REVIEW

Natural Cyclopeptaibiotics and Related Cyclic Tetrapeptides: Structural Diversity and Future Prospects

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Linearity is not considered a prerequisite anymore, and extension of the current definition of 'peptaibiotics' to cyclic, Aib-containing peptides is proposed. Sequences and bioactivities, together with ecophysiological importance of cyclopeptaibiotics and related cyclic tetrapeptides, and their fungal-taxonomic relationships, are discussed.

Introduction. – Peptaibiotics constitute a constantly growing family of peptide antibiotics [1–3]. Currently, more than 800 individual sequences of peptaibiotics, produced by members of *ca.* 20 fungal genera, are reported in the literature. Peptaibiotics are defined as linear polypeptide antibiotics which *i*) have a molecular weight between 500 and 2,200 Dalton, thus containing 5–21 amino acid residues; *ii*) show a high content of α-aminoisobutyric acid (Aib); *iii*) are characterized by the presence of other non-proteinogenic 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. The majority of Aib-containing peptides carries a C-terminal residue representing a 2-amino alcohol, and this subgroup is, therefore, referred to as *peptaibols*. In strongly lipophilic peptaibols, the N-terminus is acylated by octanoic, decanoic, or (*Z*)-dec-4-enoic acid, and these are named *lipopeptaibols* [4]. In the third subfamily of *lipoaminopeptides* (also named 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 present knowledge, the compound, 2-amino-6-hydroxy-4-methyl-8-oxodecanoic 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

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subfamilies [1]. A review introducing nine subfamilies according to structural homologies of peptaibiotics has been published [5], but these need to be revised and updated, because more than half of the currently known sequences of peptaibiotics have been discovered since then [2][3][6].

Peptaibiotics are usually classified according to their main chain length, as long-chain (17–21 residues), medium-chain (11–16 residues), and short-chain (5–10 residues) sequences. Although not yet reported in literature, *Brückner* and co-workers [1] claimed the detection of very short chain (<5 residues) peptaibiotics that may represent drastically truncated sequences.

Cyclopeptaibiotics – A New Subfamily of Peptaibiotics. – We do not consider linearity as a necessary prerequisite anymore and propose to extend the above definition of 'peptaibiotics' to encompass also cyclic, Aib-containing peptides. Currently, this small subgroup comprises seven cyclic tetrapeptides all of them displaying the same building scheme (*Fig. 1*).

Sequences and bioactivities, ecophysiological and taxonomic importance of cyclopeptaibiotics and related cyclic peptides will be discussed below. Finally, this review is aimed at linking the remarkable chemical and biological properties of linear and cyclic Aib-containing peptides.

Chlamydocin. – Originally isolated from the soil fungus *Diheterospora* [7] (now *Pochonia*) *chlamydosporia* (Clavicipitaceae, Hypocreales; teleomorph *Metacordyceps* (syn. *Cordyceps*) *chlamydosporia* [8]) S 3440, chlamydocin (**1**) is regarded as the classical paradigm of a 'cyclopeptaibiotic'. The chlamydocin-producing species is known as an egg parasite of cyst nematodes such as *Heterodera schachtii* in beet or root-knot nematodes of the genus *Meloidogyne*. As this fungus is still found to be genetically heterogeneous [8], some biochemical heterogeneity can be expected that might entail the production of structurally variable, microheterogeneous mixtures of cyclic peptides by different strains. To the best of our knowledge, chlamydocin (**1**) is the first example of a cyclic, Aib-containing peptide reported in literature. Chlamydocin (**1**) was sequenced after trifluoroacetolysis and subsequent *Edman* degradation; the configuration of the amino acids was determined by treatment of the HCl hydrolysate with L- and D-amino acid oxidase. Notably, its proline residue was assigned the D-configuration. In this context, it should be pointed out again that isovaline (Iva) is frequently reported as D- or L-isomer in peptaibiotics, whereas all other chiral α-amino acids of linear peptaibiotics known so far have been reported to possess L-configuration. Both enantiomers of Iva may even be present in the same peptaibiotic [1].

The uncommon lipoamino acid of chlamydocin (**1**) was assigned as L-2-amino-9,10-epoxy-8-oxodecanoic acid (Aoe). A minor component, **2**, carrying L-2-amino-9,10-epoxy-8-hydroxydecanoic acid (Ahe) was also detected instead of Aoe in the crude extract of *Pochonia chlamydosporia* S 3440 [7].

Based on structural homology, it was demonstrated that chlamydocin (**1**) displayed unspecific phytotoxic bioactivity [9], which is in agreement with its cytostatic activity in rodents [10]. Stereoselective total syntheses of chlamydocin (**1**) and dihydrochlamydocin have been reported [11].

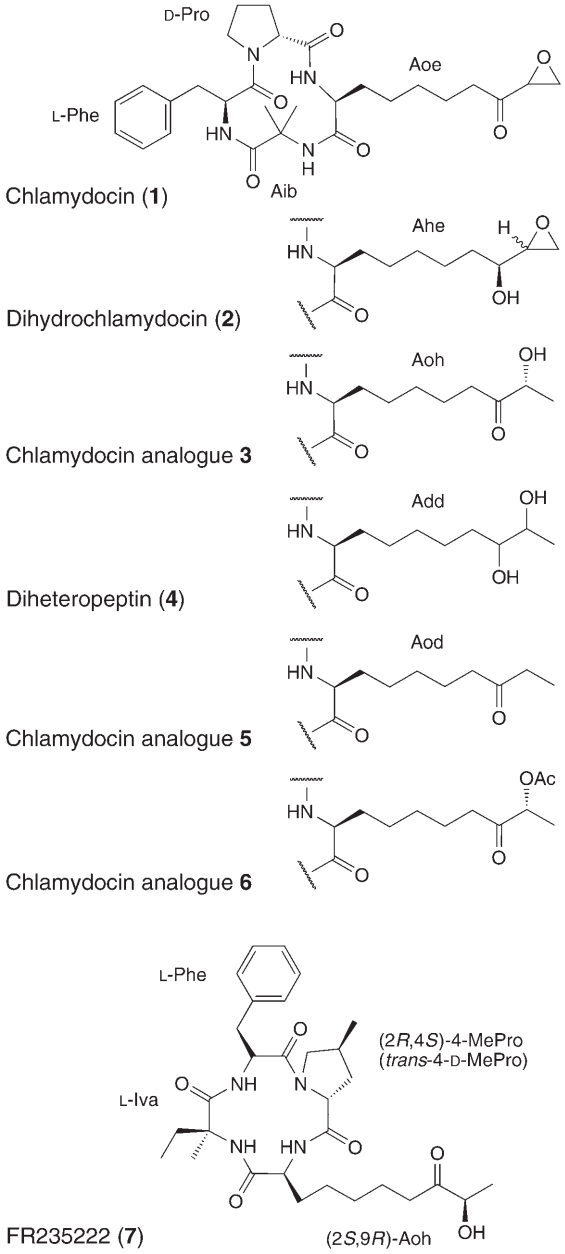


Fig. 1. *Structures of cyclotetrapeptaibiotics*

Chlamydocin Analogue from *Verticillium coccosporum*. – Twenty years after the first report on chlamydocin (**1**) had been published, a second cyclopeptaibiotic, **3**, was

isolated from a strain of '*Verticillium coccosporum*' (without accession number) obtained from the USDA-ARS collection of entomopathogenic fungi [12]. This isolate might be identical with *Pochonia suchlasporia* var. *catenata* CBS 789.85 – a strain that has originally been deposited as *Verticillium coccosporum*, subsequently preserved as *Verticillium suchlasporium* var. *catenatum*, and finally included in *Pochonia* [8]. The species *Pochonia suchlasporia* var. *catenata* is known as an egg parasite of the cereal cyst nematode *Heterodera avenae* and the Gypsy moth *Lymantria dispar*. The lipoamino acid of this analogue was assigned L-(9*S*)-2-amino-9-hydroxy-8-oxo-decanoic acid (Aoh). The analogue showed high phytotoxic activity against common Duckweed, *Lemna minor* [12].

Diheteropeptin. – In 1997, the isolation of diheteropeptin (**4**) from a *Diheterospora* sp. was reported [13]. The producing fungus was subsequently identified as *Diheterospora chlamydosporia* Q 58044 [14], thus representing the same species as the chlamydocin producer S 3440. Diheteropeptin (**4**) is distinguished from the above chlamydocin homologues by the presence of a (2*S*,8*R*,9*R*)-2-amino-8,9-dihydroxydecanoic acid (Add) residue [15]. The peptide inhibited histone deacetylase and mimicked transforming growth factor-β (TGF-β)-like activity in mink epithelial lung (M*v*1Lu) cells. It is known that TGF-β protects neuronal cells from damage caused by brain ischemia injury and *Alzheimer*'s disease. Thus, chlamydocin analogues were discussed as potential candidates for treatment of neuronal disorders [13][14]. An asymmetric total synthesis of diheteropeptin (**4**) has been elaborated [16].

Chlamydocin and Analogues from *Peniophora* cf. *nuda*. – Chlamydocin (**1**) and two new analogues, **5** and **6**, were recently isolated from a culture of *Peniophora* cf. *nuda* (Peniophoraceae, Russulales). The isolate was obtained from a soil sample collected in Tottori Prefecture, Japan [17]. The species is widely distributed and commonly associated with white rot of dead branches of woody angiosperms [18].

Electron microscopy revealed a dolipore-parenthesome septum, and sequencing of its 18S rRNA gene identified the fungus to be closely related to *Peniophora nuda* [17]. Notably, this is the first unequivocal report confirming the isolation of an Aib-containing peptide from a basidiomycete. Such a result is of chemotaxonomic importance, as all previous reports claiming the isolation of peptaibiotics from basidiomycetes [19–21] should be considered ambiguous, as they might have involved a mycoparasite of ascomycete affinity [1][22–24]. Most peptaibiotics reported to date were isolated from two families of the order Hypocreales – Hypocreaceae and Clavicipitaceae (*sensu lato*). Other Hypocrealean families have been positively screened for the presence of Aib and/or Iva, as recently reviewed [1], but, to the best of our knowledge, no basidiomycete taxa.

Two lipoamino acids, 2-amino-8-oxodecanoic acid (Aod) and its AcO derivative were found in the new chlamydocin analogues. Chlamydocin (**1**) and its two new analogues exhibited plant growth-retarding activity towards rice seedlings (*Oryza sativa* cv. 'Koshihikari'), a feature that is discussed to reduce lodging, permit higher nitrogen application rates, and increase yields. Plant growth-retarding activity was shown to be caused by decrease of the endogenous level of gibberellins (gibberellic acid 1 (GA_1)), along with a simultaneous increase of abscisic acid (ABA). Regulation of

these two plant hormones was postulated to be caused by inhibition of histone deacetylases (HDACs) that may differentially affect biosynthesis of GA_1 and ABA [25]. Similar plant growth retardation has previously reported for the HC-toxin (**12**; see the *Table* and *Fig. 3*) from *Cochliobolus carbonum*, the causing agent of Leaf Spot of Maize [26].

FR235222 – The First Iva-Containing Cyclotetrapeptaibiotic. – Isovaline (Iva) which is generally considered as the CH_2 homologue of Aib, has been reported from a number of linear peptaibiotics [1]. Notably, structure elucidation of the cyclotetrapeptide FR235222 (**7**), isolated from a culture of *Acremonium* sp. No. 27082 [27], revealed the presence of one L-Iva residue. The structure of FR235222 (**7**) as shown in *Fig. 1* was assigned cyclo(-(*2S,9R*)-2-amino-9-hydroxy-8-oxodecanoic acid-*trans*-4-D-MePro-L-Phe-L-Iva-) [28]. The producer was isolated from a soil sample in Aktia City, Japan, identified as *Acremonium* cf. *murorum* by its morphological characteristics, and finally deposited as *Acremonium* sp. FERRM BP-6539. The peptide displayed potent and selective inhibition of T-cell proliferation and lymphokine production. Furthermore, it exhibited potent inhibition of HDAC [29]. Total synthesis [30][31], NMR solution structure, and a binding model have recently been published [31].

Further Aib-Containing Cyclic Heptapeptides: Scytalidamides A and B. – Two cyclic heptapeptides (*Fig. 2*), scytalidamides A (**8**) and B (**9**) were reported from *Scytalidium* sp. CNC-310 [32]. The genus *Scytalidium*, however, is considered very heterogeneous with teleomorphs belonging to different ascomycetous genera.

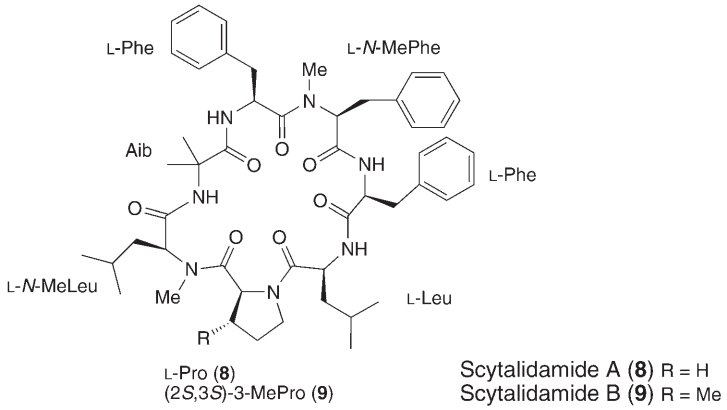


Fig. 2. *Structures of cycloheptapeptaibiotics, scytalidamides A* (**8**) *and B* (**9**)

The fungus was isolated from the surface of a sample of the green alga *Halimeda* sp. collected from a patch reef at a depth of 15 m from the northern end of Long Island, Bahamas. Thus, discovery of scytalidamides further extends the sources of Aib-containing cyclic peptides to marine habitats although the genus *Scytalidium* is generally known to be associated with terrestrial plants. Scytalidamides A and B (**8** and **9**, resp.) displayed moderate *in vitro* cytotoxicity towards HCT-116 human adenocarcinoma.

Both scytalidamides contain one Aib residue but display a building scheme different from the cyclopeptaibiotics and cyclic tetrapeptides mentioned above. The presence of further non-proteinogenic amino acids, however, supports a non-ribosomal biosynthesis: scytalidamide A (**8**) was assigned as cyclo(-L-Phe-L-*N*-MePhe-L-Phe-Aib-L-*N*-MeLeu-L-Pro-L-Leu-) and scytalidamide B (**9**) as cyclo(-L-Phe-L-*N*-MePhe-L-Phe-Aib-L-*N*-MeLeu-(*2S,3S*)-3-MePro-L-Leu-). For configuration analysis of the amino acids in the HCl hydrolysate, an advanced LC/MS method based on derivatization with L- or D-1-fluoro-2,4-dinitrophenyl-5-L-leucinamide (L-FDLA or D-FDLA, a modified *Marfey*'s reagent) [33] was used. Preparative amounts of (*2S,3S*)-3-methylproline (MePro) were isolated and further characterized by NMR, HRFAB-MS, and LC/MS of its L- and D-FDLA derivatives. Total synthesis of scytalidamide A (**8**) has been reported in [34].

Aib-Free Cyclic Tetrapeptides. – A number of cyclic tetrapeptides lacking the Aib residue have been described. These Aib-free cyclic tetrapeptides containing D-Pro, D-pipecolic acid (Pip), and D-*O*-methyltyrosine (D-OMe-Tyr) are listed in the *Table*. The structures of the apicidins are shown in *Fig. 4*, those of the azumamides and microsporins are illustrated in *Fig. 5*, and those of the remaining Aib-free cyclic tetrapeptides are depicted in *Fig. 3*.

Notably, their producers are often known from highly competitive or specialized habitats. The producer of the phytotoxic peptides Cyl-1 (**10**) and Cyl-2 (**11**), *Cylindrocladium scoparium* [35], teleomorph *Calonectria morganii* [36], acts as a plant pathogen infecting a wide range of crop and ornamental plants [18][37], and the HC-toxin (**12**)-producing *Cochliobolus carbonum* is known as the causal agent of leaf spot of maize [9][41]. The two apicidin-producing *Fusarium* isolates were reported as endophytes from acacia and White Mangroves, respectively [46][49].

The configuration of the Phe residue of WF-3161 (**13**) was originally reported to be L [42], but subsequently corrected to D by 2D-NMR, and treatment of the HCl hydrolysate with D- and L-amino acid oxidase [43]. Finally, its total synthesis was described in [52].

Treatment with amino acid oxidase was also applied to the HCl hydrolysate of HC-toxin (**12**) [9] as well as Cyl-1 (**10**) [35], and Cyl-2 (**11**) [38], respectively.

For configuration analysis of the amino acids in the HCl hydrolysates of trapoxin A and B (**14** and **15**, resp.), HPLC on a chiral *Crown Pak CR* phase was used [44]. Crystalline trapoxin A (**14**) was investigated by X-ray-analysis [45]. Total synthesis of trapoxin B (**15**) and analogues has been reported in [53].

The configuration of the amino acids in the apicidins, **16**–**22**, was determined by 2D-NMR and amino acid oxidase [46][47].

The importance of D-amino acids has recently been compiled, including protocols for their analysis [54].

Can a Sponge Produce Cyclic Tetrapeptides? – Five cyclic tetrapeptides, azumamides A–E (**23**–**27**, resp.), were recently isolated from a frozen 2.2-kg sample of the marine sponge *Mycale izuensis*. They were shown to contain unusual lipoamino acids, namely (2*S*,3*R*)-3-amino-2-methylnon-5-enedioic acid 9-amide (Amnaa), or (2*S*,3*R*)-3-amino-2-methylnon-5-endioic acid (Amnda). Depending on the isomer,

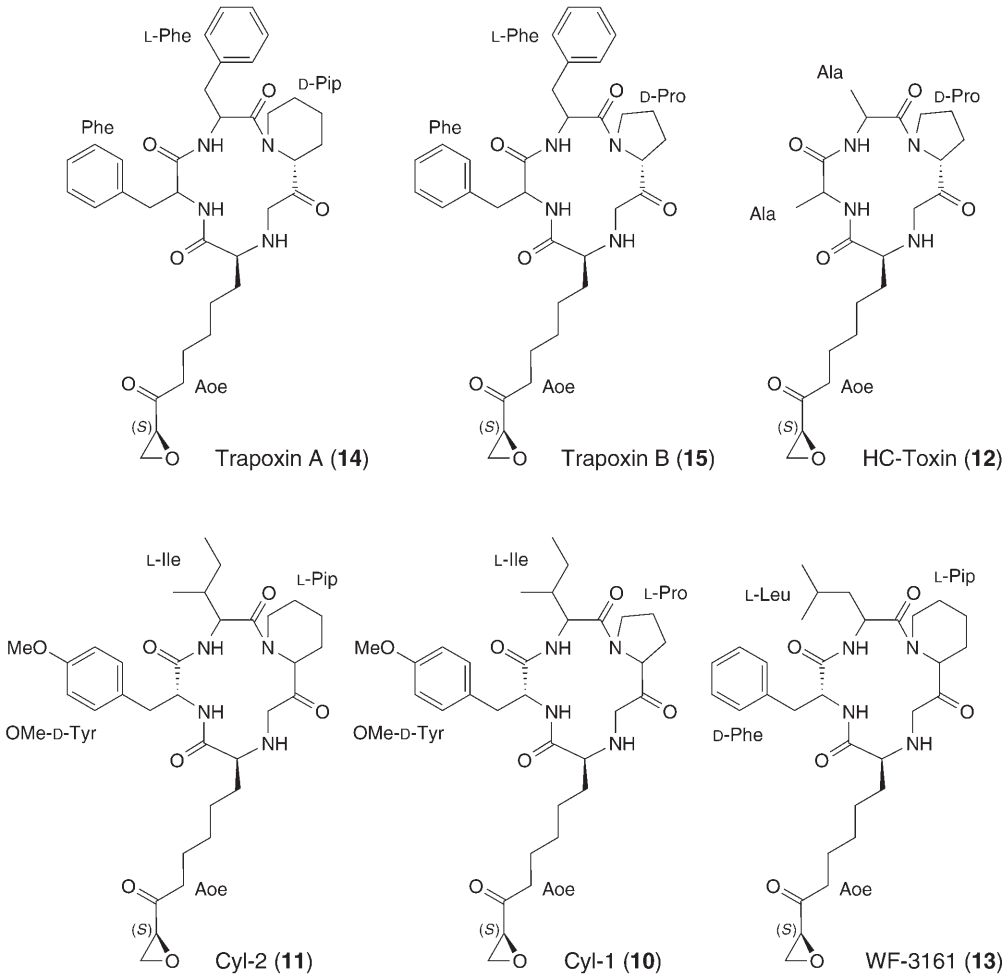


Fig. 3. *Structures of other, Aib-free cyclic tetrapeptides*

amino acid analysis revealed the presence of D-Val, D-Ala, D-Phe, and D-Tyr (*Fig. 5*). For amino acid analysis, *Marfey*'s reagent was used [50]. However, production of azumamides by a sponge, as reported, is highly questionable. Contrary to what has been stated in [50], we rather tend to assign the biosynthesis to a sponge-associated fungus whose presence has not been recognized in the material extracted.

This hypothesis is further supported by the recently reported isolation of microsporins A and B (**28** and **29**, resp.). These cyclic tetrapeptides were isolated from *Microsporum* cf. *gypseum* CNL-692. The fungus was found to be associated with the marine bryozoan *Bugula* sp. collected from the U.S. Virgin Islands [51]. The keratinophilic genus *Microsporum* (with teleomorphs in *Nannizzia*) has a worldwide distribution. Some species are known as geophilic saprotrophs, whereas others are

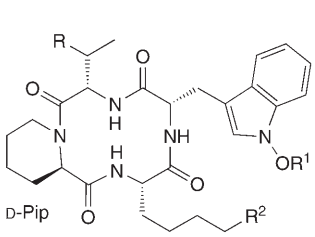
Table. *A Survey of Aib-Free Cyclic Tetrapeptides*

Cyclic tetrapeptide	Producing fungus		Geographic origin/Habitat	Sequence[a])
	Teleomorph	Anamorph (synonyms)		
Cyl-1 (**10**) [35]	*Calonectria morganii* [36] (Nectriaceae, Hypocreales)	*Cylindrocladium scoparium*	Pathogenic to a wide host range of crop plants, causing damping off, seedling root rot and seedling blight [18][37]	cyclo(-Aoe-Pro-Ile-OMe-D-Tyr-)
Cyl-2 (**11**) [37–40]				cyclo(-Aoe-Pip-Ile-OMe-D-Tyr-)
HC-toxin (**12**) [9][41]	*Cochliobolus carbonum*, race 1 (Pleosporaceae, Pleosporales)	*Bipolaris zeicola* (= *Drechslera zeicola* = *Helminthosporium carbonum*)	USA, from plants infected with leaf spot of maize (*Zea mays*)	cyclo(-Aoe-D-Pro-Ala-Ala-)
WF-3161 (**13**) [42][43]	*Petriella guttulata* No. 3161 (Microascaceae, Microascales)	*Sprothrix* sp., *Graphium* sp. (synanamorph)	Soil sample from Kamakura City, Kanagawa Prefecture, Japan.	cyclo(-L-Leu-L-Pip-L-Aoe-D-Phe-)
Trapoxins [44][45]	Not known	*Helicoma ambiens* RF-1023 (= FERM. BP-2751) (Tubeufiaceae, Pleosporales)	Not given	Trapoxin A (**14**) cyclo(-Aoe-D-Pip-Phe-Phe-) Trapoxin B (**15**) cyclo(-Aoe-D-Pro-Phe-Phe-)
Apicidins [46–48]	Not known	*Fusarium pallidoroseum* ATCC 74289 (= *Fusarium incarnatum?*)	Endophyte from branches of *Acacia* sp., Santa Rosa National Park, Guanacaste Province, Costa Rica [49]	Apicidin (**16**) cyclo(-Aod-D-Pip-Ile-OMe-Trp-) Apicidin A (**17**) cyclo(-Aod-D-Pip-Ile-Trp-) Apicidin B (**18**) cyclo(-Aod-D-Pro-Ile-OMe-Trp-)
		Fusarium sp. ATCC 74322 (Nectriaceae, Hypocreales)	Internal cortex of living roots of *Laguncularia racemosa*, Rincon River, Puntarenas Province, Costa Rica [49]	Apicidin C (**19**) cyclo(-Aod-D-Pip-Val-OMe-Trp-) Apicidin D_1 (**20**) cyclo(-Aoh-D-Pip-Ile-OMe-Trp-) Apicidin D_2 (**21**) cyclo(-Dod-D-Pip-Ile-OMe-Trp-) (Dod = 2-amino-8-dehydro-decanoic acid) Apicidin D_3 (**22**) cyclo(-Hdd-D-Pip-Ile-OMe-Trp-) (Hdd = 2-amino-8-deoxy-9-hydroxydecanoic acid)

Table (cont.)

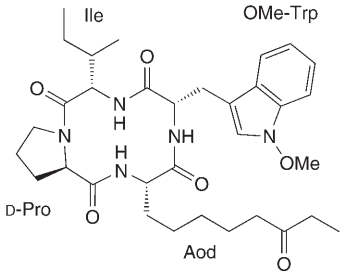
Cyclic tetrapeptide	Producing fungus		Geographic origin/Habitat	Sequence[a])
	Teleomorph	Anamorph (synonyms)		
Azumamides [50]	Azumamides were claimed to be isolated from the marine sponge *Mycale izuensis* (Porifera, Demospongiae, Mycalidae). This is highly questionable as all other cyclic peptides treated here were obtained from fungal sources. Thus, a sponge-associated fungus is predicted to be the producer.		Amakusa Islands, Southern Japan	Azumamide A (**23**) cyclo(-Amnaa-D-Phe-D-Ala-D-Val-) Azumamide B (**24**) cyclo(-Amnaa-D-Tyr-D-Ala-D-Val-) Azumamide C (**25**) cyclo(-Amnda-D-Phe-D-Ala-D-Val-) Azumamide D (**26**) cyclo(-Amnaa-D-Phe-D-Ala-D-Ala-) Azumamide E (**27**) cyclo(-Amnda-D-Phe-D-Ala-D-Val-)
Microsporins [51]	Not known	*Microsporium* cf. *gypseum*	US, Virgin Islands, associated with the marine bryozoan *Bugula* sp. (Cheilostomatida, Bugulidae)	Microsporin A (**28**) cyclo(-Aod-D-Pip-Phe-Ala-) Microsporin B (**29**) cyclo(-Ahd-D-Pip-Phe-Ala-)

[a]) Exchange positions are underlined. Abbreviations of constituents not explained in the table are given in the text.



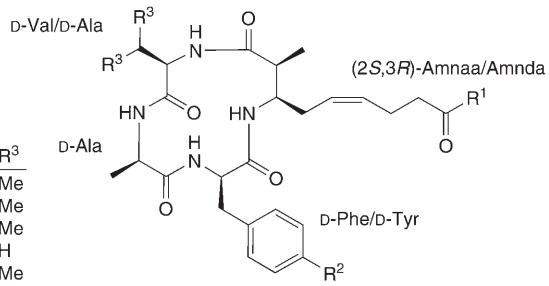
D-Pip-Containing apicidins

	R	R^1	R^2
Apicidin (**16**)	Et	MeO	$EtC(O)CH_2$
Apicidin A (**17**)	Et	H	$EtC(O)CH_2$
Apicidin C (**19**)	Me	MeO	$EtC(O)CH_2$
Apicidin D1 (**20**)	Et	MeO	$MeCH(OH)C(O)CH_2$
Apicidin D2 (**21**)	Et	MeO	$EtCH((S)\text{-}OH)CH_2$
Apicidin D3 (**22**)	Et	MeO	$MeCH(OH)CH_2CH_2$

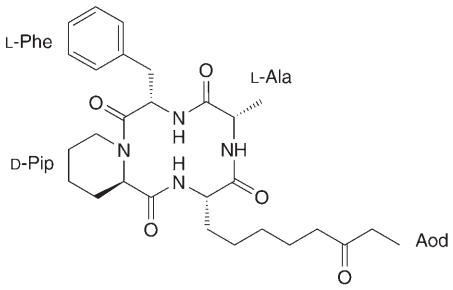


Apicidin B (**18**)

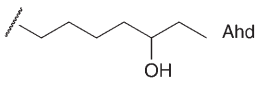
Fig. 4. *Structures of apicidins*



	R^1	R^2	R^3
Azumamide A (**23**)	NH_2	H	Me
Azumamide B (**24**)	NH_2	OH	Me
Azumamide C (**25**)	OH	OH	Me
Azumamide D (**26**)	NH_2	H	H
Azumamide E (**27**)	OH	H	Me



Microsporin A (**28**)



Microsporin B (**29**)

Fig. 5. *Structures of azumamides A–E* (**23**–**27**, resp.)*, and microsporins A and B* (**28** and **29**, resp.)

dermatophytes pathogenic to mammals, including humans [55]. Usually, they cause a single inflammatory skin or scalp lesion; invaded hairs show an ectothrix infection [56].

The sequences of microsporin A (**28**) was established as cyclo(-Aod-D-Pip-Phe-Ala-) and cyclo(-Ahd-D-Pip-Phe-Ala-), respectively. The structure of microsporin A (**28**) was further confirmed by solid-phase synthesis. Notably, the unusual lipoamino acid of microsporin B (**29**), (*2S*)-2-amino-8-hydroxydecanoic acid (Ahd), has not been described for cyclic tetrapeptides or cyclopeptaibiotics before. The configuration of the amino acids in the HCl hydrolysate was determined after derivatization with *Marfey*'s reagent and subsequent HPLC analysis of the diastereoisomers. Microsporins A and B (**28** and **29**, resp.) are potent inhibitors of histone deacetylase and showed cytotoxic activity against human colon adenocarcinoma (HCT-116) and other cancer cell lines [51].

Facts and Hypotheses on Structure–Activity Relationships. – Notably, all cyclic tetrapeptides reviewed here display the same building scheme. An uncommon, branched or unbranched lipoamino acid is linked to a Pro or Pip residue, respectively. Two additional amino acids are required to form a four-membered ring (*Figs. 1–5*). Most of the cyclic tetrapeptides contain one D-amino acid residue, which is likely to confer increased resistance to the ring system against proteolytic cleavage. This resistance is further increased by the presence of one Aib residue in the cyclopeptaibiotics. The occurrence of lipoamino and D-amino acids strongly suggests a non-ribosomal peptide biosynthesis as it is known from linear peptaibiotics [57][58] and larger cyclic peptides of fungal origin such as the L-α-aminobutyric acid (Abu)-containing cyclosporins from *Tolypocladium inflatum* [59][60].

The potent antiprotozoal activity of apicidins **16**–**22**, was demonstrated to be caused by inhibition of parasite histone deacylase [46–48]. The antitumor effects of WF-3161 (**13**) [42] and trapoxins [44] may be explained by irreversible inhibition of histone deacetylase 1 [61]. Contrary to what is known from linear peptaibiotics [1][23], ionophoric activities of cyclic tetrapeptides and cyclopeptaibiotics have not yet been reported in literature. The lumen formed by such a four-membered ring system is too small to act as a channel. Currently, the potent bioactivities of these peptides are best explained by the inhibition of histone deacetylases [61].

Synthetic, Non-Natural Aib-Containing Cyclic Peptides. – Although this review is focussed on naturally occurring cyclic peptides, it is worth mentioning that several non-natural cyclic hexa-, hepta-, and octapeptides containing a number of sterically constrained Aib-residues have been prepared by solution-phase synthesis. Crystal structures of some of them have been determined by X-ray-analysis [62–66].

Neither synthetic Aib-containing analogues of natural astins representing anti-tumor cyclic pentapeptides [67] and macrocyclic analogues of linear natural peptides such as neuropeptide Y (NPY) [68], nor the vast number of Aib-containing cyclic peptides filed in the patent literature, are treated in this review. We propose that use of the term 'cyclopeptaibiotics' should be restricted to biologically active, native peptides.

Future Prospects. – Structural diversity of cyclic tetrapeptides and cyclopeptaibiotics is expected to increase in the future. Recently, microheterogeneity has been

impressively demonstrated for apicidins [46] and azumamides [50]. Therefore, a similar structural diversity, as it is already known from linear peptaibiotics [69–71], can be postulated for cyclic tetrapeptides and cyclopeptaibiotics. Considering structural homologies known from linear peptaibiotics, it is not excluded that, for instance, HC-toxins containing Aib or Vxx (Val, and D- or L-Iva) instead of Ala will be detected. Such result would confirm previous observations for trichobrachins TB III A and TB III B: These are hexapeptides, the N-terminus of which has not been assigned yet. Remarkably, Aib could not be determined in those peptides [71][72].

The method of peptaibiomics [73] which has been successfully applied to screen peptaibiotics of *Trichoderma* sp. and its *Hypocrea* teleomorphs [3][74][75] is also recommended as a pivotal tool for screening cyclic tetrapeptides and cyclopeptaibiotics. Formation of such cyclic tetrapeptides and cyclopeptaibiotics might be interpreted as an adaptation to the highly specialized life style of the producers as plant pathogens, egg pathogens of nematodes or insects, or as plant endophytes. Therefore, screening of fungi from such ecological niches should considerably contribute to the diversification of cyclic tetrapeptide and cyclopeptaibiotic structures.

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