Chemical Nature and Sequence of Alamethicin

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An n.m.r. spectroscopy study of pure alamethicin shows it to be a linear polypeptide of 19 residues. The *N*-terminus is blocked by an acetyl group, and the eighteenth residue, glutamic acid, is linked by an amide bond on its side chain to phenylalaninol (Fig. 6). The new formula is confirmed by a comparison between pure chemical compounds and the products of partial hydrolysis.

'Alamethicin' is now known to consist of a mixture of chromatographically separable components (McMullen, 1970), the main ones being components F.30 (approx. 85%) and F.50 (approx. 12%). Previous work (Payne *et al.*, 1970; Ovchinnikov *et al.*, 1971) that has attempted to characterize the peptide has been performed almost exclusively on the unseparated mixture of these various components as supplied by the Upjohn Co., Kalamazoo, Mich., U.S.A.

Meyer & Ruissner (1967) determined the composition to be (Gln₂,Pro₂,Glu,Ala₂,Val₂,Leu,Gly,-Mea₈), where Mea is 2-methylalanine (i.e. α -amino-isobutyric acid). A complete sequence analysis was carried out by conventional chemical techniques (Payne *et al.*, 1970), and Ovchinnikov *et al.* (1971) confirmed this sequence by mass spectrometry. Both groups found only seven and not eight 2-methylalanine residues (see above but also see below) and that alamethicin was hydrolysed rapidly to give the following linear peptide:

Pro-Mea-Ala-Mea-Ala-Gln-Mea-Val-Mea-Gly-Leu-Mea-Pro-Val-Mea-Mea-Glu-Gln

The sequence of this linear peptide was unequivocally determined. The cyclic structure of intact alamethicin consisted of linking the γ -carboxylate group of the glutamic acid residue to the first proline residue in the sequence, and was proposed by Payne *et al.* (1970) predominantly on the basis of the fact that there was no *N*-terminus and only one carboxyl group, which was clearly shown to be that of the terminal glutamine residue. It is to be stressed that no peptide 'crossing' this supposed cyclizing link was ever isolated.

Our intention was to follow up the above work by finding the conformation of the peptide through the use of lanthanide probes. The work was carried out on pure F.30 and F.50 fractions. However, as shown below, it became necessary to establish the true nature of the peptide first.

Experimental

Materials

The alamethicin was supplied as a freeze-dried powder by Dr. J. Melling (Microbiological Research Establishment, Porton, Wilts., U.K.) in the form of the separated F.30 and F.50 components. Minor residual impurities were removed in the following way. The fraction, F.30 or F.50, was dissolved in the minimum quantity of unbuffered aq. 10% (v/v) ethanol and passed through a Sephadex G-25 column, which was eluted with the same solution. The elution of the component was followed by monitoring the E_{260} of the aromatic ring that it contains (see below). The procedure was repeated on a narrow 'cut' from the centre of the F.30-component peak of the u.v. trace. The separation may also be performed on a Sephadex G-50 column.

A sample of pure acetylmethylalanine (m.p. 191°C) was required for comparison purposes (see below) and was prepared (see Bell, 1958) for us by Dr. M. R. Alecio, University of Oxford. Methylalanine (100mg) was dissolved in water (0.3ml) and 2M-NaOH (0.5ml) added. Acetic anhydride (240 μ l) and 2M-NaOH (2.4ml) were added in eight equal portions, the additions being made alternately. The reaction was carried out at 0°C with stirring. After 1h at 20°C the solution was neutralized with 3M-H₂SO₄ (approx. 1.1 ml) and much of the solvent was removed at 30°C. On cooling, acetylmethylalanine crystallized. The compound was characterized by its n.m.r. spectrum at two pH values, 1.0 and 10.0.

The deuterated solvents used in the n.m.r. studies were all purchased commercially and were of high purity grades ($\geq 99\%$ ²H). The two main solvents used were ²H₂O and C²H₃O²H. The 'very dry' dimethyl sulphoxide was purchased from CIBA-GEIGY, and was dried by using molecular sieves (Union Carbide size 3A, from BDH Chemicals,

(a) NH + Aromatic region

(b) α -CH + β -CH₂ region

Fig. 1. Proton n.m.r. spectrum of alamethicin component F.30

The solvent was methanol (nominal pH 1.5), and the spectrum was obtained with convolution enhancement (Campbell *et al.*, 1973). Some of the most important regions of the spectrum are labelled. In (a) 7.4–8.7 p.p.m. covers the NH region, which includes resonances of the phenylalaninol and the extra amino acid that was found and is labelled Mea-0 to show that it occurs before the first amino acid, proline-1, of the previously determined sequence. Between 7.0 and 7.3 p.p.m. the aromatic resonances are seen. In (b) various CH and CH₂ groups are observed, and again several of them are labelled as belonging to specific residues. These assignments are based on spin-decoupling experiments (the coupling is clearly seen in the spectrum), and on probe experiments with lanthanides and chromocyanide (D. R. Martin & R. J. P. Williams, unpublished work). The 'acetyl' peak and the phenylalaninol and Mea-0 peaks (including the aromatic peaks) are all quite inconsistent with the reported structure. In (c) tetramethylsilane is a marker. Numbering of the residues is that of the previously determined sequence (see the text).

Poole, Dorset, U.K.). All other solvents were used as supplied.

Solutions were prepared by weighing an appropriate amount of freeze-dried alamethicin and adding the solvent. Care was taken to avoid contact with the atmosphere as much as possible so as to minimize the water peak in the n.m.r. spectrum. The n.m.r. spectra were independent of concentration in the 2–10 mM range.

Complete hydrolysates of alamethicin were prepared by hydrolysis with 12M-HCl at 65–70°C for about 100h. Partial-hydrolysis conditions are given in the text. Samples were recovered by freeze-drying and solutions were then made up in chosen solvents.

Instrumentation

All proton spectra were taken on a Bruker spectrometer with a field of 6.4 T (64000 G) giving a resonant frequency of 270 MHz. All spectra were run in the Fourier Transform mode. The instrument has facilities for both time-shared and gated homonuclear double irradiation. It has a deuterium field-frequency lock requiring a minimum of approx. 10% deuterium in a sample. The probe is thermostatically controlled, and the temperature is adjustable over a wide range. Spectra reported in the present paper were taken at 30°C.

Chemical characterization of component F.30

The first high-resolution proton n.m.r. spectrum of alamethicin component F.30 showed that the analytical composition of our sample was not that of the sequence given in the introduction. There were two sets of peaks in the spectrum that could not possibly be explained by the sequence or even the supposed composition of alamethicin. These 'extra' peaks were also present in identical form in the second most common component (approx. 12%) of alamethicin (F.50), and were present in the unseparated Upjohn alamethicin, on which virtually all previous work, including the sequence study (Payne *et al.*, 1970), had been performed. It was quickly found that these extra peaks could not be explained by one 'extra' moiety, and they are therefore treated here under two headings (see Fig. 1), as follows: (1) a set of aromatic peaks at approx. 7 p.p.m. (and other related but not obviously extra peaks subsequently found to belong to the same moiety); peaks from aromatic carbons were also observed in ^{13}C n.m.r. spectra; (2) a single sharp peak in the methyl region at approx. 2 p.p.m. (and another single peak at approx. 1.5 p.p.m. that was not immediately obvious as an extra peak).

Nature of the aromatic moiety

A complete hydrolysate of component F.30 was prepared, and the n.m.r. spectrum of this hydrolysate was compared with that of a quantitatively prepared

mixture of the amino acids supposedly present in alamethicin. The aromatic peak was again present in the hydrolysate, but was missing from the mixture (obviously), and several other 'extra' peaks were evident. The aromatic moiety of the hydrolysate was shown by a pH titration, followed by n.m.r. spectroscopy, to possess a single pK at approx. pH 10 but no carboxylate pK. This suggests that the group has an aliphatic amino group. Attempted isolation of this moiety by running the hydrolysate down a Sephadex G-10 column was unsuccessful. However, it was successfully isolated by solvent extraction from $^2\text{H}_2\text{O}$ into C^2HCl_3 at pH 11. The n.m.r. spectrum of the isolated aromatic moiety in $^2\text{H}_2\text{O}$ accounted for all the peaks in the n.m.r. spectrum of the complete hydrolysate that could not come from amino acids previously known to be present. The spectrum of this aromatic compound at pH 11 is shown in Fig. 2, along with an n.m.r. spin-decoupling experiment which proved that the aliphatic part of the compound was in the form of a $-\text{CH}_2-\text{RCH}-\text{CH}_2-$ unit. The positions of the resonances, together with a pK_a value of 10, and the presence of no other protons except for those of a phenyl group, indicated very strongly that the material was β -phenylalaninol. A pure sample of the latter compound (from Fluorochem, Glossop, Lancs., U.K.), gave an absolutely identical spectrum (with all chemical shifts reproduced to within less than 0.02 p.p.m.), and had the same pK value.

There was already very strong evidence at this stage to indicate that this phenylalaninol, isolated from a complete hydrolysate, was in fact bound into the intact alamethicin component F.30. The evidence was as follows.

(a) It is very unlikely that an impurity could persist in constant amount throughout the large number of column separation procedures used to purify component F.30 and in 1:1 ratio to the known components in all preparations. The 1:1 ratio was determined directly from the integration of n.m.r. spectra of pure alamethicin and of hydrolysates.

(b) This same moiety is also present in the other major chromatographic component, i.e. F.50, in a 1:1 ratio to its known components in all preparations.

(c) There is no free amino group in component F.30 [a point extensively investigated in the sequence determinations (Payne *et al.*, 1970; Ovchinnikov *et al.*, 1971)], and there is no pK_a value of 10.0. Thus the phenylalaninol must be bound by its amino group.

The binding of the compound was directly shown by n.m.r. decoupling experiments on the intact component F.30. Irradiation of the phenylalaninol ' α ' CH(1) proton (see Fig. 3 for our nomenclature) decoupled all four β, β' protons and also a peak of single proton area in the amide NH region (see Fig. 3). Thus the amino group of phenylalaninol has become an amide in component F.30. This single NH proton

Fig. 2. Proton n.m.r. spectrum in $^2\text{H}_2\text{O}$ at pH 11 of the aromatic fragment

(a) The spectrum of the whole fragment; (b) the scale-expanded version of the aliphatic region of this spectrum; (c) a typical n.m.r. decoupling experiment showing that irradiation of a peak simplifies the spectrum of groups bound directly to it. The chemical formula shown is proved directly from the relative intensities of all the peaks and the decoupling pattern.

must obviously result from an amide linkage of the phenylalaninol to a carboxyl group. The phenylalaninol was therefore taken to be bound through its amino group.

Further direct evidence about the amine binding of the aromatic moiety (and about the other 'extra' n.m.r. peaks of the *N*-terminal-blocking group mentioned below) was obtained by partial hydrolysis,

Fig. 3. Detection of the bound NH of the phenylalaninol residue

Irradiation of the α -CH of phenylalaninol removes the coupling specifically in one NH signal in intact alamethicin. This experiment completes the assignment of all the protons in phenylalaninol and gives its structure in the alamethicin molecule apart from the OH resonance. The solvent was dry dimethyl sulphoxide. (a) Without irradiation; (b) with irradiation

conducted on component F.30 in the n.m.r. tube itself. The peptide (about 15mm) in C²H₅O²H/²H₂O (3:1, v/v) and 3M-²HCl solution in an n.m.r. tube was placed in the n.m.r. spectrometer at 37°C and spectra were obtained at intervals over 4h. A single spectrum is recorded in less than 5min. The aromatic peak itself, and the peaks from other protons in the phenylalaninol of the intact component F.30, diminished in intensity with time and were replaced by sharp peaks at slightly different chemical-shift positions, showing that the phenylalaninol had been hydrolysed from the component F.30. The sharp peaks of the phenylalaninol replacing somewhat broader peaks are expected in such a hydrolysis if all other products and the initial alamethicin are of much higher molecular weight. The 'half-life' under these

conditions was about 90min. The functional groups released were also detected.

The problem of the attachment of the phenylalaninol was tackled next. The previous chemical (Payne *et al.*, 1970) and mass-spectroscopic (Ovchinnikov *et al.*, 1971) studies preclude interference with any part of the sequence, including the amide groups of the two glutamine residues. These amides of component F.30 were confirmed to be normal amide groups by n.m.r. studies in dimethyl sulphoxide, in which amide-NH₂ groups are readily recognized. The previous studies also showed that the α -carboxyl group of the terminal glutamine residue of the intact alamethicin component F.30 is free. The position of the free carboxyl group was confirmed by direct n.m.r. observation of the -CO₂H proton in dry

Fig. 4. Spectrum of alamethicin component F.30 in dry dimethyl sulphoxide showing the OH resonance and its decoupling by irradiating the β' -CH₂ of phenylalaninol (see Fig. 3)

(a) A spectrum at high pH where the OH is not seen; (b) a spectrum at low pH showing the OH triplet; (c) decoupling of the OH triplet by irradiation at f_2 , the β' -CH₂ frequency.

dimethyl sulphoxide, a solvent in which such -CO₂H protons are seen in characteristic positions. The only way in which the phenylalaninol could be attached, therefore, was to 'break' the proposed γ -carboxyl link from the glutamic acid residue to the proline residue (cycling link) and attach the phenylalaninol to this γ -carboxyl group by an amide link.

This postulate at once raises the question whether component F.30 is cyclic at all. This possibility was examined by looking at the only other functional group the phenylalaninol possesses, i.e. the hydroxyl group, to see whether it was in fact free. The proton of the hydroxyl group, because of its very rapid exchange with solvent deuterons and/or protons, is

not normally seen in $^2\text{H}_2\text{O}$ or $\text{C}^2\text{H}_3\text{O}^2\text{H}$. However, $-\text{OH}$ protons can be seen by running spectra in very dry deuterated dimethyl sulphoxide. These experiments are shown in Fig. 4. Initially and at high effective pH, Fig. 4(a), the $-\text{OH}$ is not visible because of rapid exchange with remaining traces of water. When the 'pH' was changed to a lower value by the addition of small amounts of ^2HCl in dry dimethyl sulphoxide, exchange became slow, and the proton was seen in the spectrum as a triplet. (It is a triplet as it is coupled to the two phenylalaninol $\beta\text{-CH}_2$ protons.) Irradiation at f_2 of the (identified) two $\beta\text{'-CH}_2$ protons of the phenylalaninol of component F.30 decoupled the $-\text{OH}$ triplet, as shown in Fig. 4(c). This experiment shows that the triplet is that of the phenylalaninol $-\text{OH}$. The experiment was successfully reproduced with several different samples, and shows conclusively that the $-\text{OH}$ of the phenylalaninol is free. Alamethicin component F.30 must therefore be linear, since, given our knowledge of all other functional groups, there is no possible way of closing the peptide ring. Obviously, then, some additional group must be attached to the initial proline residue blocking the presumed N -terminus of the sequence proposed. This group is in fact the moiety giving rise to the second group of extra peaks seen in the n.m.r. spectrum.

N-Terminal blocking group

It will be remembered that in the n.m.r. spectrum of intact component F.30 there was an extra peak in the methyl region as well as the extra aromatic peaks (Fig. 1). Closer inspection showed that, as well as this single sharp peak at approx. 2.0 p.p.m., there was another peak, probably associated with it, at approx. 1.5 p.p.m. and which was also a singlet. The relationship between the two peaks was discovered on adding the paramagnetic chromicyanide anion to alamethicin. This anion broadens the n.m.r. signals of nuclei close to it in space, i.e. near its binding site. The addition of chromicyanide ion to component F.30 in $^2\text{H}_2\text{O}$ solution greatly broadened the 2.0 p.p.m. peak, and broadened the 1.5 p.p.m. peak, but virtually all other peaks in the spectrum were unaffected. Campbell *et al.* (1973) showed that a difference spectrum obtained by subtracting an n.m.r. spectrum in the presence of low concentrations of chromicyanide ion from one in the absence of this ion reveals only the groups nearest to the reagent. Such a difference spectrum was obtained for alamethicin (5 mM) by using 0.2 mM-potassium chromicyanide and showed only the peaks at 2.0 and 1.5 p.p.m.

The group giving rise to these two peaks does not survive the complete hydrolysis described, since there were no unusual peaks in the hydrolysate in the methyl region. The nature of the group was studied by 'in-probe' partial hydrolysis like that described above

Fig. 5. Identification of the isolated aliphatic N -terminal fragment

This fragment of alamethicin was identified by a study of its n.m.r. spectrum in $^2\text{H}_2\text{O}$. From the top downwards the spectra are of (a) XH, the unknown fragment, (b) XH plus acetate as a marker, (c) XH plus acetate marker plus an equal amount of pure separately synthesized acetyl-2-methylalanine at low pH and (d) at high pH. Note the precise coincidence of the XH and the acetyl-2-methylalanine resonances at two pH values, which establishes identity.

for the aromatic moiety. Component F.30 was hydrolysed in 3.5 M- ^2HCl in $^2\text{H}_2\text{O}$ at 37°C. Component F.30 is not very soluble under these conditions, and hence does not yield a high-resolution n.m.r. spectrum itself, but after about 20 min of hydrolysis in the n.m.r. tube two very sharp peaks appeared in the spectrum and these increased in intensity with time. After 2 h the reaction was stopped by adding NaO^2H until the pH was approx. 1.0. Insoluble materials were spun down and the n.m.r. spectrum of the residual solution was found to be extremely simple, consisting of two peaks at approx. 2.0 and 1.5 p.p.m. A pH titration of

the sample showed that there was an ionizable group of low pK but none of high pK . A detailed but routine n.m.r. study showed that this material is *N*-acetyl-2-methylalanine and we presume that the extra methyl peaks due to this group in the intact alamethicin n.m.r. spectrum are lost on hydrolysis as acetic acid (volatile) and conventional 2-methylalanine. The structure was confirmed by addition of some pure synthetic *N*-acetyl-2-methylalanine to the n.m.r. tube containing the hydrolysed *N*-terminal group together with some sodium acetate as an n.m.r. spectral 'marker' (to enable direct intensity comparison) (see Fig. 5). It can be seen (spectrum *c*) that all absorption peaks in the spectrum superimpose precisely and remain superimposed after a change of pH changes the spectrum. The synthetic *N*-acetyl-2-methylalanine must be identical with the material from the partial hydrolysis.

These partial-hydrolysis experiments apparently indicate that both the phenylalaninol and the *N*-acetyl methylalanine are readily removed from the peptide. This might be explained by their intrinsic lability but it could also be that it is the rest of the peptide which is resistant to hydrolysis for it is noticeably very hydrophobic. In any event further experiments are required to explain our data.

The *N*-acetyl-2-methylalanine must be covalently bound to component F.30, and this is confirmed by the partial-hydrolysis experiments in $C^2H_3O^2H/H_2O$ (3:1, v/v) in $3M-HCl$ at $37^\circ C$. As well as the aromatic group that is hydrolysed from the alamethicin after 1 h or so, as reported above, both peaks of the bound *N*-acetyl-2-methylalanine group diminish in intensity while being progressively replaced by very sharp peaks at slightly different chemical-shift positions, again with a half-life of about 90 min. Hence both 'extra' groups are directly observed to be hydrolysed from alamethicin rapidly in mild conditions, leaving the rest of the alamethicin largely unaffected.

Bearing in mind all the previous chemical work (Payne *et al.*, 1970; Ovchinnikov *et al.*, 1971; our n.m.r. studies) the only possible functional group to which to attach this *N*-acetyl-2-methylalanine is the presumed *N*-terminal proline residue. Direct evidence for this is that addition of the paramagnetic chromicyanide ion not only broadens the peak of this *N*-terminal-blocking group (see above) but also broadens the proline α -CH peak that is clearly visible in the spectrum. Thus the two residues are very close in space to one another. Further, the hydrolysis of this group from component F.30 in the in-probe partial-hydrolysis experiments [$C^2H_3O^2H/H_2O$ (3:1, v/v) at $37^\circ C$] described above would be expected to leave a positively charged terminal proline residue if this were the blocked *N*-terminus, to which the negatively charged chromicyanide ion would now bind strongly. This was found to be the case. The placement of both the phenylalaninol and the *N*-acetyl-2-methylalanine is further confirmed by more detailed conformational studies with the use of the paramagnetic lanthanide cations (D. R. Martin & R. J. P. Williams, unpublished work).

In summary, alamethicin component F.30 is shown to be a linear peptide with the *N*-terminus blocked and with a phenylalaninol side chain attached to the γ -carboxyl group of the only glutamic acid residue in the peptide (see Fig. 6). The fairly strong binding of the chromicyanide ion at the *N*-terminus of component F.30 as found by the n.m.r. has led us to consider the possibility that in dry methanol there may be a small amount of a positively charged species, such as is shown in Fig. 6, which is further stabilized by the binding of the chromicyanide ion. It is worthwhile briefly reviewing the previous composition of Meyer & Ruisser (1967), and sequence studies of Payne *et al.* (1970) and Ovchinnikov *et al.* (1971) to show that their experimental results do not materially disagree with the results presented here.

Ac-Mea-Pro-Mea-Ala-Mea-Ala-Gln-Mea-Val-Mea-Gly-Leu-Mea-Pro-Val-Mea-Mea-Glu-Gln

Fig. 6. New sequence of alamethicin

There is one additional amino acid, an *N*-terminal-blocking group, and a side chain on the eighteenth residue, phenylalaninol. Below the linear sequence is shown a possible internal ring closure of alamethicin that would produce a positive charge at the *N*-terminus and so account for the anion binding in dry methanol.

The phenylalaninol was undetected previously because, although it stains with ninhydrin, it sticks very strongly to the amino acid analyser column. The *N*-acetyl-2-methylalanine would also be undetected both when intact and when split into acetic acid and methylalanine, as the latter would simply appear along with the seven other methylalanine residues present. Despite the care taken by Payne *et al.* (1970) to obtain an accurate analysis of methylalanine, they obtained only seven such residues. The analysis was made difficult by (i) weak staining with ninhydrin, (ii) microheterogeneity in impure alamethicin with alanine at position 5 as well as methylalanine, and (iii) the fact that work was carried out on a mixture of components F.30, F.50, etc., which contained further unknown impurities.

Both Payne *et al.* (1970) and Ovchinnikov *et al.* (1971) reported that a simple linear peptide was rapidly obtained on mild hydrolysis. This is confirmed by our n.m.r. partial-hydrolysis experiments (referred to above) where the rapid hydrolysis of the two blocking groups from component F.30 was directly observed. Once these blocking groups have been 'lost' the alamethicin will obviously give sequence results entirely consistent between our data and the earlier work, apart from the supposed cyclic linkage. Ovchinnikov *et al.* (1971) passed the peptides from a partial hydrolysate down a cation-exchange resin at low pH to separate them before mass spectrometry and detected them with ninhydrin. The blocking groups would easily go undetected.

The linear gramicidins A, B and C were originally thought to be cyclic, but were subsequently found to have the C-terminus blocked by ethanolamine (compare with phenylalaninol) and the N-terminus blocked by formyl (compare with acetyl) (Synge, 1945, 1950; Sarges & Witkop, 1964). Thus the histories of the study of the two groups of peptides, gramicidins and alamethicins, are closely parallel.

Sequence of component F.50

The second most common component of 'alamethicin', namely component F.50 (approx. 12%), was also studied. We have shown that the amino acid compositions of components F.30 and F.50 are identical (except for a glutamic acid/glutamine distinction; see below). All the experiments described above for component F.30 have also been performed on component F.50 with identical results, except that, in place of the terminal glutamine $-\text{CO}_2\text{H}$ group, component F.50 has an amide group ($-\text{CONH}_2$), which was proved by n.m.r. experiments in dimethyl sulphoxide. The two blocking groups are present in identical manner with those in component F.30.

The identity of the $\text{Pro}_{(2)}\text{-Glx}_{(19)}$ (new numbering) sequences for components F.30 and F.50 has not been conclusively proved. But the virtual 'fingerprint' identity of the high-resolution 270 MHz n.m.r.

spectra of components F.30 and F.50 in all regions and the difficulty of separating these components show that the sequences in these two peptides must be very similar if not identical.

Membrane properties of alamethicin

The membrane conduction properties of alamethicin (Mueller & Rudin, 1968*a,b*; Cherry *et al.*, 1972; Gordon & Haydon, 1972) are most intriguing in a biological context. Under the right conditions alamethicin can give artificial-membrane ion-permeability properties such that potentials across the membrane mimic nerve action potentials. The linearity of alamethicin is of great importance in understanding the nature of this function. Models of our sequence show that alamethicin is long enough to stretch across a lipid bilayer, and so 'pores' may be formed that consist of alamethicin molecules laid lengthways side by side. This could result in co-operativity between molecules in pores much as has been reported (Gordon & Haydon, 1972). The previous suggestions of stacked-ring pores must evidently be rejected. The only other series of peptides which form similar pores are the gramicidins. From our work the alamethicins and linear gramicidins are shown to share common structural features which differentiate them from ionophores which are cyclic or smaller linear molecules. It seems probable that these two groups of linear peptides can generate aqueous channels through the membrane.

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