Journal of Peptide Science
J. Peptide Sci. **9**: 666–678 (2003)
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/psc.497

JOURNAL OF PEPTIDE SCIENCE

Review

The Occurrence of Peptaibols and Structurally Related Peptaibiotics in Fungi and their Mass Spectrometric Identification via Diagnostic Fragment Ions‡

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Received 28 February 2003
Accepted 25 April 2003

Abstract: Peptaibols and related peptide antibiotics (peptaibiotics) display diagnostically useful fragmentation patterns during mass spectrometry (FAB-MS, ESI-CID-MS/MS and CID-MS^n). The paper compiles fragmentation data of pseudo-molecular ions reported in the literature as a guide to the rational identification of recurrently isolated and new peptaibols and peptaibiotics. Taxonomic and ecological aspects of microorganisms producing peptaibols and peptaibiotics are discussed. Copyright © 2003 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: peptaibols; lipopeptaibols; lipoaminopeptides; peptaibiotics; fungicolous fungi; ESI-CID-MS/MS; CID-MS^n; b-type fragmentation

INTRODUCTION

Peptaibols and peptaibiotics (lipopeptaibols, lipoaminopeptaibols, lipoaminopeptides) constitute a constantly growing family of peptide antibiotics of fungal origin.

They show interesting physico-chemical and biological activities depending on particular structural properties, such as the formation of pores in bilayer lipid membranes as well as antibacterial, antifungal, occasionally antiviral [1] and antiparasitic activities [2]. Inhibition of mitochondrial ATPase, uncoupling of oxidative phosphorylation, immunosuppression [3–7], inhibition of platelet aggregation [8], induction of fungal morphogenesis and neuroleptic effects [9–12] have been reported.

The broad-spectrum antibiotic and membrane-disrupting activities of peptaibiotics could, to some extent, account for their action against plant and fungal hosts of the producing microorganisms.

Peptaibols and related peptaibiotics are composed of 5–20 amino acids, and amongst them there are several α-aminoisobutyric acid (Aib) moieties as a characteristic of these structures. Their structural diversity is caused by the varying amount and nature of the constituting amino acids, and different substitutions of the *N*- and *C*-terminus (see below). The name peptaibol was originally proposed by Benedetti *et al.* [13], but also by Brückner and co-workers [14] — independently of each other.

We recommend that the name should be used for linear peptide antibiotics that exhibit the following characteristics, thus expanding the original definition [15 and tables 3–4]:

(i) have a molecular weight between 500 and 2200 Dalton; (ii) show a high content of α-aminoisobutyric acid; (iii) are characterized by the presence of non-proteinogenic amino acids and (iv) possess an acetylated *N*-terminus, whereas the *C*-terminus is reduced to an amino alcohol.

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In 1991, Brückner *et al.* [16,17] introduced the comprehensive term peptaibiotic to include such components lacking the amino alcohol. To further expand their definition, considering recent findings in this area of research, we suggest the term peptaibiotic for linear peptide antibiotics that

(i) have a molecular weight between 500 and 2200 Dalton; (ii) show a high content of α-aminoisobutyric acid; (iii) are characterized the presence of non-proteinogenic amino acids and/or lipoamino acids and (iv) possess an acylated *N*-terminus, whereas the *C*-terminus may consist of a free or methoxy-substituted 2-amino alcohol, amine, amide, free amino acid, diketopiperazine or sugar alcohol [see tables 3–4].

A common feature of this heterogeneous class of substances is their biosynthesis via non-ribosomal pathways following the so-called 'thio-template mechanism'. This mode of biosynthesis enables the presence of unusual, non-proteinogenic amino acids, amino alcohols, lipoamino acids, amines and fatty acids in these structures [18,19].

Presently, the family of peptaibols and related peptaibiotics amounts to more than 300 members. For information on special compounds 'The Peptaibol Database' can be recommended which is available on the World Wide Web at: http://www.cryst.bbk.ac.uk/peptaibol/welcome.html [20,21]. However, searching for structures and producers of peptaibols and peptaibiotics is enabled, too, by other databases, such as the Chapman & Hall, Dictionary of Natural Products on CD ROM, and Antibase 2.0 [22]. An excellent review, summarizing related sub-families of peptaibols, has recently been presented by Chugh and Wallace [23]. Lipopeptaibol antibiotics were reviewed by Toniolo *et al.* [24]; and detailed information on mass spectrometry of leucinostatin antibiotics was given by Isogai *et al.* [25]. Due to the still growing number of peptaibiotics, recurrent isolations may occur frequently. Therefore, assistance in preventing re-isolations or disclosure of new structures may be welcome.

Mass spectrometric methods such as fast atom bombardment ionization (FAB-MS) including linked B/E-MIKES scan approaches, ESI-CID-MS/MS, ESI-CID-MS^n and MALDI-TOF-PSD-MS have been indispensable tools for the determination of molecular weights via the pseudo-molecular ions such as $[M+H]^+$ and $[M+Na]^+$ as well as sequence analysis via the generation of diagnostic fragment ions. Such ions can be generated by **c**ollision-**i**nduced **d**issociation (**CID**, synonymous: **c**one-**v**oltage **f**ragmentation, **CVF**) of the selected parent ion(s) using triple quadrupole or ion-trap mass spectrometers.

The present paper compiles data on the mass spectrometric behaviour of peptaibols and peptaibiotics including pseudo-molecular ions and the formation of diagnostic fragment ions. This compilation is intended to serve as a guide to a more rational identification of recurrent as well as novel representatives of these fungal peptides.

MICROORGANISMS PRODUCING PEPTAIBIOTICS

Peptaibols and related peptides (peptaibiotics) are biosynthesized exclusively by fungi, mainly soil-borne and plant-pathogenic, as well as fungicolous (also known as mycophilic) taxa. The latter occur on, as well as within, the fruit-bodies of asco- and basidiomycetes. Genera containing fungicolous species or fungicolous species themselves mentioned in this paper are marked with a superscript #.

More detailed information on modern taxonomic aspects of the producers of peptide antibiotics has been given [26–29].

Most of the structures reviewed here were isolated from *fungi imperfecti* belonging to the genera *Trichoderma*#, *Acremonium*#, *Paecilomyces*# and *Emericellopsis*#. Less commonly reported producers are listed in Table 1.

The occurrence of peptaibols such as boletusin [30] as well as the tylopeptins A and B [31] has been reported for a few basidiomycete fruit-bodies such as *Boletus* ssp. and *Tylopilus neofelleus*.

There are doubts, however, about the isolation of a peptaibiotic from the fruit-bodies of basidiomycetes. The authors making this claim pointed out the obvious structural homologies with the even co-isolated chrysospermins A–D [32], but they did not mention whether the fresh (in the case of boletusin) or dried material (in the case of the tylopeptins), used for extraction, showed any sign of decay or infection by mycophilic fungi.

In some cases, an infection of an asco- or basidiomycete fruiting-body by the genus *Sepedonium*# is easily recognized by the presence of large quantities of yellow aleurioconidia (synonym: chlamydospores), the colour of which is mainly due to the presence of sepedonin and its derivatives as well as skyrin [33]. However, the colour starts to appear relatively late, when masses of chlamydospores become mature. For that reason, it cannot be excluded that accidentally younger stages of infection were

Table 1 A Survey of the Less Frequently Reported Fungal Producers of Peptaibiotics

Name of peptaibiotic	Isolated from	Reference(s)
Chrysospermins	*Sepedonium chrysospermum*[#] (Teleomorph: *Hypomyces chrysospermus* (Bull.) Tul. Synonym: *Apiocrea chrysosperma*)	[32]
Peptaibolin	*Sepedonium* sp.[#]	[89]
Ampullosporins	*Sepedonium ampullosporum*[#]	[9,10]
Hypomurocins A and B	*Hypocrea muroiana*[#] Hino & Katsumoto	[90]
Some of the hypelcins	*Hypocrea peltata*[#] (Jungh.) Sacc.	[91]
Lipohexin, Texenomycin A	*Acremonium lindtneri* (Kirschstein) Samuels & Rogerson (Synonyms: *Moeszia lindtneri* (Kirschstein) G. Arnold *Cylindrocarpon lindtneri*); Teleomorph: *Sporophagomyces chrysostomus* [92] (Synonym: *Hypomyces chrysostomus* Berk. & Broome [93])	[67,68]
Clonostachin	*Clonostachys* sp.	[8]
Aibellin	*Verticimonosporium ellipticum* Matsushima	[63,64]
LP237-F5, -F7, -F8	*Tolypocladium geodes* W. Gams	[42,43]
Some of the Antiamoebins	*Stilbella fimentaria* [94] (*Synonym*: Stilbella erythrocephala) *Gliocladium catenulatum*[#] *Gilman & Abbott*	[94–96]
Stilboflavins	*Stilbella flavipes* (Peck) Seifert	[96]
Antiamoebin I	*Verticillium epiphytum* Hansford [97] (Synonym: *Cephalosporium pimprina*[#])	[76]
Antiamoebin I	*Clonostachys rosea* f. *catenulata* Gilman and Abbott [98] (Synonym: *Gliocladium catenulatum*[#])	[77]
Gliodeliquescin	*Gliocladium deliquescens*	[99]
Efrapeptins	*Tolypocladium inflatum* W. Gams (*Synonym: Tolypocladium niveum* (Rostrupp) Bisset) and other *Tolypocladium* species	[100]

not recognized macroscopically, thus leading to the extraction of infected material.

Moreover, it is well known from the literature [34] that a primary infection by *Sepedonium chrysospermum*[#], attacking its preferred hosts in the order *Boletales*, e.g. *Boletus, Paxillus, Tylopilus* and *Scleroderma* species, [for a review see: 33,35] normally causes total necrosis of the infected host cells. However, if 'weak pathogens', such as *Botrytis cinerea*[#] or *Trichothecium roseum*[#], invade fruit-bodies, previously colonized by *Sepedonium chrysospermum*, the latter can grow biotrophically within the mycelium of these so-called 'secondary parasites' [36].

NOMENCLATURE OF PEPTAIBOLS AND PEPTAIBIOTICS

The name peptaibol was introduced originally for small fungal peptides containing a high portion of the non-proteinogenic amino acid Aib (α-aminoisobutyric acid or α-methyl alanine; Figure 1). The Aib residues may number two, as in the pentameric peptaibolin from *Sepedonium* strains, or up to nine in some of the stilboflavins.

The term 'peptaibol' should be applied only to peptides having an acetylated *N*-terminus, and an 2-amino alcohol as *C*-terminus (see above).

X-Aaa-$(Aaa)_n$-AaaY

Figure 1 A general, simplified building scheme of the peptaibiotics. n = in the range of 3 [89] to 19 [106] residues, containing a high proportion of Aib as well as other non-proteinogenic amino acids; Xaa = *N*-terminal amino acid substituted with unbranched, or 2- or 4-methyl branched, saturated or unsaturated fatty acid; Yaa = substituent covalently linked to the *C*-terminal amino acid: free or methoxy-substituted amino alcohol, amine, amide, free amino acid, diketopiperazine, or sugar alcohol. A peptaibol synthetase from *Trichoderma virens* has recently been cloned [107].

Structurally varying compounds such as lipopeptaibols have been named 'peptaibiotics' [16,17]. Variations of *N*-acetylated peptaibol-type substances concern a heterologous *C*-terminal moiety, such as a mannitol residue [clonostachin: 8], a free carboxy group formed by glycine [XR 586: 37], glutamine, valine [16,17] or serine [cephaibols P and Q: 2], a methoxylated Aib [NA VII: 38], L-prolineamide, or a diketopiperazine such as *cyclo*-L-Pro-Aib [pseudokonins KL III and KL VI: 39].

In the so-called lipopeptaibols, the *N*-terminus is substituted by a fatty acid with more than four carbons [38] and the *C*-terminus is substituted by an amino alcohol. This group comprises trichogin GA IV [38], the trikoningins KB I and KB [40], trichodecenins TD-I and TD-II [41] as well as LP237_F7 and LP237_F8 [42,43].

In the subfamily of lipoaminopeptides (also reported as aminolipopeptides) the *N*-terminus is substituted by long-chain, α-methyl-branched fatty acids (Figure 1). An L- proline-, L-4-hydroxy-proline or *cis*-4-methyl-L-proline residue is found in position 2 of the peptide chain, and in most cases it is followed by a lipoamino acid residue in position 3. To our present knowledge, this compound, 2-amino-4-methyl-6-hydroxy-8-oxo-decanoic acid (AHMOD), has only been recorded from this subfamily.

In some of the leucinostatins the AHMOD moiety is replaced by L-Leu or L-Val [25,44–46]. The *C*-terminus of leucinostatin is substituted, too, by an amino alcohol (cf Table 2).

It should be mentioned that the primary structures of some leucinostatins remain uncertain, because the assignment of structures by LC-Frit-FAB-MS/MS was not entirely conclusive [25].

Up to now, the following lipoaminopeptides have been published in the literature:

- 22 leucinostatins from different strains of *Paecilomyces marquandii*[#] (Massee) Hughes and *Paecilomyces lilacinus*[#] (Thom) Samson [5,25,47–53]

Table 2 Reference Masses of the Amino Acid Residues Occurring in Peptaibiotics ($[M + H - H_2O]^+$)

Amino acid	Abbreviation		Monoisotopic molecular weight	Molecular formula of the residue
	3-letter code[a]	1-letter code[b]		
Alanine	Ala	A	71.0371	C_3H_5NO
α-Aminoisobutyric acid	Aib	U	85.0528	C_4H_7NO
2-Amino-4-methyl-6-hydroxy-8-oxo-decanoic acid	AHMOD	—	213.1365	$C_{11}H_{19}NO_3$
Asparagine	Asn	N	114.0429	$C_4H_6N_2O_2$
Aspartic acid	Asp	D	115.0269	$C_4H_5NO_3$
α-Ethyl-norvaline	EtNor	Z	127.0997	$C_7H_{13}NO$
Glutamine	Gln	Q	128.0586	$C_5H_8N_2O_2$
Glutamic acid	Glu	E	129.0426	$C_5H_7NO_3$
Glycine	Gly	G	57.0215	C_2H_3NO
β-Hydroxyleucine	Hyleu	—	129.0790	$C_6H_{11}NO_2$
4-Hydroxyproline	Hyp	O	113.0477	$C_5H_7NO_2$
Isoleucine	Ile	I	113.0841	$C_6H_{11}NO$
Isovaline	Iva	J	99.0684	C_5H_9NO
Leucine	Leu	L	113.0841	$C_6H_{11}NO$
4-Methylproline	MePro	—	111.0684	C_6H_9NO
Phenylalanine	Phe	F	147.0684	C_9H_9NO
Pipecolic acid	Pip	—	111.0684	C_6H_9NO
Proline	Pro	P	97.0528	C_5H_7NO
Serine	Ser	S	87.0320	$C_3H_5NO_2$
Tryptophan	Trp	W	186.0793	$C_{11}H_{10}N_2O$
Tyrosine	Tyr	Y	163.0633	$C_9H_9NO_2$
Valine	Val	V	99.0684	C_5H_9NO

[a] In some cases, the abbreviations commonly used for non-proteinogenic amino acids do not match the 3-letter code.
[b] Note, that there is no 1-letter code for some of the non-proteinogenic amino acids.

Table 3 Modifications of the *N*- and *C*-terminus in Peptaibol-type Antibiotics

N-terminus			*C*-terminus		Name of compound (selected examples)	MW of compound $(M)^+$
Fatty acid moiety (abbr., MW)	Modified amino acid	MW (m/z)	Structure (abbr.)	MW[a] (m/z)		
Acetyl- (Ac; 43)	Ala	114	Phenylalaninol (Pheol)	150	Trichoaureocin 1a [101]	
	Aib	128	Phenylalaninol (Pheol)	150	Longibrachins LGA, LGB; atroviridins	
			Valinol (Valol)	102	Harzianin Pc_u4	1401
			Tryptophanol (Trpol)	190	Trichorzins PA	
			Esterified mannitol	165	Clonostachin	1667
			Leucinol (Leuol)	117	Trikoningin KA V, other hypelcins	
			Isoleucinol (Ileol)	117	Hypelcins A-V, -VI; Stilboflavins SF B 9, 10; TrichovirinTV_XIIa [102]	1927; 1913
			Prolineamide (Pro-NH_2)	114	Pseudokonin KL III	1074
			cyclo-Aib-L-Prolinal	183	Pseudokonin KL VI	1045
			Aib-OCH_3	99	NA VII	
			2-[(2-Amino-3-phenylpropyl)amino]ethanol	183	Aibellin	2000
			Glutamine (Gln)	128	Trichobrachin TB I A, B, C and D; Trichobrachin TB IIa C and D [109][a]	
			Valine (Val)	99	Trichobrachin TB IIa A and B [109][a]	
	Val	142	Phenylalaninol (Pheol)	150	Bergofungins; antiamoebins XII, XIV	
	Iva	142	Leucinol (Leuol)	117	Hypomurocin HM A-2	1174
	Leu	156	Phenylalaninol (Pheol)	150	Peptaibolin; antiamoebins XV, XVI	589; 1622; 1636
	Phe	190	Tryptophanol (Trpol)	190	Chrysospermins; boletusin; peptavirin B	
			Phenylalaninol (Pheol)	150	Other cephaibols; other antiamoebins	
			Serine (Ser)	104	Cephaibols P, Q	1872; 1856
	Trp	230	Leucinol (Leuol)	117	Ampullosporins; tylopeptins	
			Phenylalaninol (Pheol)	150	Zervamicins	
			Glycine	71	XR586	1878
	Pip	154	Pipecolic acid (Pip)	Different	Efrapeptins [108]	

(*continued overleaf*)

Table 3 (*Continued*)

N-terminus			*C*-terminus		Name of compound (selected examples)	MW of compound $(M)^+$
Fatty acid moiety (abbr., MW)	Modified amino acid	MW (m/z)	Structure (abbr.)	MW[a] (m/z)		
n-Octanoyl- (Oc, 127)	Aib	212	Leucinol (Leuol)	117	Trichogin GA IV; trikoningin KB I; LP237_F5, LP237_F8	1065; 1037; 1341; 1325
	Iva	226	Leucinol (Leuol)	117	Trikoningin KB II	1051
n-Decanoyl- (Dec, 155)	Aib	240	Leucinol (Leuol)	117	LP237_F7	1311
cis-4-Decenoyl- (155)	Gly	210	Leucinol (Leuol)	117	Trichodecenins-TD_I and—TD_II	751
(4S,2E)-4-Methylhex-2-enyl- (MeHA, 111)	Pro	208	MPD, DPD or DPD-N-oxide[b]	87; 101 or 117	Leucinostatins L; T	1189; 1089
	cis-4-methyl-L-Pro	222	MPD, DPD or DPD-N-oxide[b]	87; 101 or 117	Most of the other leucinostatins	
2-Methyloctanoyl- (MOA, 141)	Pro	238	AAE or AMAE[b]	119/133	Helioferins; Roseoferins D_2, D_3, E, F	1121; 1135
2-Methyldecanoyl- (MDA, 169)	Pro	266	AMAE (trichopolyns); AAE or AMAE (roseoferins)	119/133	Trichopolyns I (or A), II (or B); Roseoferins A, B, C, D_1, G[a]	1206; 1192
	L-4-Hyp	282	AAE or AMAE	119/133	Roseoferins H; I; K	1179; 1165;1151
	cis-4-methyl-L-Pro	280	MPD, DPD or DPD-N-oxide		Acremostatins A; B; C	1262; 1276; 1292
3-Hydroxy-2-methyldecanoyl- (HMDA, 185)	Pro	282	AMAE (trichodiaminol)	133	Trichopolyns III; IV; V	1192; 1178; 1222
β-Keto-2-methyl-tetradecanoyl- (MOTDA, 239)	Pro	336	Free β-alanine (β-Ala)	89	Lipohexin	765
	Pro	336	Arginol (Argol)	160	Texenomycins A, B	2028

[a] The molecular weight is calculated from the $[M + H]^+$ ion (m/z). All *C*-terminal residues tabulated were demonstrated to possess the *S*-configuration (L-isomers).
[b] Trichobrachins TB III A and TB III B are hexapeptides, the *N*-terminus of which has not been assigned yet. Remarkably, they no longer contain Aib.
[c] MPD: N^1-methyl-propane-1,2-diamine; DPD: N^1,N^1-dimethyl-propane-1,2-diamine; DPD-NO: (2S)-N^1,N^1-dimethyl-propane-1,2-diamine-N-oxide;
AAE: 2-(2′-aminopropyl)-aminoethanol; AMAE: 2-(2′-aminopropyl)-N-methylamino-ethanol. For detailed information on structure assignment of selected compounds see [19,53–55]. In such cases where no $[M]^+$ peak is tabulated the reader is directly referred to the original reference(s).

Table 4 Non-proteinogenic amino acids[a] found in peptaibiotics

Non-proteinogenic amino acid (Abbr.)	Name of the peptaibiotic
α-Aminoisobutyric acid (Aib)	Characteristic feature of all peptaibiotics
L-Isovaline (Iva)	Bergofungins A, B and D; peptavirins A and B; clonostachin in position 7 and 10; some efrapeptins
D-Isovaline (Iva)	Harzianins HC III, HC VIII, HC IX, HC XII, HC XIII, HC XV; atroviridins; tylopeptin A; boletusin; XR586; trichokindins I–VII [91], clonostachin in position 4 and 13, antiamoebins in different positions (except for antiamoebin VI, which does not contain Iva)
cis-4-L-Hydroxyproline (Hyp)	Bergofungins [103,104], clonostachin; XR586; zervamicins; Roseoferins H, I and K
trans-4-L-Hydroxyproline (Hyp)	Heptaibin [105], antiamoebins, emerimicins
cis-4-L-Methylproline (MePro)	Most of the leucinostatins;[b] acremostatins[c]
β-Hydroxy-L-leucine (Hyleu)	Most of the leucinostatins; acremostatins
β-Alanine (β-Ala)	Efrapeptins [109], leucinostatins, acremostatins, lipohexin, texenomycins
L-Pipecolic acid (Pip)	Efrapeptins A–H
α-Ethyl-norvaline (Etnor)	LP237_F5 and LP237_F8
2-Amino-4-methyl-6-hydroxy-8-oxo-decanoic acid (AHMOD)	All lipoaminopeptides, exceptions are mentioned in the text (see above)

[a] Unless otherwise indicated amino acids possess *S*-configuration (L-isomers).
[b] The structure of a number of leucinostatins has not been completely assigned, plausible sequences were tabulated by Isogai *et al.* [25].
[c] The optical configuration of the amino acids of the acremostatins could not be analyzed.

as well as the conjugate structure leucinostatin-β-O-di-glucoside [54] isolated from the endophyte *Acremonium* sp. Tbp-5 [55].

- five trichopolyns from *Trichoderma polysporum*[#] (Link ex Pers.) Rifai TMI 60146 [56–58];
- two helioferins from *Mycogone rosea*[#] Link DSM 8822 [59].
- and 16 roseoferins from *Mycogone rosea*[#] Link DSM 12973 [60]. Another nine novel roseoferins (H_1–H_4; I_1,I_2; K_1–K_3) were obtained by replacing the L-Pro moiety of the corresponding MDA-containing roseoferins by L-Hyp via 'precursor-directed biosynthesis' [61].
- and three acremostatins formed by co-cultivation of *Mycogone rosea* DSM 12973 and *Acremonium* sp. Tbp-5 [62].

There are five compounds that build a bridge between peptaibols and lipoaminopeptides at present.

Aibellin [63,64], one of these compounds, carries an *N*-terminal Aib, which is acetylated, whereas the *C*-terminus is an amino alcohol, 2-[(2-amino-3-phenylpropyl)amino]ethanol. Lipohexin [65,66], and texenomycins A and B [67,68], are *N*-terminally substituted by a 2-methyl-3-oxo-tetradecanoyl moiety (MOTDA). The *C*-terminus of lipohexin representing a partial structure of texenomycins is formed by β-alanine. The 20meric texenomycins A and B carry an L-arginol residue as the *C*-terminus. Both lipohexin and texenomycin were co-isolated from cultures of *Acremonium* (*Moeszia*) *lindtneri* DSM 11119; but texenomycin A and its C-2-epimer texenomycin B (which is rapidly converted into A under basic conditions) by contrast, were originally claimed to be obtained from *Scleroderma texense* [62]. This puffball, which was recently classified as a member of the *Boletales* [69–71], is occasionally infected by species of *Sepedonium* (W. Gams, personal observation).

The occurrence of a free serine residue as the *C*-terminus was reported for cephaibols P and Q [2].

The basic amino acids histidine and lysine as well as the sulphur-containing amino acids cysteine and methionine, have not been found as yet in the peptaibiotics.

L-Serine occurs in a few peptaibols and related structures. Examples are harzianin HC I, III, XI, XII, and XIII [72]; trichorzin PA II, IV, V, VI, VII, VII and XI; PA_U4 and PC_U4 [73,74]; trichokindins I–VII [75]; chrysospermins [32] and tylopeptins [30,31].

L-Threonine has to date only been described as a constituent of some emerimicins and zervamicins, and as occurring in the cephaibols P and Q [2; 76,

77]. However, to date, L-tyrosine has been found only in LP237_F5 [41].

The chirality of amino acids in the peptaibols and peptaibiotics was determined to be L in most cases. A common method for making this determination is derivatization of amino acids in the hydrolysate with N_α-(2,4-dinitro-5-fluoro-phenyl)-1-L-alanine-amide (FDAA, Marfey's reagent), followed by HPLC separation and comparison of the retention times with those of derivatized standard amino acids [78]. However, classical GC/MS approaches have also been used in amino acid analysis [37,39,72].

MASS-SPECTROMETRIC METHODS FOR STRUCTURAL CHARACTERIZATION

Sequencing of peptaibols and peptaibiotics with a molecular weight up to 2000 Dalton can be furnished by classical (FAB-MS) and modern methods of tandem mass spectrometry (ESI-CID-MS/MS, ESI-CID-MS^n, MALDI-TOF-PSD-MS).

In the case of high-energy-ionization (FAB-MS), a remarkable number of rather non-specific fragment ions are observed in addition to $[M + H]^+$, $[M + Na]^+$ and $[M + K]^+$. It appears important to mention that different CID methods will generate different types of fragments. Hence, the simultaneous use of different MS methods is recommended.

According to the original Roepstorff nomenclature [80] which was revised by Biemann [81], six series of fragment ions can be generated during cleavage of a peptide bond, corresponding to *C*-heteroatom- and α-bond cleavages. The resulting positive fragment ions are classified as *a*-, *b*-, and *c*-type if the positive charge remains on the *N*-terminal fragment. However, if it remains on the *C*-terminus, the fragments generated are characterized as *x*, *y* and *z*. Even under high energy ionization conditions, *b*-type fragments are the most commonly produced while the others are weak or even not observable.

The interpretation of spectra generated by high-energy ionization (EI, FAB) is often hampered by the presence of an additional series of secondary or tertiary fragment ions, thus complicating the subsequent structural assignment.

Soft ionization techniques such as electrospray ionization (ESI or MALDI) will usually not generate fragments. Such spectra almost exclusively show pseudo-molecular ions such as $[M + H]^+$, $[M + Na]^+$, $[M + K]^+$, $[2M + H]^+$ and $[2M + Na]^+$. These single ions can be activated subsequently by collision with argon or helium gas in collision cells. Some of the modern HR-ESI-Qq-TOF instruments use highly purified nitrogen. In the case of FT-ICR/MS instruments the collision gas is sometimes substituted by soft-laser pulses inducing fragmentation. This method causes a low-energy fragmentation, which is referred to as CID-MS/MS and CID-MS^n.

Fragments of the B_n-type- ($[M - H_2O]^+$) are thereby produced with a bias toward the positive ion mode, i.e the charge remains on the *N*-terminus after cleavage of the peptide bond. The presence of leucine or isoleucine will lead, for example, to a *b*-type fragment of m/z 113, whereas the occurrence of valine or isovaline in the peptide chain will be signalled by a corresponding fragment of m/z 99. Thus, in comparison with FAB-MS, CID-MS/MS enables a more precise assignment of fragments arising from a defined pseudo-molecular ion. However, there is an obvious disadvantage of ESI-CID-MS/MS of larger peptides in comparison with FAB-MS: In most cases, not all of the possible *b*-type fragments will be visible.

The possibility of generating further generations of daughter ions (MS^n) using electric or magnetic ion trap (IT) analysers enables both the detection and the subsequent sequencing of positional isomers (see below). In contrast to what occurs in linear quadrupole analysers, but much like that seen in ICR, the ions within an ion trap rotate for microseconds on stable orbits in a high-frequency field of alternating current, adjacent to a ring electrode. By varying the amplitude of the current a selective measurement of the mass of ions can be achieved, which thus are forced to leave their orbits in a stepwise manner. The real advantage of ion trap mass spectrometers is the generation of up to ten generations of daughter-ions. For instance, every MS^2 daughter ion generated by collision (mostly with helium) can be fragmented separately, thus generating MS^3 grand-daughter ions. The further fragmentation of these ions will result in MS^4 great-grand-daughter ions and so on [82–86].

DISCUSSION

Tandem-MS methods, especially spray ionization or MALDI coupled to quadrupole, ion trap or TOF analysers have been proven useful for assignment of structure in studies of peptaibols and peptaibiotics. In spite of the remarkable structural diversity of these substances a series of general and repeating

structural elements will often be present enabling the identification of a given structure as a member of the peptaibiotics.

The analysis of saturated fatty acyl moieties occurring in lipopeptaibols and lipoaminopeptides often requires additional ESI-'In-Source'-fragmentation experiments.

1D- and 2D-NMR studies are also needed to distinguish between isoleucine or leucine as a part of a given molecule. For fatty acid analysis, GC/MS-, EI/MS and NMR investigations of the etheric or $CHCl_3$ extracts of the hydrochloric acid hydrolysate are indispensable.

In some cases, *b*-type fragmentation is rather weak. For instance, the presence of an Aib-Pro bond often suppresses formation of b_n-acylium ions. According to Rebuffat *et al.* [72], this tertiary amide bond undergoes a preferential cleavage, leading to an *N*-terminal acylium ion N^+, and a di-protonated *C*-terminal ion. This pattern of cleavage results in the absence of fragment ions at higher masses and the superimposition of two independent b_n-type series at lower masses, starting from the N^+ and also from the di-protonated *C*-terminal ions. To overcome this problem, the use of the so-called 'pulsar function' was proven to be beneficial. This function was recently developed for ESI-QqTOF-MS/MS instruments to collect, firstly, ions of a defined m/z range or even a single mass and to subsequently release the 'ion package', thus remarkably enhancing the sensitivity of the instrument. The superiority of this technique was recently shown for the peptaibols of the trichofumin family [87]. Also, the presence of a saturated *N*-terminal fatty acid moiety next to a proline or proline-derived residue will generally induce the formation of the corresponding a-type fragment while suppressing the b_1-fragment ion [59–62]. The presence of a β-hydroxy amino acid within the peptide chain may favour the formation of rather intense $[b_n\text{-}H_2O]^+$-ions.

No cleavage between the β-alanyl residue and the *C*-terminal propane-amine moieties was observed in the case of leucinostatins (reviewed by Isogai *et al.*, [25]) and acremostatins [62]. This could be ascribed to the presence of a non-α-peptide linkage.

Last but not least, the mass spectrometric behaviour of 2-amino-4-methyl-6-hydroxy-8-oxo-decanoic acid (AHMOD) [49,88] as a constituent of leucino- and acremostatins, trichopolyns, helioferins and roseoferins must be discussed briefly. ESI-MS/MS and ESI-MS^n of these lipoaminopeptides are characterized by the loss of a side chain from the AHMOD moiety (m/z 72) as a result of an α-cleavage. This diagnostic feature leads to the formation of a rather intense fragment ion $[M + H\text{-}C_3H_5O]^+$. Acid hydrolysis of lipoaminopeptides with HCl or H_2SO_4 and subsequent analysis of the hydrolysate by ESI-MS produces 4-methyl-6-(2-oxobutyl)-2-piperidine carboxylic acid (MOBPA) from AHMOD by dehydration followed by a Michael-addition and subsequent cyclization. Thus, a characteristic fragment with m/z 214 is visible in the positive ESI mass spectrum [52,56–58,59–62,88].

Acknowledgement

We would like to thank Willibald Schliemann (Institute of Plant Biochemistry, Halle [Saale]) for critical reading, Richard C Summerbell (CBS, Utrecht) for careful linguistic corrections and Hans Brückner (Justus-von–Liebig-University, Gießen) for valuable comments on the manuscript.

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