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Structures of Polysporins A-D, Four New Peptaibols Isolated from *Trichoderma polysporum*[♦].

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Abstract: *The structures of four new peptides isolated from the fungus Trichoderma polysporum have been ascertained by LC/MS and NMR experiments. All of them are composed of 20 residues with a high ratio of α-aminoisobutyric acid.* Copyright © 1996 Elsevier Science Ltd

Peptaibols are a class of peptides of fungal origin that share some special features. The main common characteristic is the presence in variable proportions of a special aminoacid, the α-aminoisobutyric acid (abbreviated as Aib and is in the origin of the family name peptaibols). Other similarities are the acetylation of the N terminus and the reduction to alcohol of the C terminus. In addition, this last reduced aminoacid is usually phenylalaninol (Fol). The most common families similar to these are alamethicins[1], hypelcins[2], trichosporins[3], and, more recently, trichocellins[4], trichokindins[5] and aibellin[6]. They present a variety of biological activities, derived from their membrane modifying properties, such as the formation of voltage-gated ion channels[7], hemolysis[8], Ca^{2+}-dependent chatecholamine secretion from bovine adrenal chromaffin cells[9], uncoupling of oxidative phosphorilation[10], and, for aibellin, enhanced propionate production and reduced methanogenesis by rumen microorganisms[11].

In this paper we report the structural elucidation of polysporins A-D, four new members of this family isolated from the mycelium of *Trichoderma polysporum*[12]. The presence of aminoacid Aib in the sequence prevented the use of automated Edman degradation to obtain sequence information. Thus, we turned to LC/MS and LC/MS/MS to get sequence information. By using LC/MS[13] we obtained a Reconstructed Ion Chromatogram (RIC) that showed 4 different peaks[14], the major component being the third (Peak C). The techniques used to sequence these peptaibols are exmplified using the data obtained from Peak C. From the ^{1}H-NMR, the presence of the acetyl moiety along with the Fol residue were clearly established. The LC/MS spectrum of Peak C is shown in Figure 1. This spectrum presents peaks corresponding to the protonated molecule at m/z 1992.0 along with the double and triple charged species (signals at m/z 996.4 and 664.8 respectively). Also two fragment ions are obtained at m/z 1217.8 and 774.4. Since the information we could get from the product ion MS/MS experiments on the $(M+H)^+$ ion at m/z 1992.0 was very limited, we turned to

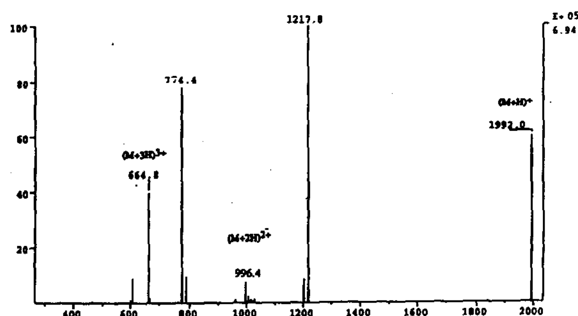

Figure 1

experiments on the fragment ions at m/z 1217.8 and 774.4. The results obtained are presented on Figures 2 and 3 respectively. Since the Aib-Pro bond is known to be particulaly labile in this type of compounds[15], the fragmentation pattern arises from the rupture of this bond in positions 13-14. Then, sequence information is obtained on residues 14-20 from the ion at m/z 774.4 and from the ion at m/z 1217.8, on residues 1-13. The sequence thus deduced is as follows:

Ac-Aib^{1}-Pro^{2}-Aib^{3}-Ala^{4}-Aib^{5}-Aib^{6}-Glx^{7}-Aib^{8}-Leu/Ile^{9}-Aib^{10}-Gly^{11}-Leu/Ile^{12}-Aib^{13}-Pro^{14}-Val^{15}-Aib^{16}-Aib^{17}-Glx^{18}-Glx^{19}-Fol^{20}.

The assignment of residues 1-3 are based here on previous experiments on alamethicin type materials[1,15]and the ions at m/z 310 and 224.9 are consistent with this fragment. Residues Pro-Val at positions 14 and 15 were determined via the single ion at m/z 196.8 but their order can not be obtained from the product ion MS/MS data obtained on the original sample. However, previous experiments have suggested the order shown and our data are consistent with these findings[1,15]. Two major uncertainities remained that could not be determined using this technique: the nature of Glx aminoacids and Leu/Ile discrimination. This second could be resolved by using NMR experiments such as HOHAHA and ROESY, shown in Figures 4 and 5.

Figure 4 shows the HOHAHA spectrum used for the identification of the individual aminoacids present in the peptaibol. From it we could identify the presence of one Ile and one Leu. The positions of these aminoacids were determined by ROESY experiments as shown in Figure 5. Using the glycine as the marker (since there is only one in the petaibol) it is possible to rebuild the sequence to see that the leucine is in position 12 and the isoleucine is in position 9. From the HOHAHA and ROESY experiments it was also possible to clearly discern two glutamines. The third Glx could not be ascertained as we were not able to detect any

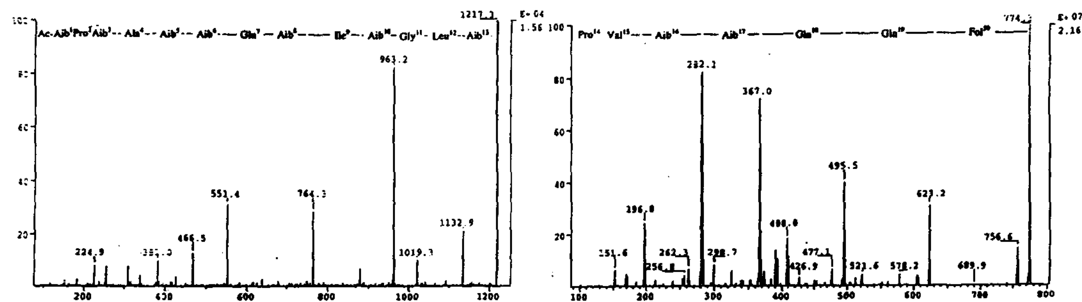

Figure 2

Figure 3

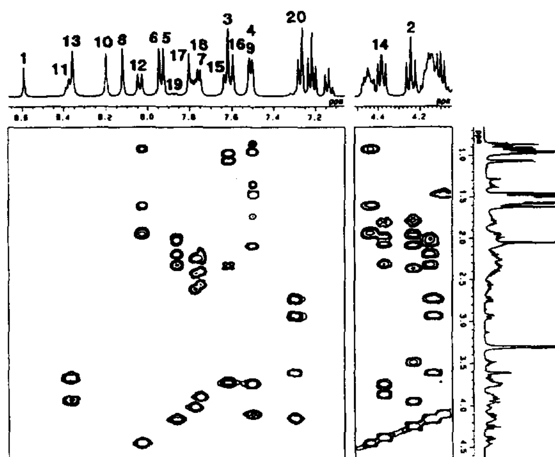

Figure 4

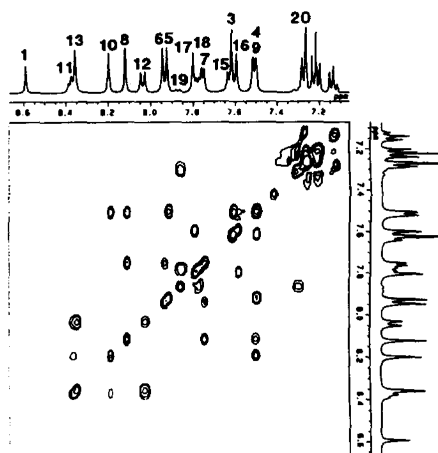

Figure 5

protons from the free amide, nor an acidic one. This problem was addressed by the use of FT ion cyclotron resonance mass (FT-ICR) spectrometry with an electrospray ionisation source to record high resolution mass spectra. This type of mass spectrometer has previously been used for high resolution characterisation of biomolecules[16]. The spectra were collected with the original mixture. For peak C the results are summarised in the following Table (calculated mass is for three Gln):

	Calculated mass	Observed mass	ΔM (ppm)
$(M+Na)^+$	2013.1513	2013.1590	3.8
$(M+H)^+$	1991.1693	1991.1754	3.1
$(M+2Na)^{2+}$	1018.0706	1018.0578	12.5
$(M+Na+H)^{2+}$	1007.0796	1007.0667	12.6

With these results it can clearly be seen that the third Glx is another glutamine[17]. The three remaining peptaibols were elucidated similarly and the sequences are as follows:

Ac-Aib-Pro-Aib-Ala-Aib-Aib-Gln-Aib-Val-Aib-Gly-Val-Aib-Pro-Val-Aib-Aib-Gln-Gln-Fol (Polysporin A).
Ac-Aib-Pro-Aib-Ala-Aib-Aib-Gln-Aib-Val-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-Gln-Gln-Fol (Polysporin B).
Ac-Aib-Pro-Aib-Ala-Aib-Aib-Gln-Aib-Ile-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-Gln-Gln-Fol (Polysporin C).
Ac-Aib-Pro-Aib-Ala-Aib-Aib-Gln-Aib-Ile-Aib-Gly-Leu-Aib-Pro-Val-Aib-Val-Gln-Gln-Fol (Polysporin D).

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REFERENCES AND NOTES

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12.-Details on the fermentation and isolation processes will be published elsewhere.

13.-HPLC conditions: Instrument: HP 1090L; eluent A: Water/0.05% TFA; eluent B: Acetonitrile/water 70:30 +0.04% TFA; gradient: 50 to 100% B in 50 minutes; flow: 200 μl/min; injection volume: 25 μl; detector wavelength: 210 nm; column: ABI Brownlee Aquapore RP-300 7 micron 220x2.1 mm. Samples were investigated using an Electrospray Ionisation source fitted to a Finnigan TSQ 700 triple stage quadrupole mass spectrometer.

14.-There are shoulders on the peaks suggesting minor components present under them. The work has been carried out on the major component of each peak.

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17.-There is some evidence that one of the Gln at positions 18 or 19 is partially hydrolysed to Glu.

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