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The Structures of the Frenatin Peptides from the Skin Secretion of the Giant Tree Frog *Litoria Infrafrenata*

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Abstract: The granular dorsal glands of the giant tree frog *Litoria infrafrenata* contain five peptides including caerulein (a known neuropeptide), and four new peptides named frenatins 1 ($MH^+ = 1140$ Da), 2 (1423), 3 (2180) and 4 (2493). The amino acid sequences of the frenatins are detailed: their structures do not correspond to those of peptides isolated from other amphibians or animals. Frenatin 3, Gly-Leu-Met-Ser-Val-Leu-Gly-His-Ala-Val-Gly-Asn-Val-Leu-Gly-Gly-Leu-Phe-Lys-Pro-Lys-Ser-(OH), has wide spectrum antimicrobial properties.

Keywords: peptides; neuropeptide; antimicrobial agent; skin secretion; frogs

Amphibians live in environments where there are numerous micro-organisms and animal predators, and their dermal secretions contain a variety of peptides [1,2]. Some of these have been identified as host defence chemicals, i.e. antibiotics, hormones, opioids and toxins, but many of the others have functions which have not, as yet, been determined. During the last three decades, a number of families of amphibian peptides have been identified by other research groups (particularly that of Erspamer) including the angiotensins, bombesins, bombinins, bradykinins, caeruleins, dermorphins, magainins, pipinins, tachykinins and tryptophyllins [1, 2].

Testing for pharmacological activity in the skin secretions of Australian frogs commenced in the 1960s [3]; this led to the isolation of the hypotensive peptides caerulein (**1**) from *Litoria caerulea* [3] and uperolein from *Uperoleia* species [4]. Caerulein is a potent analgesic, a hypotensive peptide, probably a hormone, and it has been used clinically [1,2]. The green tree frogs *Litoria caerulea* [5, 6]. *Litoria gilleni* [7] and *Litoria splendida* [8] collectively contain more than thirty peptides in their skin secretions. They all contain caerulein (**1**) [3], but the major peptide families are the caerins (e.g. caerin 1.1 (**2**), and the caeridins (e.g. caeridin 1.1 (**3**)). The cationic peptide caerin 1.1 is a wide-spectrum antimicrobial agent, while the anionic caeridin 1.1 exhibits neither hypotensive nor antibiotic activity, and its function in the amphibian integument is not known.

Pyr-Gln-Asp-Tyr(SO_3H)-Thr-Gly-Trp-Met-Asp-Phe(NH_2) (**1**)

Gly-Leu-Leu-Ser-Val-Leu-Gly-Ser-Val-Ala-Lys-His-Val-Leu-Pro-His-Val-Val-Pro-Val-Ile-Ala-Glu-His-Leu(NH_2) (**2**)

Gly-Leu-Leu-Asp-Gly-Leu-Leu-Gly-Thr-Leu-Gly-Leu(NH_2) (**3**)

The giant or white lipped tree frog, *Litoria infrafrenata* [9], is the largest tree frog in Australia, measuring up to 14 cm in length. Unlike other Australian tree frogs, *L. infrafrenata* is distributed extensively throughout the lowland areas of New Guinea and its associated islands. In Australia, it is confined to the Cape York Peninsula of northern

Abbreviations: FAB-MS, fast atom bombardment mass spectrometry; MIC, minimum inhibitory concentration.

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Queensland, and is regarded as a species which entered Australia from the north during one of the several periods when a land bridge connected the two land masses. *Litoria infrafrenata* is morphologically quite different from the other Australian tree frogs that we have studied, e.g. *L. caerulea*, *L. gilleni* and *L. splendida*, in that it does not possess massive parotoid glands. Instead, its granular glands are distributed over the whole dorsal surface. Its colour may range from green to brown, but one feature is constant and diagnostic; it has a broad white stripe extending along the lower lip to beyond the insertion of the arm. It is unique amongst *Litoria* species in having a haploid chromosome number of twelve: all others have thirteen [10]. The fact that there are very significant morphological differences between *Litoria infrafrenata* and other Australian tree frogs led us to anticipate that its peptide profile would be unique.

We have reported that mild electrical stimulation of the skin of amphibians effects the release of skin secretions, and that this can be done periodically (generally monthly) without harming the animal [11]. This is very important at a time when frog populations are in decline [12]. Each 'milking' of a single specimen of *L. infrafrenata* gives, on average, some 25 mg of solid material following lyophilization. An HPLC separation of 0.1 mg of this material is shown in Figure 1. The skin secretion contains five major peptide components, designated **a**–**e** in Figure 1. Combination of individual fractions from ten such separations gave sufficient material for the purpose of structure determination, i.e. some 480 μg of **a**, 320 μg of **b**, but only 20–40 μg of each of **c**–**e**.

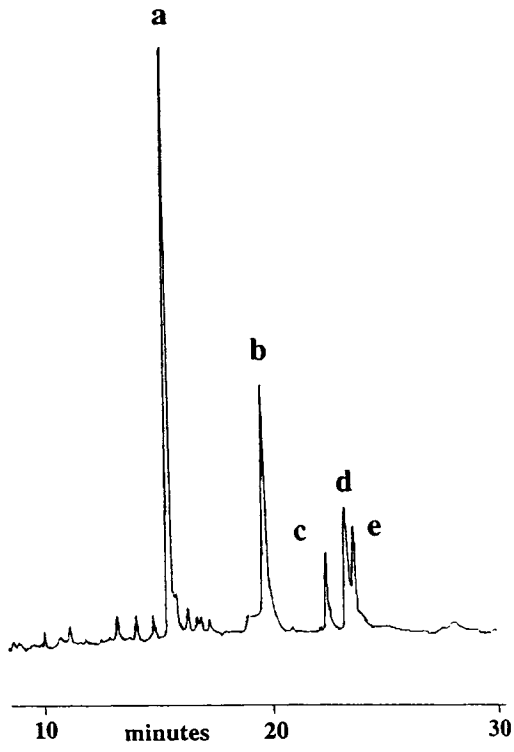

Figure 1 HPLC separation of the peptides from the glandular secretion of *Litoria infrafrenata*. For experimental details see Experimental Part.

Amino Acid Sequence Determination of the Frenatin Peptides

Peptide **a** is the hypotensive peptide caerulein (**1**), identical to the same compound isolated from other *Litoria* species. Fast atom bombardment mass spectrometry (FAB-MS) indicates that the other components have the following MH^+ values: **b** (2493 Da), **c** (1140), **d** (1423) and **e** (2180). (Masses given are nominal masses, i.e. the mass produced by summation of the integral masses of individual amino acid residues.) We name the peptides frenatins 1–4, in ascending order of their molecular weights. Their sequences are listed in Scheme 1.

Scheme 1. The amino acid sequences of the frenatins.

Frenatin 1 ($MH^+ = 1140$) (**4**)
Gly-Leu-Leu-Asp-Ala-Leu-Ser-Gly-Ile-Leu-Gly-Leu-(NH_2)

Frenatin 2 ($MH^+ = 1423$) (**5**)
Gly-Leu-Leu-Gly-Thr-Leu-Gly-Asn-Leu-Leu-Asn-Gly-Leu-Gly-Leu-(NH_2)

Frenatin 3 ($MH^+ = 2180$) (**6**)
Gly-Leu-Met-Ser-Val-Leu-Gly-His-Ala-Val-Gly-Asn-Val-Leu-Gly-Gly-Leu-Phe-Lys-Pro-Lys-Ser-(OH)

Frenatin 4 ($MH^+ = 2493$) (**7**)
Gly-Phe-Leu-Asp-Lys-Leu-Lys-Lys-Gly-Ala-Ser-Asp-Phe-Ala-Asn-Ala-Leu-Val-Asn-Ser-Ile-Lys-Gly-Thr-(OH)

The sequence of each of the frenatin peptides was determined by a combination of automated Edman sequencing [13] and mass spectrometric techniques (cf. [3]) or as follows. The MH^+ value of each peptide is determined by FAB-MS. The number of CO_2H and $CONH_2$ groups present in each peptide is determined by allowing the peptide to react with acidic methanol which converts all carboxylic acid and amide groups to their methyl esters. FAB-MS is then used to determine the MH^+ value of each modified peptide. The difference between the mass of the peptide and its methyl ester derivative provides the required information, e.g. frenatin 1 methyl ester ($MH^+ = 1169$ Da; mass difference between frenatin 1 and its methyl ester is 29 Da (i.e. $CO_2H + 14$ and $CONH_2 + 15$), therefore frenatin 1 has one CO_2H and $CONH_2$ group). Manual Edman/FAB-MS [14] is used

to determine as many amino acid residues from the N-terminal end of the peptide as possible. The FAB tandem MS/MS method is then used to provide sequencing data [15]. In the case of the larger frenatins 3 and 4 (see Scheme 1), Lys-C and/or chymotrypsin digest produced smaller peptides which were identified by FAB-MS/MS. Combination of these data generally provided the sequence of the peptide, with the exception that isomeric Leu and Ile cannot reliably be distinguished by the MS/MS technique. The MS structure determinations for frenatins 1–3 and 4 are detailed in Schemes 2 and 3 respectively, while an example of the use of the MS/MS method for sequencing is shown for illustrative purposes in Figure 2 for frenatin 1.

Finally, each peptide was sequenced by the automated Edman procedure [13] which differentiates between Leu and Ile and confirms the overall structure. The combined data provide the sequences of frenatins 1–4 listed in scheme 1.

Scheme 2. MS data for Frenatins 1–3 and enzymic digestion products.[a]

Frenatin 1 [$MH^+ = 1140$ Da]

(i) Methylation[a] gives [$MH^+ = 1169$] – one CO_2H group and one $CONH_2$ group.

(ii) Manual Edman/MS[b] Gly-Leu[c] Leu-Asp-Ala

(iii) Tandem MS/MS sequencing data[d(i)] (see also Figure 2).

B cleavages[d(ii)] m/z 1010, 953, 840, 727, 670, 583, 470, 399, 284
[Asp-Ala-Leu-Ser-Gly-Leu-Leu-Gly-Leu(NH_2)]

Y + 2 cleavages[d(ii)] m/z 1083, 742 588
[Gly-(Leu-Leu-Asp) (Ala-Leu)]

Sequence = Gly-Leu-Leu-Asp-Ala-Leu-Ser-Gly-Leu-Leu-Gly-Leu-(NH_2)

Frenatin 2 [$MH^+ = 1423$ Da]

(i) Methylation gives [$MH^+ = 1148$] – three $CONH_2$ groups.

(ii) Manual Edman/MS Gly-Leu-Leu

(iii) MS/MS sequencing data.

B cleavages m/z 1293, 1236, 1123, 1066, 952, 839, 726, 612, 555, 341, 284, 171.
(Leu (3) Gly [Leu Thr] Gly-Asn-Leu-Leu-Asn-Gly-Leu-Gly-Leu-(NH_2))

Sequence = Gly-Leu-Leu-Gly-Thr-Leu-Gly-Asn-Leu-Leu-Asn-Gly-Leu-Gly-Leu-(NH_2)

Frenatin 3 [$MH^+ = 2180$ Da]

(i) Methylation gives [$MH^+ = 2209$] – one CO_2H and one $CONH_2$ group.

(ii) Manual Edman/MS Gly-Leu-Met-Ser-Val-Leu-Gly-His-Ala

(iii) MS/MS sequencing data of Edman products and enzymic digest products.

(1792) (MH^+) B cleavages m/z 1687, 1559, 1462, 1334, 1187, 1074, 1017, 960, 478.
product from {[382] Gly (15) Gly-Leu-Phe-Lys-Pro-Lys-Ser(OH)}
4th Edman Y + 2 cleavages m/z 1694 and 1581
cycle. [Val (5) Leu]

Partial sequence = Val (5) Leu (265) (382) Gly-Gly-Leu-Phe-Lys-Pro-Lys-Ser(OH)

Lys-C digest of frenatin 3 gave peptide, $MH^+ = 2093$, indicating loss of a C-terminal Ser (OH) adjacent to Lys.

Chymotrypsin digest gave peptides (**A**, $MH^+ = 619$), (**B**, $MH^+ = 766$) and (**C**, $MH^+ - 1140$).

Peptide **A** B cleavages m/z 488, 389, 302, 171
(619) [Met-Ser-Val-Leu]
Y + 2 cleavages m/z 562, 449
[Gly-Leu]
Sequence = Gly-Leu-Met-Ser-Val-Leu

Peptide **B** B cleavages m/z 649, 535, 478, 379, 308
(766) [Ala-Val-Gly-Asn-Val]
Y + 2 cleavages m/z 653, 596
[Leu-Gly]
Sequence = Leu-Gly-(His)-Ala-Val-Gly-Asn-Val

Frenatin 3 = Gly-Leu-Met-Ser-Val-Leu-Gly-His-Ala-Val-Gly-Asn-Val-Leu-Gly-Gly-Leu-Phe-Lys-Pro-Lys-Ser-(OH)

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[a] For an explanation of esterification data on peptides see text.

[b] See [14], also [5].

[c] The MS method does not differentiate between isomeric Leu and Ile. Leu is depicted (for simplicity) in the Scheme. Automated Edman sequencing [13] distinguishes Leu and Ile.

[d] (i) For a recent review of peptide cleavages see [15].
(ii) For an explanation of B and Y + 2 cleavages see legend to Figure 2.

Scheme 3. MS data for Frenatin 4 and enzymic digestion products.

Frenatin 4 $[MH^+ = 2493]$

(i) Methylation gives $[MH^+ = 2565]$ – three CO_2H and two $CONH_2$.

(ii) Manual Edman/MS – Gly Phe Leu.

(iii) Lys-C digest gives peptides **(D)** $[MH^+ = 820]$, **(E)** [1692], **(F)** [1564], **(G)** [1534] and **(H)** [1406].

Peptide **D** $[MH^+ = 820]$

(i) Manual Edman/MS – Gly-Phe-Leu.

(ii) MS/MS sequencing

B cleavages	*m/z* 674, 561, 433, 318, 205.
	[Leu(3)-Asp-Lys-Leu-Lys-(OH)
Y + 2 cleavages	*m/z* 763, 616, 503, 388, 260
	[Gly(1)-Phe-Leu-Asp-Lys-(OH)]

Sequence of **D** = Gly(1)-Phe-Leu-Asp-Lys-Leu-Lys(OH)

Peptide **E** $[MH^+ = 1692]$

(i) Manual Edman/MS – Lys Gly Ala

Peptide **F** $[MH^+ = 1564]$

(i) Manual Edman/MS – Gly-Ala-Ser-Asp-Phe-Ala

(ii) MS/MS sequencing

B cleavages	*m/z* 1445, 1388, 1260, 1147, 1060
	[Ser-Leu-Lys-Gly-Thr-(OH)

Peptide **G** $[MH^+ = 1534]$

(i) Manual Edman/MS – Lys-Gly-Ala

Peptide **H** $[MH^+ = 1406]$

(i) Manual Edman/MS – Gly-Ala-Ser-Asp

(ii) MS/MS sequencing

B cleavages	*m/z* 1260, 1147, 1060, 946, 847, 734, 663
	[Ala-Leu-Val-Asn-Ser-Leu-Lys]

Sequence of **E** = Lys(8) Gly-Ala-Ser-Asp-Phe-Ala-Asn-Ala-Leu-Val-Asn-Ser-Leu-Lys-Gly-Thr-(OH)

Frenatin 4 = Gly-Phe-Leu-Asp-Lys-Leu-Lys-Lys-Gly-Ala-Ser-Asp-Phe-Ala-Asn-Ala-Leu-Val-Asn-Ser-Leu-Lys-Gly-Thr-(OH)

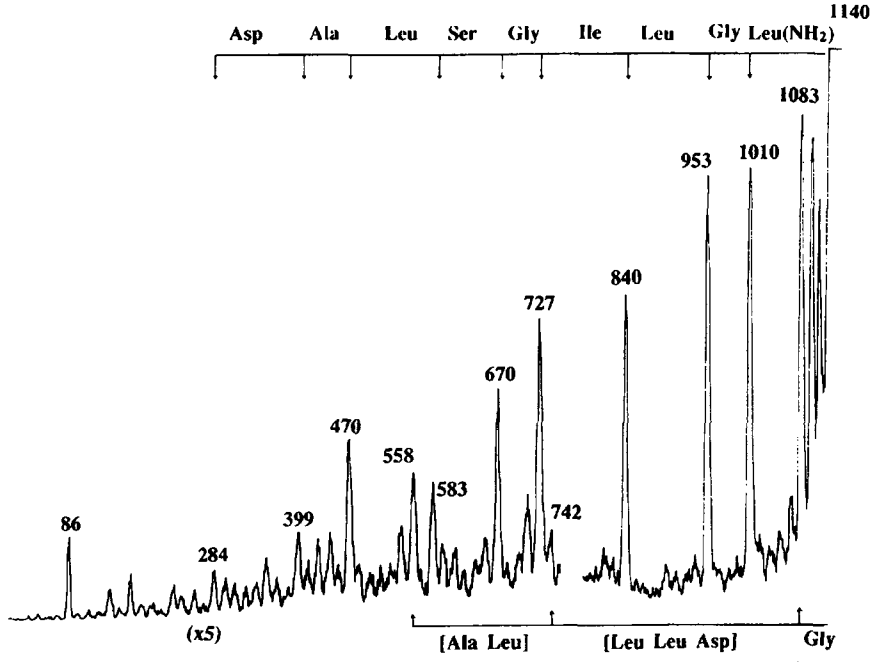

Figure 2. FAB collision induced mass spectrum (MS/MS) of the MH^+ ion of Frenatin 1. Schematic arrows on the top of the figure give the sequence data provided by B cleavages, while those at the bottom of the figure indicate the data provided by Y + 2 cleavages. (B cleavages are those that form $R-C\equiv O^+$ ions (e.g. $RCO^+NH_2CH_2CO_2H \rightarrow RCO^+ + NH_2CH_2CO_2H$), while Y + 2 cleavages form R^+NH_3 ions (e.g. $NH_2CH_2CO^+NH_2R \rightarrow R^+NH_3 + NHCH_2CO$)). See [15] for full details of fragmentation types.

Structure and Bioactivity Relationships of the Frenatin Peptides

Four of the five peptides from *L. infrafrenata* are post-translationally modified. Caerulein (**1**) is modified at both the C- and N-terminal positions and also on Tyr, while frenatins 1, 2 and 4 have $CONH_2$ groups at the C-terminal end of the peptide.

The frenatin peptides are different from any amphibian peptides thus far reported. In particular, apart from caerulein (**1**), which is present in all of the Australian green tree frogs studied to date, there is no direct correspondence with the peptides of other tree frogs. The larger peptides, frenatins 3 and 4, are unique and are quite different in structure from the caerin peptides (e.g. caerin 1.1 (**2**) of other Australian tree frogs. This constitutes a further difference between *L. infrafrenata* and other *Litoria* species.

There is a view [16] that the structures of bioactive amphibian peptides should correlate with those of peptides from other organisms, in particular with mammalian peptides. Thus, we carried out a GEN-INFO (R) BLAST Network Service (Cruncher) data bank search (Australian National Genomic Information Service) (cf. 17) for the frenatin 3 and 4 structures. Several unrelated proteins, which have only their association with membranes in common, showed some degree of identity with frenatin 3, but this was not sufficient to be statistically significant. For example, a sucrose transporter [18] contained a unit which showed 50% residue identity with frenatin 3. No significant correlation was found between reported peptides and frenatin 4.

Amphibian peptides which show antibiotic activity often have the following general features in common, viz they (i), are cationic, (ii) have molecular weights greater than 1500 Da, and (iii) can be drawn as full or partial α-helical structures, using the Edmundson wheel projection, in which the hydrophilic and hydrophobic side chains are in defined regions of the projection. Such amphipathic peptides are considered to interact with the outer lipid layer of the bacterial cell, penetrate the bacterial membrane, form ion channels, and ultimately kill the cell [1, 2]. Frenatin 3 conforms to all of the three features listed above, in particular, the Edmundson projection shows an α-helix with reasonably well-defined hydrophilic and hydrophobic regions (Figure 3(A)). In contrast, and Edmundson projection for frenatin 4 (Figure 3(B)) shows hydrophilic and hydrophobic character scattered throughout the α-helical representation.

We did not have enough of the purified natural peptides to measure their antimicrobial activities, so we had frenatins 1–4 synthesized (see Experimental part for details) for this purpose. Automated Edman sequencing showed each synthetic peptide to have the same sequence as its natural counterpart: in addition, the natural and synthetic peptides had identical mass spectra and co-eluted on HPLC. The synthetic peptides were tested for antimicrobial activity by Ciba-Geigy, Australia. Synthetic frenatin 3 is active, exhibiting medium to good activity against the following test organisms: *Bacillus cereus* (MIC value = 12.5 μg/ml), *Escherichia coli* (50), *Leuconostoc mesenteroides* (25), *Micrococcus luteus* (1.5),

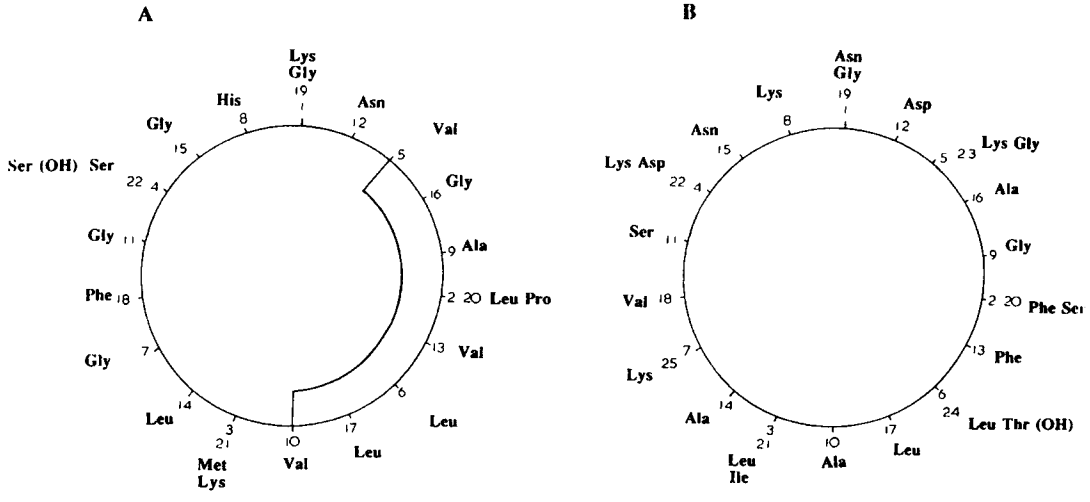

Figure 3 Edmundson wheel projection of (A) Frenatin 3 and (B) Frenatin 4. The hydrophilic and hydrophobic zones are indicated for Frenatin 3. The flat Pro residue at position 20 in Frenatin 3 will distort the α-helix beyond that position.

Pasteurella haemolytica (0.8), *Staphylococcus aureus* (< 100), *Streptococcus faecalis* (< 150) and *Streptococcus uberis* (50). Synthetic frenatin 4 shows no activity below 100 μg/ml against any of the microorganisms listed above. This latter result was so unusual that we had a new sample synthesized and retested – it also showed no activity below 100 μg/ml against the test organisms.

The smaller peptides, frenatins 1 and 2 are not efficient antimicrobial agents. The only test organism that they are active against is *Micrococcus luteus* (frenatin 1 at 25 μg/ml and frenatin 2 at 50 μg/ml). These two peptides show some resemblance to the caeridin peptides (see, e.g., **3**) isolated from other *Litoria* species [5–8]. The role of such peptides in frog skin is not known. However, frenatin 1 is an anionic peptide and frenatin 2 a neutral peptide: it is possible that such peptides may be spacer components of the pre-pro-antimicrobial peptide (see [19] for a discussion of the biosynthesis of antimicrobial peptides).

In conclusion, the skin secretion of *L. infrafrenata* contains two powerful host defence compounds, the known neuropeptide caerulein, and the antimicrobial agent frenatin 3. The functions of the other peptides, particularly the abundant frenatin 4, in the amphibian integument are not understood at this time.

EXPERIMENTAL PART

Preparation of Skin Secretions

Specimens of *Litoria infrafrenata* were held by the back legs, and the skin was moistened with deionized water, and stimulated by means of a bipolar electrode of 21G platinum attached to a Palmer Student Model electrical stimulator. The electrode was rubbed gently in a circular manner on the skin of the animal, using 10 volts and a pulse duration of 3 ms [11]. The resulting secretion was washed from the frog with deionized water (50 cm^3), the mixture diluted with an equal volume of methanol, centrifuged, filtered through a Millex HV filter unit (0.45 μm), and lyophilized. This procedure provides, on average, some 25 mg of solid material.

HPLC Separation of the Glandular Secretion

HPLC separation was achieved using a VYDAC C18 HPLC column (5 μ, 300 A, 4.6 × 250 mm) (Separations Group, Hesperia, CA, USA) equilibrated with acetonitrile/aqueous trifluoroacetic acid (0.1% TFA) (10% acetonitrile). The lyophilized mixture (generally *ca.* 1 mg), was dissolved in deionized water (50 μl), of which a 5 μl fraction was injected into the column. The elution profile was generated using a linear gradient produced by an ICI DP 800 Data Station controlling two LC1 100 HPLC pumps, increasing from 10–75% acetonitrile over a period of 30 min at a flow rate of 1 cm^3/min. The eluant was monitored by ultraviolet adsorbance at 214 nm using an ICI LC-1200 variable wavelength detector (ICI Australia, Melbourne, Australia). The HPLC trace (Figure 1) shows five major components (**a** to **e**) with respective retention times of 15.5, 19.6, 22.4, 23.5 and 23.9 min. Fractions were collected, concentrated and dried *in vacuo* for subsequent analysis. Combination of appropriate fractions from ten such separations were required to produce workable amounts of each peptide. The fractions are identified as follows: **a** [caerulein (**1**, 480 μg obtained following the procedure outlined above)], **b** [frenatin 4 (**7**, 320 μg)], **c** [frenatin 1 (**4**, 20 μg)], **d** [frenatin 2 (**5**, 40 μg)] and **e** [frenatin 3 (**6**, 40 μg)]. The above HPLC data indicate that of the 25 mg of solid material produced by each milking, 22 mg is peptide material, comprising frenatins 1 (0.5 mg), 2 (1 mg), 3 (1 mg), 4 (8 mg) and caerulein (12 mg).

Mass Spectrometric Investigation

Fast atom bombardment (FAB) mass spectra were recorded with a Vacuum Generators ZAB 2HF reverse sector mass spectrometer equipped with an Ion Tech FAB gun operating with xenon at 8 kV with a current of 1 μA. Routine spectra for molecular weight data were obtained by scanning the magnet over a mass range from m/z 200 to 3500 using 1500 mass resolution. Collisional activation mass spectra (MS/MS) were obtained by setting the magnet to focus the particular ion under study, adding argon to the second collision cell to a pressure of 2×10^{-5} torr (to produce a reduction in the ion beam of some 50%), and scanning the electric sector.

Samples, normally 2–5 μl, were dissolved in water (25 μl) and dried on the FAB probe tip *in vacuo*. A eutectic mixture of 1,4-dithiothreitol/1,4-dithioerythritol (5:1) was added to the probe tip, and mixed thoroughly with the sample before insertion into the ion source.

Manual Edman Degradation (14)

To the peptide (typically 20 μmole) in aqueous pyridine (50%, 30 μl), was added a solution of phenylisothiocyanate in heptane (5%, 25 μl) in a 3 cm^3 screw cap glass vial. The solution was purged

with oxygen free nitrogen for 1 min, the vial capped and incubated at 50°C for 30 min, the mixture extracted with heptane/ethyl acetate (2:1, 2 × 30 μl), blown dry under nitrogen, trifluoroacetic acid (25 μl) was added, the solution purged with oxygen free nitrogen, the vial capped, incubated at 50°C for 30 min, and blown dry using nitrogen. The resultant solid was suspended in distilled water (50 μl) and extracted with *n*-butyl acetate (2 × 10 μl). A 2 μl aliquot of the aqueous layer was then removed for FAB-MS analysis to determine the molecular weight of the residual peptide, and the remaining solution was blown dry and subjected to a further cycle of the degradation process.

Enzymic Digestion (Lys-C or Chymotrypsin)

Methods are similar, for example, a solution of endopropease Lys-C (from *Lysobacter enzymogenes*, Sigma, St Louis, MI, USA) was prepared by dissolving three units in water (100 μg). The peptide (generally 10–25 μg) was dissolved in aqueous ammonium hydrogen carbonate (0.1 M, 25 ml), and protease Lys-C (1 μl) was added. The solution was heated at 40–45°C for 2 h, and the products analysed directly by FAB-MS.

Automated Peptide Sequencing

Peptide sequencing was performed by a standard procedure [13] on an applied Biosystem 470A sequencer equipped with a 900A data analysis module. The best results were obtained using a disc of immobilon film treated with bioprene in ethanol, onto which the peptide was absorbed from aqueous acetonitrile (90%). The disc was pierced several times with a razor blade in order to aid the flow of solvent.

Synthetic Peptides

The four frenatin peptides were synthesized commercially (by Chiron Mimotopes, Clayton, Victoria, Australia) using L-amino acids via the standard *N*-α-Fmoc method (full details including protecting groups and deprotection have been reported recently [20]. Each reference compound was routinely checked by HPLC and mass spectrometry by Chiron, the sequence was guaranteed, and the purity was >85% in each case. On receipt, we further purified the peptide by HPLC (see HPLC section above for full details including retention times) and confirmed the sequence by automated sequencing and mass spectrometry.

Methyl ester formation was performed as described previously [8].

Antimicrobial testing on synthetic peptides was carried out by Ciba-Geigy (Australia) as part of their standard screening program. The method used involved the measurement of inhibition zones produced by the applied peptide on a thin agarose plate containing the microorganisms under study. Avilomycin was used as standard for all measurements. The microorganisms used are listed in the text. Activities are recorded as MIC values; i.e. the minimum inhibitory concentration of peptide per ml required to totally inhibit the growth of the named microorganism.

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