent drastically truncated sequences is basically possible. Notably, sequences comprising more than 21 amino acids have not been reported yet. The number of Aib residues found may range from one as in some of the trichocompactins [5] or two as in the five-residue peptaibolin [6] up to nine in some of the 20-residue stilboflavins [7], or even ten in the 21-residue peptaibiotic SCH 643432 [8]. Trichobrachins TB III A and TB III B are peptides, the N-termini of which have not been assigned yet [9]. Nonribosomal peptide synthetases from *Trichoderma virens* [10] and *Sepedonium ampullosporum* [11] have recently been characterized.

Peptaibiotics show interesting physicochemical and biological activities depending on particular structural properties, such as formation of pores in bilayer lipid membranes as well as antibacterial, antifungal, occasionally antiviral, and antiparasitic activities. Inhibition of mitochondrial ATPase, uncoupling of oxidative phosphorylation, immunosuppression, inhibition of platelet aggregation, induction of fungal morphogenesis, and neuroleptic effects have been reported and reviewed in [2].

From April 2003 until present, *ca.* 300 new individual sequences of peptaibiotics were published in the literature, but most of them have not yet been included in databases such as the '*Peptaibol Database*' [12]. To summarize these new sequences and novel constituents, as well as to introduce fungal species hitherto unknown as producers of peptaibiotics, the relevant literature is reviewed. Furthermore, ecophysiological and taxonomic aspects of the producing fungi are discussed.

2. New Sequences, Constituents, and Producers of Peptaibiotics: General Remarks. – The first three paragraphs, *i.e.*, *2.1–2.3*, of this section comprise these new peptaibiotics that have been isolated from their fungal producers as pure individual substances, or mixtures of homologues and/or positional isomers in preparative amounts. In contrast, those new peptaibiotics that have been exclusively discovered and sequenced by a so-called '*peptaibiomic*' approach [5][13][14] will be introduced separately in *2.4* of this section. Representative sequences of new peptaibiotics are listed in the *Table*, whereas uncommon and new constituents of peptaibiotics are illustrated in the *Figure*.

2.1. *Peptaibols. Alamethicin*. The 20-residue alamethicins (ALM) can be considered as the most thoroughly investigated peptaibol antibiotics. The first report on the isolation and partial structural characterization of a polypeptide antibiotic (*Upjohn Company*, Kalamazoo; U-22,324) from the culture broth of the fungal strain NRRL 3199, having originally been misidentified as *Trichoderma viride*, was published almost 40 years ago [15]. The taxonomy of ALM-producing *Trichoderma* species has recently been revised. All strains investigated were assigned to belong to the so-called *T. brevicompactum* complex [5]. An investigation by TLC on silica *H* (*i.e.*, acidic silica

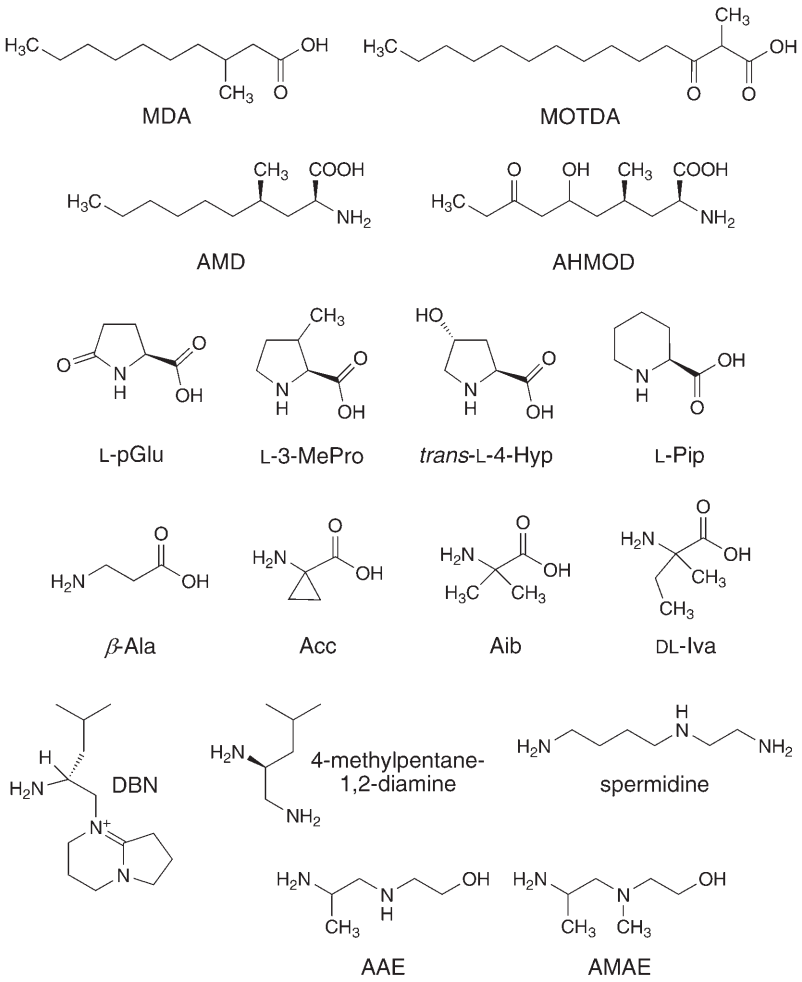

Figure. *Selected uncommon constituents of peptaibiotics*

gel) of the *Upjohn* ALM revealed that this material was composed of two major groups named, according to R_f 100 values: ALM F30 (*ca.* 85%), ALM F50 (*ca.* 12%), and a minor component ALM F20 (2%). However, minor or trace components of ALMs, numbered accordingly F40, F60, and F70, were also detected by TLC [16]. In 1984, a sequence of the acidic ALM F30 and a sequence of the neutral ALM F50 were published [17]. The former was recognized to carry a Glu-Gln residue in positions 18 and 19, which is exchanged by Gln-Gln in the latter. In 2003, the sequences of ALM F30 and ALM F50 were thoroughly reconsidered and reconciled [18].

HPLC Separation of ALM F30 using an acidic gradient revealed ten individual sequences of ALM F30/1–10. Basically, the same gradient was used to separate ALM F50 but omitting the addition of TFA (CF_3COOH) to the eluents. As a result, 13

Table. *Representative Sequences of New Peptaibiotics*[a])

Peptaibiotic		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
ALM F30/3	Ac	Aib	Pro	Aib	Ala	Aib	*Ala*	*Gln*	Aib	*Aib*	Aib	Gly	*Val*	Aib	Pro	Val	Aib	Aib	*Glu*	Gln	Pheol	
ALM F50/5	Ac	Aib	Pro	*Aib*	Ala	Aib	*Ala*	Gln	Aib	*Aib*	Aib	Gly	*Val*	Aib	Pro	*Val*	Aib	Aib	*Gln*	Gln	Pheol	
[desAA(1–6),Pyr] ALM F30								**Glp**	Aib	Val	Aib	Gly	Leu	Aib	Pro	Val	Aib	Aib	*Glu*	Gln	Pheol	
[desAA(1–6),Pyr] ALM F50								**Glp**	Aib	Val	Aib	Gly	Leu	Aib	Pro	Val	Aib	Aib	*Gln*	Gln	Pheol	
SZ-A4	Ac	*Aib*	Ala	Aib	Ala	Aib	*Ala*	Gln	Aib	*Lxx*	Aib	Gly	Leu	Aib	Pro	Vxx	Aib	*Vxx*	Gln	Gln	Pheol	
Cervinin I	**Ac**	**Leu**	Aib	Aib	Leu	Aib	Pro	Ala	Aib	Val	Leu	*Leuol*										
Cervinin II	**Ac**	**Leu**	Aib	Aib	Leu	Aib	Pro	Ala	Aib	Val	Leu	*Leuol-OAc*										
Culicinin D	**BTA**	Pro	**AHMOD**	*Aib*	*Aib*	**AMD**	Leu	Aib	Leu	*β*Ala	**AAE**											
SCH 643432	**MOTDA**	Pro	Aib	Aib	Aib	*Aib*	Ala	Ala	Aib	Leu	Ala	Aib	Ala	Ala	Aib	**Arg**	Ala	Aib	Gly	Aib	Aib	Ala
EFR-G	Ac	**Pip**	Aib	**Pip**	**L-*Iva***	*Aib*	Leu	*β*Ala	Gly	*Aib*	Aib	***Pip***	Aib	Ala	Leu	**L-*Iva***	**PIHPPE**					
ACR-2	Ac	**Pip**	Aib	**Pip**	**D-*Iva***	**D-*Iva***	Leu	*β*Ala	Gly	***Acc***	Aib	***Pip***	Aib	Gly	Leu	*Aib*	**PIHPPE**					
Neoefrapeptin E	Ac	**Pip**	Aib	**Pip**	**D-*Iva***	**D-*Iva***	Leu	*β*Ala	Gly	***Acc***	**L-*Iva***	***Pip***	Aib	Gly	Leu	**L-*Iva***	**PIHPPE**					
Neoefrapeptin F	Ac	**Pip**	Aib	**Pip**	*Iva*	*Aib*	Leu	*β*Ala	Gly	***Acc***	Aib	***3MePro***	Aib	Gly	Leu	**L-*Iva***	**PIHPPE**					
Neoefrapeptin H	Ac	**Pip**	Aib	**Pip**	**D-*Iva***	**D-*Iva***	Leu	*β*Ala	Gly	***Acc***	*Aib*	***Pip***	Aib	Gly								
Cicadapeptin1	**Dec**	**Hyp**	**Hyp**	Val	Aib	Gln	Aib	*Leu*	**DAMP**													
Integramin B	**Ac**	**D-Iva**	**Hyp**	Ile	**L-Iva**	Leu	Aib	*Aib*	***Iva***	Hyp	Leu	**D-Iva**	Aib	Hyp	**Iva**	**Iva**	Gly					
TCP-IIa/-IIb	Ac	Aib	Gly	Ala	Lxx	*Aib*	*Gly*	*Lxx*	**Vxx**													
TCT-AIIa/-IIb	**Ac**	***Lxx***	Aib	Pro	*Vxx*	Aib	Pro	*Aib*	*Lxx*	Aib	Pro	Lxxol										
TCT-BIVa/-IVb	**Ac**	***Lxx***	Aib	Pro	Vxx	*Lxx*	Aib	Pro	*Aib*	*Lxx*	Aib	Pro	Lxxol									
TFR-A	**MDA**	Pro	***AHMOD***	Ala	Aib	*Aib*	*Lxx*	*Ala*	Aib	Aib	***AMAE***											
TFR-B	**MDA**	Pro	***AHMOD***	Ala	Aib	*Aib*	*Lxx*	*Ala*	Aib	Aib	***AAE***											
TFR-E	**MDA**	Pro	***Desmethyl-AHMOD***	Ala	Aib	*Aib*	*Lxx*	*Ala*	Aib	Aib	***AMAE***											

[a]) Abbreviations: ALM, alamethicin; SZ, suzukacillin; EFR, efrapeptin; ACR, acretocin; TCP, trichocompactin; TCT, trichocryptin; TFR, trichoferin. Exchangeable positions are italicized, new and uncommon constituents are highlighted in bold. Abbreviations of uncommon constituents are explained in the text and the *Figure*. Vxx, valine/isovaline; Lxx, leucine/isoleucine. Note, that only peptaibiotics displaying new or uncommon constituents are listed.

individual peptides, ALM F 50/2, 3a–3c, 4a, 4b, 5, 6a, 6b, 7, and 8a–8c, could be sequenced.

Recently, the same material used in [18] was further analyzed by nonaqueous capillary electrophoresis/mass spectrometry (NACE/MS): eleven amino acid sequences were identified, characterized by the exchange of Ala to Aib in position 6, Gln to Glu in position 7 or 19. To continue, two novel ALM were detected, which are characterized by the loss of the C-terminal Pheol residue, thus terminating in Gln. Notably, two truncated 14-residue sequences carrying an N-terminal pyroglutamyl group (commonly abbreviated as Glp, pGlu, or Pyr) were found. Overall, seven new minor sequences are reported compared to [18]. To the best of our knowledge, this is the first report on the occurrence of Glp as a constituent of fungal peptides in nature. Literature search did not reveal any previous publication regarding the isolation of peptide-bound Glp from a fungal source. To exclude the artificial formation of the Glp-peptaibols during workup or long-term storage, degradation studies were performed. Although neither treatment increased the amount of the truncated Glp peptides compared to untreated samples, further studies have to be performed in order to unequivocally prove the origin of the truncated Glp peptaibols [19].

Suzukacillin. Suzukacillin (SZ) was isolated from the culture broth of *Trichoderma viride* strain 63C-I and separated by TLC into two fractions, suzukacillins A (R_f 0.18) and B (R_f 0.70) [20][21]. Using the material provided by Dr. *T. Ooka*, the major fraction, SZ-A, was further characterized and a preliminary sequence published [22]. Although it appeared to be uniform in TLC, SZ-A could be further separated by HPLC, yielding 13 individual peaks. Nevertheless, two of them, SZ-A10 and -11, still represented a microheterogeneous mixture. Thus, 15 individual 20-residue peptaibols, SZ-A1–A9, -A10a/b, -A11a/b, and SZ-A12–A13, were shown to be present in total. Despite the microheterogeneity, complete sequences of all SZ-A peptaibols and chirality of the individual amino acids have been determined by HPLC/MS^n and GC/EI-MS approaches [23]. In this context, the previously established presence of D-Iva [24] was confirmed.

Trichobrachin. Trichobrachin (TB) has been isolated from *Trichoderma longibrachiatum* CBS 936.69 – a strain that is now reclassified as *Trichoderma ghanense*. Three major groups designated TB I, TB II, and TB III could be separated and isolated by preparative TLC on silica gel [9]. Recently, the formation of peptaibiotics by *Trichoderma parceramosum* (*ghanense*) CBS 936.69 was thoroughly reinvestigated [25]. The trichobrachin mixture comprises ten 19-residue peptides with free C-terminal Gln residues (TB I peptides), two 18-residue peptides with free C-terminal Gln residues (TB II 1 and TB II 2), seven 20-residue peptides with C-terminal amide-bonded Pheol (TB II 3–10), and thirty-four eleven-residue peptides with either C-terminal Leuol, Ileol, or Valol (TB III 1–34). TLC Analysis of the dynamics of TB formation and degradation unequivocally demonstrated that those two 18-residue TB I and TB II peptides with a free carboxy terminus resulted from enzymatic C-terminal degradation of 20-residue TB II peptides.

Antiamoebin. The 16-residue peptaibols antiamoebins (AAM) I–XVI, known for their antihelminthic and antiprotozoal activities had previously been reported from cultures of *Emericellopsis synnematicola*, *Emericellopsis poonensis*, and *Verticillium epiphytum* (syn. *Cephalosporium pimprina*), but have also been isolated from

Clonostachys rosea f. *catenulata* (syn. *Gliocladium catenulatum*) CBS 511.66, as well as *Stilbella fimetaria* (syn. *Stilbella erythrocephala*) CBS 548.84 and ATCC 28144 [26]. Notably, most of the above producers of AAM are fungicolous or coprophilous fungi. Fruiting bodies of mushrooms and toadstools, but also dung, are generally regarded as highly competitive substrates. In an impressive study [27], it has unequivocally been proven that antiamoebins were responsible for antibiosis in colonized herbivore dung.

Four strains of *Stilbella fimetaria* (syn. *S. erythrocephala*) were isolated from dung of wild rabbits (D 99026, D 01024, and D 03001) or of the tortoise *Testudo hermanni* (D 03012). Furthermore, dung pellets naturally colonized by *S. fimetaria*, were collected in the field, lyophilized, and extracted. The same was carried out with pre-sterilized dung that has been artificially inoculated with the four strains mentioned above. As expected, AAM could be detected in all liquid cultures. To continue, the total AAM concentration – both in wild and artificially inoculated dungs – was 126–624 μg/g fresh weight, with minimum inhibitory concentrations against most other coprophilous fungi being at or below 100 μg/ml. It should be pointed out that this is the first report describing the detection and isolation of peptaibiotics from natural substrates. The diterpene antibiotic myrocin B, not previously described from *S. fimetaria*, was also produced, but only at low, nonfungicidal levels (5.3 μg/g). As no other antifungal substances could be detected, a decisive role of the broad-spectrum antimycotic AAM during colonization of dung was proposed.

Cervinins I and II. The genus *Mycogone* exclusively comprises fungicolous fungi. *M. perniciosa* and *M. rosea* are known as devastating, nonspecific parasites of agarics. These two species have been reported to cause economically relevant losses in commercial mushroom growing [28]. *M. rosea* is known as the producer of nine-residue lipoaminopeptides: strain DSM 8822 was reported as the producer of helioferins A and B [29], whereas strain DSM 12973 produced both helioferins and the closely related roseoferins A–G [30]. Other species of *Mycogone* infect a rather narrow range of host fungi: *M. calospora*, for instance, exclusively parasitizes coral fungi of the genus *Ramaria* (Gomphales, Ramariaceae), whereas *Mycogone cervina* has been reported on the Glazed Cup, *Mycolachnea hemisphaerica* (Pezizales, Pyronemataceae), and on false morels (Pezizales, Helvellaceae) so far [31].

Recently, *M. cervina* strain A09–02 was isolated as a parasite of the Vinegar Cup *Helvella* (*Paxina*) *acetabulum* and shown to produce two twelve-residue peptaibol antibiotics both exhibiting Ac-Leu as a new N-terminal motif [32]. Moreover, Leuol is found as the C-terminal residue of cervinin I. Notably, no attempts were made to determine the configuration of the amino acids. Cervinin II, however, has the same sequence of amino acids, but its C-terminal Leuol residue is acetylated [32]. As no comments were made with respect to the origin of the latter C-terminus it cannot be completely excluded that cervinin I could have been partly acetylated during workup, thus generating cervinin II. Furthermore, the authors stated that peptaibols exclusively contain L-amino acids. The latter assertion is not acceptable because a number of well-known counter-examples concerning the occurrence of D-Iva in peptaibiotics has been published and discussed in [2]. To the best of our knowledge, this is the first report on the isolation and structure elucidation of secondary metabolites from *M. cervina*. Surprisingly, no lipoaminopeptides have yet been recorded for that species.

2.2. *Lipoaminopeptides. Culicinin.* Four ten-residue lipoaminopeptides, culicinins A–D, were recently characterized from an Australian isolate of *Culicinomyces clavisporus* (Hypocreales, Clavicipitaceae). Strain LL-12I252 was isolated from larvae of the biting midge *Forcipomyia marksae* (Diptera, Ceratopogonidae). Culicinins exhibit a number of common structural features: their N-terminal Pro residue is protected by butanoic acid (BTA). In 2-position, 2-amino-6-hydroxy-4-methyl-8-oxodecanoic acid (AHMOD) is found. Another lipoamino acid, 2-amino-4-methyl-decanoic acid (AMD), is situated in position 5, while β-alanine (3-aminopropanoic acid: APA) is located in position 9 of the peptide chain. The C-terminus is substituted by 2-(2′-aminopropyl)aminoethanol (AAE), here referred to as APAE [33]. The lipoamino acid AHMOD is present in position 2 of almost all lipoaminopeptides, being the characteristic structural element of this subgroup of the peptaibiotics. C-Terminal AAE has previously been described for trichopolyns, helio- and roseoferins (as reviewed in [2]). Notably, BTA and AMD have been detected in peptaibiotics for the first time, thus representing novel structural elements. Selective inhibitory activity against PTEN-negative MDA468 tumor cells has been observed.

2.3. *Other Peptaibiotics. SCH 466457 and SCH 466456*. Two antifungal peptaibiotics carrying an N-terminal 2-methyl-3-oxotetradecanoyl (MOTDA) residue were isolated from a taxonomically unidentified fungus [34]. The first compound, SCH 466457, represents a 17-residue peptaibiotic which terminates in free Ala. The second compound, SCH 466456, exhibits the same amino acid sequence in positions 1–16, and another Ala residue follows in position 17. Notably, a free Aib residue is found in the C-terminal position 18. To the best of our knowledge, this is the first report in literature describing the occurrence of free Aib residue as the C-terminal constituent of a peptaibiotic. Both compounds were active against *Candida* ssp., *Aspergillus* ssp., and *Trichophyton* dermatophytes.

SCH 643432. Two antifungal 21-residue peptaibiotics, the N-termini of which are also blocked by a MOTDA residue, were isolated from *Paecilomyces variotii* SCF 1559 [8]. They exhibit some structural homology with the 21-residue texenomycins A and B [35], as well as the six-residue lipohexin which represents a deletion sequence of the texenomycins. However, the configuration of the amino acids has not been determined. Both peptaibiotics are produced by *Acremonium lindtneri* (syn. *Moeszia lindtneri*, teleomorph: *Hypomyces chrysostomus*) DSM 11119, *Mariannaea elegans* CBS 120677 (i265), and *M. elegans* CBS 120687 (i910), the latter two strains originally being misidentified as *Paecilomyces* sp. [36]. As in the case of lipohexin, the C-terminus of SCH 643432-(1) is composed of free alanine or β-alanine, respectively. The primary structure of the isobaric compound SCH 643432-(2) has not yet been published. There are some examples of peptaibols, the C-terminus of which are composed of a free amino acid: Gly is known as a constituent XR586 from *Acremonium persicinum* X21488 [37], from both integramides A and B, and from two neoefrapeptins (see below). Ser has been described for cephaibols P and Q from *Acremonium tubakii* DSM 12774 [38], Val is known from the trichobrachins TB IIa A and B [9], and Gln has recently been detected in two minor compounds of alamethicin ([19], see above). Furthermore, the presence of Vxx has recently been reported for trichocompactins I–XII from *Trichoderma brevicompactum* and *T.* cf. *brevicompactum* [4] as well as *T. stromaticum* [14].

Efrapeptins/Elvapeptins. The 16-residue efrapeptins (EFR) [39] have been isolated from *Tolypocladium inflatum* (syn. *T. niveum*, teleomorph: *Cordyceps subsessilis:* [40]). The material referred to as tolypin by *Matha et al.* [41] mainly consists of efrapeptin F. The potent antifungal and insecticidal activity of these peptaibiotics is best explained by inhibition of mitochondrial ATPase [42]. In 1982, a partial sequence of efrapeptin D, the major compound, was published [43]. However, the structure of the C-terminus was not elucidated until 1991, when efrapeptins C, D, E, F, G, and H were shown to be blocked by *N*-peptido-1-isobutyl-2-(2,3,4,6,7,8-hexahydro-1-pyrrolo[1,2-*a*]pyrimidinio)ethylamine [39], subsequently abbreviated as PIHPPE. Furthermore, production of efrapeptins has been demonstrated for *Tolypocladium geodes* [44] and the two entomopathogenic species *T. parasiticum* [45] and *T. cylindrosporum*, as well as for *T. nubicola* and *T. tundrense* [46]. It was suggested to use the pattern of efrapeptins as a chemotaxonomic marker to distinguish between morphologically similar species of *Tolypocladium*. However, efrapeptin production seems to be not only species-, but strain-specific. The remarkable antifungal and insecticidal activity of the efrapeptins may contribute to the ecophysiology of their fungal producers as their *in vivo* formation has also been demonstrated [45]. Further minor compounds designated efrapeptins A, H, and I have been reported, but no attempts were made to elucidate their structures [47].

Recently, two highly methylated linear peptides, as well as efrapeptin G, were isolated from the marine fungus, strain 021172cKZ, associated with the sponge *Teichaxinella* sp. (Halichondria, Axinellidae). The taxonomy of the producer remains, however, confusing: it was identified as *Leucosphaerina indica* (Hypocreales, Sordariomycetes, Ascomycota) *via* molecular methods, but classified as an atypical *Acremonium* sp. through morphology-based examination [48].

To clarify the biosynthetic relationship between efra- and elvapeptins (ELV) that have been obtained as co-metabolites from *Tolypocladium inflatum* IMI 202309 [49], a study was conducted which demonstrated the *in vitro* conversion of elvapeptins to efrapeptins by oxidative cyclization with CuCl/pyridine. From this observation, the authors concluded that elvapeptins may contain a C-terminal spermidine residue that might also be converted to the amine residue of efrapeptins *in vivo* [50].

Acretocin. Another group of peptaibiotics, acretocin (ACR), that is very closely related to the efrapeptins has been detected in *Acremonium croticinigenum* CBS 217.70 – a species that is known as a facultative parasite of a number of polypores (= bracket fungi) and agarics. Unlike efrapeptins which carry L-Iva ((*S*)-Iva), the presence of D-Iva ((*R*)-Iva) has been established for acretocins. Remarkably, acretocins I–VI differ from the efrapeptins C–G by a mass difference of 2 Da, an observation that led to the assumption that an α,β-didehydro amino acid, presumably α,β-didehydro-α-aminobutanoic acid, might be present [51]. Although α,β-didehydro amino acids have not yet been reported as constituents of peptaibiotics, they are frequently found in peptide antibiotics from *Gram*-positive bacteria. The most well-known examples are lantibiotics, such as gallidermin which is produced by the *Gram*-positive bacterium *Staphylococcus gallinarum* (F16/P57) Tü 3928 [52]. Apart from those antibiotics, the presence of α,β-didehydro-α-aminobutanoic acid has recently been established for hassallidin A [53] and B [54] – two glycosylated *cyclo*-lipopeptides from the epiphytic cyanobacterium *Hassallia* sp. (Nostocales, Microchaetaceae). Comparing the sequences of the neoefrapeptins [55], the presence of 1-aminocyclopropane-1-carboxylic acid

(Ac_3c) in acretocins instead of an α,β-didehydro amino acid was established recently by us (to be published).

Neoefrapeptins. Recently, ten 16-residue and two 13-residue neoefrapeptins A–N with insecticidal activity [55] were isolated from *Geotrichum candidum* SID 22780 (Saccharomycetales, Endomycetaceae; teleomorph: *Galactomyces candidus*). Notably, strains of *G. candidum* are of relevance in smear-ripened cheeses. It should be pointed out that this is the first report in the literature regarding the isolation and structural elucidation of a peptaibiotic from a yeast-like fungus. A nonribosomal peptide synthetase (NRPS) has recently been assigned as the key enzyme for production of the cyclohexapeptide siderophore ferrichrome by the fission yeast *Schizosaccharomyces pombe* [56]. Thus, the detection of further peptaibiotics from yeast-like organisms could be postulated.

Neoefrapeptins revealed a number of uncommon structural elements that are newly described for peptaibiotics: Ac_3c (abbreviated as Acc in the *Table* and in [55]) and (2*S*,3*S*)-3-methylproline (*cis*-L-3-MePro) were identified. Configuration analysis of the 16-residue neoefrapeptins revealed L-Iva[15]. Except for the Aib-containing neoefrapeptins D and N, Iva is present in position 4. Some of the neoefrapeptins also contain Iva[5] and Iva[10]. If present in position 4 and/or 5, the Iva residue was assigned the D-configuration, whereas the Iva residues in positions 10 and 15 were shown to possess the L-configuration. The L-configuration was assigned to all pipecolic acid (Pip) residues found in neoefrapeptins.

Cicadapeptins. Some species of *Cordyceps* (Hypocreales, Clavicipitaceae) are widely used in traditional Chinese medicine. Structures of known bioactive secondary metabolites and their pharmacological effects have thoroughly been reviewed [57]. The genus contains mostly entomopathogenic (*C. sinensis*, *C. militaris*, *C. pruinosa*) and fungicolous fungi (*C. ophioglossoides*, a parasite of underground deer truffles, *Elaphomyces* spp.). Recently, two eight-residue peptaibiotics, cicadapeptins I and II, were isolated from *Cordyceps heteropoda* ARSEF#1880, a parasite of the Australian cicada *Cicadetta puer* (Homoptera, Cicadaceae). The N-terminus of these peptaibiotics is blocked by a decanoic acid residue, and the C-terminus is amidated by 1,2-diamino-4-methylpentane (DMAP) [58]. Decanoic acid as N-terminal substituent of peptaibiotics has previously described for the lipopeptaibol LP237/F 7 [59] obtained from the soil-borne *Tolypocladium geodes* LP237, whereas 1,2-diamino-4-methylpentane represents a new structural element of peptaibiotics. Another distinguishing feature is the presence of two consecutive *trans*-L-4-Hyp residues. Notably, such Hyp-Hyp motif has been described for the first time to occur in peptaibiotics.

Integramides. Two novel inhibitors of HIV-1 integrase, the 16-residue integramides A and B, were isolated from extracts of *Dendrodochium* sp. MF 6888 [60]. The taxonomic position of the anamorphic fungal genus is yet unclear but some representatives seem to belong to the family Bionectriaceae of the order Hypocreales. Remarkably, integramides contain an acetylated N-terminal D-Iva and a total of five (A) or six (B) D- or L-Iva residues. Among the natural peptaibiotics, integramides are those containing the largest proportion of Iva described to date. Free Gly is found at the C-terminus of both peptaibiotics.

2.4. *Peptaibiomics – A Novel Methodical Approach in the Search for Peptaibiotics.* Recently, the technical term '*peptaibiomics*' was proposed, describing, in analogy to

proteomics, the approach to analyze the entirety of peptaibiotics, the so-called 'peptaibiome', produced by a certain strain under defined conditions. An advanced, rapid, and selective method was introduced that comprises solid-phase extraction (SPE) of peptaibiotics on C_{18}-cartridges followed by on-line reversed-phase (RP) HPLC coupled to an ion trap electrospray tandem mass spectrometer (ES-MS). The presence of peptaibiotics is indicated by the characteristic mass differences of m/z 85.1 Da, representing Aib residues which can be observed in the *b*-series of acylium fragment ions resulting from ESI-MS. This method was used to analyze the peptaibiome of recently described species of *Hypocrea* and *Trichoderma*. Peptaibiotics produced by the following strains were partially sequenced and compared to published sequences: *Hypocrea muroiana* MUCL 28442, *H. nigricans* MUCL 28439, *H. gelatinosa* CBS 724.87, *H. dichromospora* CBS 337.69, H. *vinosa* CBS 247.63, *H. semiorbis* CBS 244.63, and *H. citrina* (syn. *H. lactea*) CBS 853.70; *Trichoderma asperellum* CBS 433.97, *T. aggressivum* f. *europaeum* CBS 100526, *T. inhamatum* CBS 345.96, and *T. stromaticum* CBS 101875 [13].

Three strains of *Trichoderma brevicompactum* (CBS 109720, CBS 119569, CBS 119570) and another four which are closely related to that species (*Trichoderma* cf. *brevicompactum* CBS 112445, CBS 119576, CBS 119577, and NRRL 3199) were analyzed for the formation of peptaibiotics. These isolates were selected because of an antagonistic potential against Eutypa dieback and Esca, fungal diseases of grapevine (*Vitis vinifera*) trunks, that have not yet been investigated for the production of peptide antibiotics. All strains were found to produce membrane-active alamethicins F 30 (see above). In addition to that, novel peptaibols were detected, namely fourteen twelve-residue trichocryptins B, twelve eleven-residue trichocryptins A, nineteen eleven-residue trichobrevins A and B, six ten-residue lipoaminopeptides – trichoferins, and seventeen eight-residue trichocompactins, the latter terminating in free Vxx [5]. Taken together, the differential patterns of alamethicin production as well as the production of different trichothecene-type mycotoxins clearly support DNA-sequencing results. Both molecular and chemotaxonomic approaches indicate the existence of two species within what has been called *Trichoderma brevicompactum*. The taxonomy of *Trichoderma brevicompactum* is the subject of an ongoing study because the species, as presently circumscribed, is still heterogeneous.

The same approach has been applied to another eight grapevine-protective strains of *Trichoderma* species (*T. strigosum*, *T. erinaceus*, *T. pubescens*, *T. stromaticum*, and *T. spirale* as well as *T.* cf. *strigosum*, *T.* cf. *pubescens*): new seven-, ten-, and eleven-residue lipopeptaibols, with N-terminal alkanoyl, and C-terminal Leuol or Ileol residues were found, and named lipostrigocins and lipopubescins. Furthermore, new 18-residue peptaibols named trichostromaticins, and 19-residue peptaibols, named trichostrigocins, were discovered. One peptaibiotic carrying a free C-terminal Vxx, named trichocompactin XII, was also sequenced. In summary, these compounds may partially be responsible for plant-protective action of the producers [5][14].

3. Discussion. – The number of peptaibiotics published in the literature remarkably increased during the last three years. A first major reason explaining this phenomenon is that the analytical equipment used for screening and structural elucidation of peptide antibiotics is becoming more and more sophisticated [61]. New analytical approaches,

as illustrated by the method of peptaibiomics, target-oriented selection, and optimization of separation techniques, as well as the use of state-of-the-art methods in mass spectrometry, enable the detection of homologues and positional isomers, even if present in trace amounts. In principle, only one fully-grown *Petri* dish or slope is required for a first routine analysis of the peptaibiome of a fungal culture. In this context, it should be clearly pointed out again that Iva is frequently found as D- or L-isomer in peptaibiotics [2]. To continue, both enantiomers of Iva can be present in the same peptaibiotic, as was reported for neoefrapeptins and integramides (see above) as well as for the 15-residue peptaibol clonostachin: the latter contains D-Iva in positions 4 and 13, whereas L-Iva is found in positions 7 and 10 [62]. On total hydrolysis, an apparently racemic mixture (*RS*)-DL-Iva is released. Separation of DL-Iva and DL-Pip, analyzed as *N*-trifluoroacyl amino acid propyl esters on *Chirasil*-L-*Val*™ (*N*-propionyl-L-valine-*tert*-butylamide polysiloxane), is not always satisfactory, as it depends both on the age and quality of the capillary columns provided by various manufacturers. However, Iva enantiomers can be resolved on *Chirasil*-L-*Val*™ after conversion into *N*-acetylisovaline propyl esters [63]. Instead of *Chirasil*-L-*Val*™, a *Lipodex*™ *E* column, representing a functionalized γ-cyclodextrine, can be used providing excellent resolution of Iva and Pro enantiomers [64]. Notably, all eight stereoisomers of 3- and 4-Hyp can be resolved on *Chirasil*-L-*Val*™ [65]. Alternatively, pre-column derivatization with the chiral N^{α}-(5-fluoro-2,4-dinitrophenyl)-L-alanine amide (FDAA; *Marfey*'s reagent), and subsequent HPLC or HPLC/ESI-MS analysis of the resulting diastereoisomers is recommended [66]: by this method, DL-Iva and DL-Pip are very well resolved. Last but not least, methanolysis of peptaibiotics, followed by trifluoroacetylation and analysis of the resulting dipeptides by GC/EI-MS, has been introduced as a method to solve the problem to assign the positions of isobaric amino acids Val/Iva and Leu/Ile, respectively [26]. Recent advances in quantitation of DL-amino acids are summarized in [67].

Second, screening of fungi from highly competitive habitats such as fungicolous, coprophilous, and entomopathogenic species remarkably enhances the probability to find new sequences of peptaibiotics. For instance, trichostromaticins A–E [14] may contribute to the potent bioactivity of *Trichoderma stromaticum* [68] (teleomorph: *Hypocrea stromatica* [69]). Hyperparasitic strains of this species that is known as a biologically active cacao endophyte have successfully been introduced in field control of the causal agent of Witches' Broom Disease of cacao (*Theobroma cacao*) in South America. They were shown to effectively suppress basidioma formation of the plant pathogen *Crinipellis perniciosa* (Tricholomataceae, Agaricales) [68][70]. High selection pressure in such environment favors strains capable of producing secondary metabolites with interesting biological activities that may effectively eliminate competitors. Owing to their exceptional membrane-penetrating action as exemplified in [71–74], peptaibiotics may play a decisive role in natural habitat as clearly demonstrated [27]. This observation supports the hypothesis of a parallel formation of hydrolytic enzymes and peptaibiotics, as their synergistic action was attributed an important role in mycoparasitism between *T. harzianum* and its fungal hosts such as *Botrytis cinerea* [75][76]. Depending on the *Trichoderma* strain/species studied, other antibiotic metabolites such as α-pentyl pyrones (6-PAP: 6-pentyl-2*H*-pyran-2-one and related structures [77]), as well as trichothecenes [78], may play an important role.

It can be hypothesized that the number of peptaibiotics described in the near future will increase considerably. For instance, the genus *Trichoderma* (with teleomorphs in *Hypocrea* [79]) is generally regarded as the richest source of peptaibiotic-producing species [80]. At the beginning of 2005, it was shown to comprise 88 species [81]. Two new species, for example, have been reported as endophytes of cocoa [82]. In April 2006, more than 100 *Trichoderma* species could be distinguished by molecular methods [83]. Many more – detected in sapwood of trunks of *Theobroma* spp., *Cola* spp., *Fagus sylvatica* (beech), *Scalesia pedunculata* (Daisy tree, Asteraceae), and in the woody liana *Ancistroderma korupensis* – have recently been described [84][85]. To continue, two new species of *Hypocrea*, *H. voglmayrii* [86] and *H. cristalligena* [87], have also been published.

Modern methods of molecular taxonomy have already led to significant changes in fungal systematics. The contribution of chemotaxonomy to this process is yet unclear but should not be overestimated. Nevertheless, the differential patterns of alamethicin production, as well as the production of different trichothecene-type mycotoxins, clearly supported necessary subdivision that is evident from the DNA-sequencing results in the *Trichoderma brevicompactum* complex. Both molecular and chemotaxonomic approaches indicate the existence of two species within what has been called *Trichoderma brevicompactum*.

With the exception of neoefrapeptins (see above), peptaibiotics have only been reported and sequenced in three families, Hypocreaceae, Clavicipitaceae, and Bionectriaceae of the order Hypocreales. Publications regarding the isolation of peptaibiotics from basidiomycetes should be considered very critically as outlined in [2]. In some cases, an infection of an asco- or basidiomycete's fruiting-body with mycoparasites might not be visible to the naked eye. For that reason, it cannot be excluded that younger stages of infection have accidentally not been recognized macroscopically, thus leading to extraction of infested material. This was first pointed out in [88]. The authors isolated chrysospermins A–D from the new Vietnamese species *Xerocomus langbianensis* (Boletaceae, Boletales) [89]. In contrast, in [35][90][91], the production of the respective peptaibiotics texenomycin, boletusin, and tylopeptin were attributed to the extracted fruiting bodies of the Potato Earthball *Scleroderma texense* (syn. *Scleroderma bovista*, Sclerodermataceae, Boletales), *Boletus* sp., and the Bitter Bolete *Tylopilus neofelleus* (Boletaceae, Boletales). It is likely that an unrecognized infection of *X. langbianensis* with *Sepedonium* sp. was the reason for detection of the four chrysospermins [88]. It should be considered that chrysospermins have originally been described from *S. chrysospermum* [92], a widespread parasite of the order Boletales [31].

To date, 18 genera of imperfect and ascomycetous fungi have been recognized to produce *ca.* 700 sequences of peptaibiotics. Most of the structures reviewed here and in [2] were isolated from the genera *Trichoderma* and its *Hypocrea* teleomorphs, from *Acremonium*, *Tolypocladium*, *Paecilomyces*, *Emericellopsis*, and *Sepedonium*. Less commonly reported producers were found in *Verticimonosporium*, *Stilbella*, *Mycogone*, *Mariannaea*, *Myrothecium*, *Clonostachys*, *Culicinomyces*, *Cordyceps*, *Geotrichum*, and *Dendrodochium*. Regardless of a number of attempts undertaken to validate the previously reported detection of Aib in hydrolyzed extracts of *Penicillium roquefortii* [93] and *P. nalgiovense* [94], both species could not be confirmed as producers of

peptaibiotics. Nevertheless, the abundance of data fully confirm our predictions expressed almost two decades ago [93][94].

By GC-FID and GC/EI-MS approaches, Aib, and pure enantiomers or mixtures of D- and L-Iva were found to occur in HCl hydrolysates of organic extracts of a number of ascomycetes. This comprises the following strains [95]: *Lecythophora mutabilis* CBS 303.62, *Nectriopsis candicans* CBS 627.72 (Bionectriaceae, Hypocreales), *Clonostachys candelabrum* (syn. *Sesquicillium candelabrum*) CBS 205.69 (Bionectriaceae, Hypocreales), *Bionectria pityrodes* CBS 322.78 (syn. *Nectria pityrodes*, Bionectriaceae, Hypocreales), *Niesslia aemula* CBS 556.75, *N. exigua* CBS 152.68, *N. exilis* CBS 560.74, as well as *Niesslia* sp., strains CBS 236.74 and CBS 477.74 (Niessliaceae, Hypocreales), and the related anamorphs *Monocillum mucidum* CBS 306.70 and *M. nordinii* CBS 101.63 (Niessliaceae, Hypocreales), *Wardomyces columbinus* CBS 233.66, *W. humicola* CBS 368.62, and *W. inflatus* CBS 367.62 (Microascaceae, Microascales). From these observations, the formation of peptides containing these nonproteinogenic α-alkyl amino acids can be concluded. Consequently, those strains should be regarded as particularly promising sources for future screening approaches. To continue, free Aib and enantiomeric mixtures of D- and L-Iva have actually been detected to occur in aqueous extracts of cultures of *Nectria cinnabarina* (Nectriaceae, Hypocreales) and a number of *Hypomyces* sp. (personal observation). To understand the importance of the latter finding, additional investigations would be required.

Generally, formation of peptaibiotics should rather be discussed as an adaptation to highly specialized life styles of the producers occasionally being marine symbionts [48][96], but mostly facultative or obligate plant pathogens, fungicolous or entomopathogenic fungi occupying some particular ecological niches.

Overall, fungal biodiversity creates differences in chemical structures that may exhibit new bioactivities, thus leading to the development of new drugs for use in human and veterinary medicine, and in agriculture and forestry.

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Note added in Proof. Four nine-residue peptaibiotics, MS-681a, b, c, and d, which act as inhibitors of myrosin light chain kinase, were isolated from *Myrothecium* sp. KY6568 [97]. Notably, the configuration of the Iva^{8} and the Iva^{2} residue was shown to be 'L', whereas Iva^{5} was assigned the 'D'-configuration. The C-terminus of all four homologues is protected by the same polyamine, (2′*RS*)-N^{1}-(2-amino-3-phenylpropyl)spermidine, being another novel constituent of peptaibiotics. [98]. Two 20-residue peptaibiotics, septocylindrin A and B, have been isolated from the fungus LL-Z1518 [99]. Amino acid residues 1–19 and configurations of the amino acids are identical with those reported for alamethicin F30/3 of alamethicin F50/5, respectively.

Septocylindrins differ from alamethicins only in the structure of the C-terminal alcohol, which is 2-(2-amino-3-phenylpropylamino)ethanol (Phaol). Such C-terminus has only been described for aibellin, a 20-residue peptaibiotic from *Verticimonosporium ellipticum* D1528 before [100]. However, the identification of the producer as *Septocylindrium* sp. is questionable as the authors did not provide taxonomic data to verify its identity [101].

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