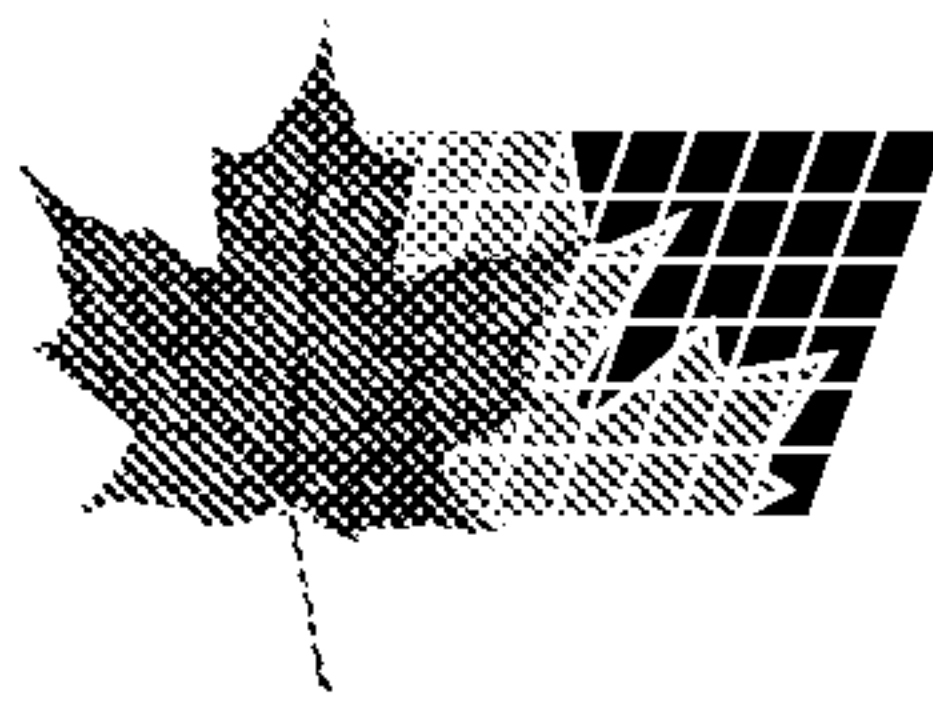




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(54) **PEPTIDES ANTIBACTERIENS ET ANTI-MALARIA**
(54) **ANTIBACTERIAL AND ANTIMALARIAL PEPTIDES**

(57) On prépare des peptides hybrides présentant une activité antibiotique et/ou antipaludéenne, contenant environ 20 à environ 40 résidus d'acides aminés, par redistribution de séquences sélectionnées de peptides connus.

(57) Antibiotically and/or antimalarially active hybrid peptides containing from about 20 to about 40 amino acid residues are prepared by rearranging selected sequences of known peptides.

PCT

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(54) Title: ANTIBACTERIAL AND ANTIMALARIAL PEPTIDES		
(57) Abstract Antibiotically and/or antimalarially active hybrid peptides containing from about 20 to about 40 amino acid residues are prepared by rearranging selected sequences of known peptides.		

ANTIBACTERIAL AND ANTIMALARIAL PEPTIDES

5

BACKGROUND OF INVENTION

10 Several naturally occurring, antibiotically
active peptides with useful therapeutic activity
against pathogenic bacteria and other classes of
microorganisms have recently been identified and
isolated from insects, frogs and other animals.
These include cecropins, attacins, magainins,
sarcotoxin, sapecin, battenecins, alamethicins,
15 defensins and PGLa.

Other naturally occurring peptides from
microorganisms, from insects and from higher
animals are generally known as toxins that lyse
red blood cells as well as other eukaryotic cells.
20 These toxins include different hemolysins such as
streptolysins, melittin, barbatolysin, paradaxins
and delta hemolysin. It is known but it is not
widely recognized that some toxins like melittin
will also kill bacteria. Therefore, for purposes
25 of this description they will be described as
antibiotically active peptides.

The invention described and claimed herein is
based on the unexpected discovery that novel
antibiotic molecules can be constructed by joining

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5 together at least two sequences from different antibiotics. One advantage of such hybrid molecules is that they may be constructed to be shorter and therefore easier to synthesize than the natural peptides from which they are derived.

10 The hybrid peptides, in addition to their antibiotic activity, appear to have other features in common. For example they all contain about 20 to 40 amino acids and often they are more effective if their C-terminals are amidated or blocked in other ways. They are therefore potential candidates for commercial preparation by solid phase synthesis. Additionally, they all appear to contain certain sequences of amino acids
15 which impart specific secondary characteristics to portions of the molecule. Often the N-terminus is hydrophilic and basic, and the C-terminus is hydrophobic. Some portions of the molecule have a tendency towards helicity, others do not. Some
20 molecules contain relatively long sequences which are flexible, thus forming hinge regions in the molecule. Often the helical portions are amphipathic, i.e., they are characterized by a hydrophilic and a hydrophobic surface.

25 The antibiotics appear to function by rupturing the cell membrane of the bacteria or other organism. Binding of the peptides to the membrane permits entry of ions into the cellular fluid thus increasing the osmotic pressure and causing more
30 fluid to enter the cell which increases the internal pressure and force the cell to burst.

The differing secondary characteristics of the different portions of the antibiotic peptides appear to be associated with their mode of action in piercing the cell membrane.

5 A very important current medical problem is to
find antibiotics with enhanced potency against
human pathogens especially those for which no
suitable antibiotic is now available or to which
10 resistant organisms have emerged by mutation of
the original pathogenic organisms. One response
to the emergence of organisms resistant to
antibiotics has been to prepare synthetic
derivatives of these compounds, but this approach
has been limited by the availability of functional
15 groups on the parent molecule that can be utilized
as foci for preparing derivatives.

 It would be useful if a pool of antibiotics of
comparatively simple structure were available
which could be synthesized with relative ease and
20 which at the same time would be susceptible to a
variety of readily accomplished structural
variations to produce synthetic antibiotically
active peptides useful against specific organisms
for which no non-toxic antibiotic is presently
25 available, or of improved activity against other
organisms for which the presently available
antibiotics are toxic to the host. Such compounds
should also have sufficient in vivo stability to
resist degradation by mammalian enzymes.

It has been discovered that naturally occurring peptides such as those mentioned above, and others like them constitute such a pool.

BRIEF DESCRIPTION OF DRAWING

5 Fig. 1 shows peptide concentration (μM) as a function of inhibition of reinvasion for assays tested for activity against Plasmodium falciparum.

THE INVENTION

10 Novel, synthetic antibiotically and/or antimalarially active, non-toxic peptides containing from about 20 to about 40 amino acid residues have now been discovered. These peptides are of improved pharmaceutical activity for any of
15 a number of reasons. Some have improved activity against known pathogens. Some, unlike their naturally occurring counterparts are non-toxic and do not cause lysis of red blood cells. Still others are active against pathogens for which no
20 completely satisfactory treatment is presently available.

 The therapeutically useful peptides of this invention are characterized as hybrids containing at least one amino acid region containing from
25 about 10 to about 15 amino acid residues in a sequence which is substantially similar to a

corresponding segment on a natural antibiotic peptide, although variations in the length and sequence of the amino acid residues are possible.

5 This invention will be better understood by consideration of the application of the invention to cecropins and melittin.

10 The cecropins are a family of basic antibacterial peptides produced by the humoral immune response of certain insects as described in U.S. Patent 4,355,104. Cecropins, together with attacins and lysozyme, are induced in the hemolymph of the pupae of the giant silk moth Hylophora cecropia following injection of live
15 bacteria. There are three major cecropins, A, B and D. There is a high degree of homology between them, and all are of about the same size (cecropin A: 37 residues, cecropin B: 35 residues, cecropin D: 36 residues). They each contain a hydrophilic amino terminal chain and a hydrophobic amidated
20 carboxyl terminus.

25 The amino acid sequences of cecropin A, B and D are shown in Table 1 which also includes the sequence of melittin. For convenience and ease of analysis the cecropin molecules have been divided into three sections: residues 1-11, 12-24 and 25 to the end. Those skilled in the art will recognize the high degree of homology and that cecropins A and B will be quite similar in their secondary structures. Both would be expected to
30 have a strong potential in a polar environment to

form an N-terminal amphipathic α -helix. The C-terminus will also have a tendency towards α -helix formation. In the central segment 12-24, there is some tendency for β -turns, for instance at residues 12-15, 15-18, 21-24. The N-terminus of cecropin D is less basic than either A or B. However, the central region of cecropin D has a higher potential for an α -helix than the A and B forms and also a strong preference for a helix in the C-terminal region.

In summary, the cecropins have a strongly hydrophilic amphipathic α -helix at the N-terminus, a more hydrophobic α -helix at the C-terminus, and a rather flexible, structurally less defined central region with some potential for β -turns.

The structure of melittin, an antibacterial peptide isolated from bee venom is shown in Table 1. It is a cationic amphipathic peptide in which residues 1-20 are predominantly hydrophobic and residues 21 to 26 are hydrophilic and basic. It will be noted that the major regions are arranged opposite from the cecropins. In melittin, they are hydrophobic/hydrophilic, and in the cecropins they are hydrophilic/hydrophobic. In the middle of the molecule there is a Gly-Leu-Pro region which may act as a hinge. Melittin has antibiotic activity, but is not useful for mammals because it is lytic for leukocytes, red blood cells and a wide variety of other cells.

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5 It has now been discovered that it is possible
to improve the pharmaceutical utility of naturally
occurring, low molecular weight, antibiotically
active peptides by rearranging selected regions or
sequences of the peptides or, in some instances,
10 adding a wholly new region to an intact region of
a naturally occurring peptide. Thus a peptide
formed by uniting the first 13 amino acid residues
of cecropin A as the amino terminus with the first
13 amino acid residues of melittin; CA(1-13) M(1-
13) has a lower lethal concentration and is more
active against Staphylococcus aureus or Bacillus
subtilis than cecropin A. Additionally, the novel
26 residue peptide is not lytic to sheep red blood
15 cells even at concentrations greater than 200 uM.
In contrast, melittin is lytic at 4-6 uM.

The term "improving the pharmaceutical
activity" as used herein means that the novel
peptide is less toxic to mammalian cells and/or
20 more active against a spectrum of pathogens or
against a specific pathogen than a naturally
occurring peptide from which it is derived. A
peptide of the invention is said to be "derived"
from a naturally occurring peptide if it contains
25 at least one region which is identical or
substantially homologous to a region on a
naturally occurring peptide. Thus CA(1-13) M(1-
13) can be considered as derived from both
cecropin A and melittin. Other peptides within
30 the scope of the invention may contain, for
example, sequences from a magainin and an attacin,
rearranged regions of one antibiotic, for example

melittin, or one region from a cecropin and another wholly unnatural region designed to be more or less hydrophobic, hydrophilic, or helical than the "naturally occurring" sequence.

5 It will be apparent to those skilled in the art that the selected region of the "naturally occurring" peptide in the final novel products of the invention does not need to be identical with the region in the natural peptide. One or more of
10 the amino acid residues of the natural peptide may be replaced with another selected amino acid to increase the basicity, to interrupt the helicity or for any other useful reason. The sequence of amino acid residues in the novel product will,
15 however, be substantially similar to natural sequence.

 The peptides of the invention will normally contain from about 20 to about 40 amino acid residues. One reason is that antibiotically
20 active low molecular weight peptides usually contain a minimum of about 20 amino acids. Another, is that peptides with more than about 40 amino acids are relatively difficult to synthesize in pure form by chemical synthesis, and may be
25 best prepared by fermentation or recombinant DNA procedures. A particular advantage of the useful peptides of this invention is that they are readily synthesized by solid phase methods and a variety of combinations are possible to achieve
30 specifically required results. An advantage of

the use of solid phase techniques is that the product can be directly synthesized with the C-terminus amidated or otherwise blocked.

5 The term "region" as used herein is similar to
"segment" or "fragment". It refers to amino acid
sequences containing at least 5 and normally from
about 5 to about 20 amino acids. A "region" is
usually selected or constructed to be flexible,
10 basic, hydrophobic, hydrophilic, amphipathic or
helical, and that will be a characteristic of the
region. A molecule may be constructed to have at
least two regions and may or may not contain a
hinge region. The region does not need to be
derived from a naturally occurring antibiotically
15 active peptide containing 20 to 40 amino acid
residues. It may be derived from a peptide
containing less than 20 or more than 40 such
residues.

20 The invention, then, comprises antibiotically
and/or antimalarially active peptides of improved
antibiotic and/or antimalarial activity containing
from about 20 to about 40 amino acid residues
including at least one region with from 5 to 20
amino acid residues which is substantially similar
25 to a corresponding sequence on a naturally
occurring antibiotically and/or antimalarially
peptide from which the novel peptide is derived,
and may be combined with one or more other regions
of the same or another natural antibiotic and/or
30 antimalarial peptide to form a hybrid molecule.

In another aspect, the invention is a method for improving the pharmaceutical activity of antibiotically and/or antimalarially active peptides which comprises synthesizing novel peptides containing from about 20 to about 40 amino acid residues containing at least one amino acid sequence or region containing from about 5 to 20 amino acid residues which is substantially identical to a corresponding sequence on a naturally occurring antibiotically and/or antimalarially active peptide.

Typical compounds within the scope of this invention may be represented by the following list wherein C represents cecropin, CA, CB and CD represent the A, B and D forms of cecropin, M represents melittin and Mag represents magainin. The numbers represent the sequence of amino acid residues in the corresponding region of the natural peptide. The notations define the characteristics of the region

	CA(1-13)Mag(13-23)	- hydrophilic/hydrophobic
	M(15-26)Mag(13-23)	- hydrophilic/hydrophobic
	Mag(13-23)CA(1-13)	- hydrophobic/hydrophilic
	Mag(13-23)M(15-26)	- hydrophobic/hydrophilic
25	M(1-13)CB(1-13)	- hydrophobic/hydrophilic
	M(1-12)ProCA(1-13)	- hydrophobic-Pro-
		hydrophilic
	M(1-15)C(1-11)	- hydrophobic/hydrophilic
	M(16-26)CA(14-37)	- hydrophilic/hydrophobic
30	CA(25-36)ProCA(1-13)	- hydrophobic-Pro-
		hydrophilic

	CA(25-37)CA(1-13)	- hydrophobic/hydrophilic
	CA(1-24)M(1-13)	- hydrophilic/hydrophobic
	CA(1-13)M(1-13)	- hydrophobic/hydrophilic
	M(16-26)M(1-13)	- hydrophilic/hydrophobic
5	M(16-26)CA(23-37)	- hydrophilic/hydrophobic
	CA(1-24)M(16-26)	- hydrophilic/hydrophilic
	CB(25-35)M(14-26)	- hydrophobic/hydrophilic
	CA(1-11)CD(12-37)	- hydrophilic/hydrophobic
	CA(1-8)M(1-18)	- hydrophilic/hydrophobic
10	CA(1-9)M(1-17)	- hydrophilic/hydrophobic
	CB(1-13)M(1-13)	- hydrophilic/hydrophobic
	CA(1-17)M(1-9)	- hydrophilic/hydrophobic
	CA(1-18)M(1-8)	- hydrophilic/hydrophobic
	M(1-13)CA(1-22)	- hydrophobic/hydrophilic
15	M(1-13)CA(1-13)	- hydrophobic/hydrophilic

Most of the above products, in addition to being hydrophobic/hydrophilic or vice-versa will also have regions of helicity or amphipathicity. Proline (Pro) is often employed to interrupt a helix although other amino acids may be similarly employed. The above peptides can also be constructed to include a flexible or hinge region.

The compounds of this invention are synthesized by standard solid phase procedures using the protection, deprotection and cleavage techniques and reagents appropriate to each specific amino acid or peptide. A combination of manual and automated (e.g., Applied Biosystem 430A) solid phase techniques can be used to synthesize the novel peptides of this invention. For background

on solid phase techniques, reference is made to Andreu, D., Merrifield, R.B., Steiner, H. and Boman, H.G., (1983) Proc. Natl. Acad. Sci USA 80, 6475-6479; Andreu, D., Merrifield, R.B., Steiner, H. and Boman, H.G., (1985) Biochemistry 24, 1683-1688; Fink, J., Boman, A., Boman, H.G., and Merrifield, R.B., (June 1989) Int. J. Peptide Protein Res. 33, 412-421; Fink, J., Merrifield, R.B., Boman, A. and Boman, H.G., (1989) J. Biol. Chem. 264, 6260-6267;

The in vivo stability of the compounds of the invention can be improved by adding a D-amino acid to the N- and C-termini.

Since the products of the invention are amphoteric they may be utilized as free bases, as acid addition salts or as metal salts. The salts must, of course, be pharmaceutically acceptable, and these will include metal salts particularly alkali and alkaline earth metal salts, suitably potassium or sodium salts. A wide variety of pharmaceutically acceptable acid addition salts are available. These include those prepared from both organic and inorganic acids, preferably mineral acids. Typical acids which may be mentioned by way of example include citric, succinic, lactic, hydrochloric and hydrobromic acids. Such products are readily prepared by procedures well known to those skilled in the art.

5 A further aspect of the present invention provides pharmaceutical compositions which comprises one or more compounds of the invention and a pharmaceutically acceptable carrier. The compositions may be made up of any pharmaceutical form appropriate for the desired route of administration. Examples of such compositions include solid compositions for oral administration such as tablets, capsules, pills, powders and granules. Liquid compositions for oral administration such as solutions, suspensions, syrups or elixirs and preparations for parenteral administration such as sterile solutions, suspensions or emulsions. The compositions may also be manufactured in the form of sterile solid compositions which can be dissolved in sterile water, physiological saline or some other sterile injectable medium immediately before use. Topical compositions, typically in the form of emulsions, suspensions, creams, lotions or foams which may contain emollients, suspending agents, chelating agents, stiffening agents, buffering agents, and other components conventionally used with topical compositions containing antibiotics may also be provided.

In all such compositions the antibiotic and/or antimalarial will normally be the principal physiologically active ingredient.

30 Optimal dosages and regimens for a given mammalian host can be readily ascertained by those skilled in the art. It will, of course, be

5 appreciated that the actual dose used will vary
according to the particular composition
formulated, the particular compound used, the mode
of application and the particular site, host and
disease being treated. Many factors that modify
the action of the drug will be taken into account
including age, weight, sex, diet, time of
administration, route of administration, rate of
excretion, condition of the patient, drug
10 combinations, reaction sensitivities and severity
of the disease.

The following non-limiting examples are given by
way of illustration only and are not to be
considered limitations of this invention, many
15 apparent variations of which are possible without
departing from the spirit or scope thereof.

EXAMPLES

EXAMPLES 1-6 AND COMPARISON WITH CA(1-37), CD(2-37), AND M(1-26)

20 Novel peptides of this invention and melittin
were synthesized by a combination of automated
(Applied Biosystem 430A) solid phase techniques
(see articles regarding synthesis, cited supra).
In particular, melittin and the novel peptides
25 CA(25-37)CA(1-13), M(1-13)CA(1-13), CA(1-
11)CD(12-37), CA(1-24)M(1-13), CA(1-13)M(1-13),
and M(16-26)M(1-13) were prepared by the following
standard double coupling protocol, based on 2.5 g
of starting resin (0.21 mmol/g): (1) CH₂Cl₂, 50

mL, 4 x 1 min; (2) 50% TFA/CH₂Cl₂, 50 mL, 2 x 1 min; (3) 50% TFA/CH₂Cl₂, 50 mL, 1 x 20 min; (4) CH₂Cl₂, 50 mL, 6 x 1 min; (5) 5% DIEA/CH₂Cl₂, 50 mL, 2 x 2 min; (6) CH₂Cl₂, 50 mL, 6 x 1 min; (7) protected amino acid, 4 eq in 20 mL of CH₂Cl₂, add to reaction vessel, rinse with 4 mL of CH₂Cl₂, and shake at room temperature for 5 min; 4 eq of DCC in 3 mL of CH₂Cl₂, add to reaction vessel, rinse with 2 mL of CH₂Cl₂ and shake for 100 min at room temperature; (8) CH₂Cl₂, 50 mL, 4 x 1 min; (9) 5% DIEA/CH₂Cl₂, 50 mL, 1 x 2 min; (10) CH₂Cl₂, 50 mL, 4 x 1 min; (11) DMF, 50 mL, 2 x 2 min; (12) protected amino acid, 8 eq in 3 mL of CH₂Cl₂, 0°C, add DCC, 4 eq in 1 mL of CH₂Cl₂, 0°C, rinse with 1 mL of CH₂Cl₂, 0°C, after 10 min. at 0°C, filter, add 25 mL of DMF, 0°C, add to reaction vessel, rinse with 5 mL of DMF, 0°C, shake for 1 h at room temperature; (13) DMF, 50 mL, 2 x 2 min; (14) CH₂Cl₂, 50 mL, 4 x 1 min; (15) 5% DIEA/CH₂Cl₂, 50 mL, 1 x 2 min; (16) CH₂Cl₂, 50 mL, 4 x 1 min; (17) 3- to 5-mg sample for ninhydrin analysis. This protocol is repeated for subsequent amino acids to complete the assembly of the desired peptide.

The fully protected peptide on the resin is then treated with TFA to remove the N Boc group and dried. Cleavage of the peptide from the resin supports is accomplished by the low/high HF method, Tam et al (1983) J. Am. Chem. Soc. 105 6442-6455. Low HF is accomplished with 5 mL of HF/dimethyl sulfide/p-cresol/p-thiocresol (25:65:7.5:2.5), at 0°C for 2 hr. High HF is accomplished with 10 mL of HF/p-cresol/p-

thiocresol (95:3.75:1.25), at 0°C for 1 hr. After
evaporation of HF, the product is first washed with
anhydrous ether to remove the scavengers, and then
dissolved in 10% HOAc in water. The crude material
5 is obtained by lyophilization.

The peptides of the invention are then
partially purified by gel filtration on a Sephadex
G-25 column in 1 M HOAc to remove low molecular
weight impurities. After lyophilization the
10 products are normally obtained in a yield of about
80%, based on the first amino acid. Reverse phase,
low pressure, preparative liquid chromatography on a
Vydac* C18 column (218TPB10) and elution with linear
gradient of 25-65% acetonitrile, in certain
15 instances, followed the Sephadex* gel filtration.

The products of the invention were tested for
activity against a variety of test organisms
selected to represent a number of different types of
pathogens some of them known to be particularly
20 virulent by the inhibition zone assay of Hoffmann et
al. (1981) Insect Biochem. 11 537-548. Thin agar or
agarose plates (8.5 cm diameter) were prepared with
6 ml of rich medium containing 100 ug/ml of
streptomycin and 2×10^5 viable cells of a test
25 organism resistant to streptomycin. Wells of 35 mm
diameter were punched in the plates and 3 ul of
serially diluted samples were placed in the wells.
The diameters of the zones of inhibition around the
wells were measured after overnight incubation

30 * Trade-mark

at 30°C or 37°C. For each peptide the squares of the zone diameters were plotted against the logarithm of the concentration, and from the slopes and intercepts the lethal concentrations were
5 calculated as described by Hultmark (1983) *EMBO J.* 2, 571-76 or Hultmark et al. (1982) *Eur. J. Biochem.* 127 207-217. The results of the assays with some of the products of the invention are shown in Table 2.

The Table 2 also shows the concentrations at
10 which the compounds will lyse sheep red blood cells. These values were obtained by either the lysis assay normally used for melittin or by an adaptation of the antibacterial inhibition zone assay of the
15 above-cited Hultmark et al. articles to plates with sheep red cells (SRC). With respect to the latter, sterile agarose plates contained 6 ml of medium with 1% agarose, 0.9% NaCl and 10% SRC suspended in
Alsevers solution. A dilution series of peptide was applied in 3 mm wells each loaded with 3 ul of the
20 respective samples. The plates were incubated at 30°C for 24 hr, the zones were recorded and LC values calculated as described in the Hultmark et al. articles. Clear zones were recorded after a few
hours of incubation and the use of such data gave an
25 LC value of the same order as readings after 24 hr. This plate assay is faster and more convenient than the lysis assay normally used for melittin.

5 It was observed during testing that basic peptides often bind to agar plates, but not so much to agarose. Therefore those lethal concentrations determined on agar may, in fact, be substantially higher than the actual lethal concentrations.

10 It will be seen that all of the hybrid peptides are less toxic than melittin as measured by ability to lyse sheep red blood cells. It will be seen also that against specific organisms most of them are more active than at least one of the naturally occurring peptides from which it is derived. CA(1-11)CD(12-37) is more active than either cecropin A or cecropin D against the
15 organisms tested. The most profound increases in antibiotic activity were with CA(1-13)M(1-13) and CA(1-24)M(1-13) against Staphylococcus aureus and Bacillus subtilis where increases of 40 to 200 fold over cecropin A were observed. The potency
20 of these two hybrid peptides against a yeast Sacchromyces cerevisiae was also greater than cecropin A. Furthermore, these two compounds were not toxic to sheep red blood cells even at 100 uM and higher.

25 One interesting embodiment of this invention is the hybrid M(16-26)M(1-13) in which the two principal regions of melittin are reversed. This product did not lyse red cells even at 240 uM, whereas the natural melittin molecule caused lysis
30 at 4-6 uM, a greater than 40-60 fold improvement.

EXAMPLES 7-8 and COMPARISON WITH
Mag, CB, CA and PGLa

Hybrid peptides CA(1-13)M(1-13) and CA(1-8)M(1-18)
were prepared as described above (see Examples 1-6) and
5 were assayed for activity against the blood stream form
of Plasmodium falciparum. For comparison the test
included cecropins A and B and the frog skin peptides
magainin 2 and PGLa.

The blood stream forms of the malaria parasite
10 Plasmodium falciparum, primarily late trophozoites and
early schizonts, were assayed by recording the inhibition
of the reinvasion of human red cells by the method of
Wahlin, B., et al, (1984) Proc. Nat'l Acad. Sci. USA 81,
7912-16. Quadruplicate microcultures of strain F32
15 (Tanzania) were incubated for 20 hrs at 37°C in a
complete tissue culture medium, with or without different
concentrations of the peptides to be assayed. After
acridine orange staining, the percentage of newly
infected red cells was scored in a fluorescence
20 microscope. For each culture 40,000 red cells were
analyzed for parasitic infection. Duplicate assays were
run for CA(1-13)M(1-13).

Figure 1 shows the results for CA(1-13)M(1-13), CA,
CB, Mag and PGLa. From Figure 1, CA has only negligible
25 activity; CB is about as potent as Mag; hybrid CA(1-13)
is of an order more potent than CB; and, PGLa has
intermediate activity.

CA(1-18)M(1-18) is even more potent than CA(1-13)M(1-13). CA(1-8)M(1-18) is in fact about four times more potent than CA(1-13)M(1-13): CA(1-8)M(1-18), at a concentration of approximately 2.2 μ M yields a 50% inhibition of reinvasion. Only a trace of hemolytic activity was observed with the hybrids of the present invention.

EXAMPLES 9-15 AND COMPARISON
WITH CA, CB AND M

Novel peptides CA(1-8)M(1-18), CA(1-9)M(1-17), CB(1-13)M(1-13), CA(1-17)M(1-9), CA(1-18)M(1-8), M(1-13)CA(1-22) and M(1-13)CA(1-13) were prepared as described above (see Examples 1-6). These novel peptides and CA, CB, and M were tested for activity against a variety of organisms selected to represent a number of different types of pathogens, some of them known to be particularly virulent by the inhibition zone assay of Hoffmann et al (1981), Insect Biochem. 11 537-48. As in Examples 1-6, thin agarose plates were prepared with the respective organisms. The diameters of the zones of inhibition were measured after overnight incubation at 30°C. The lethal concentrations were determined as described above (see Examples 1-6; Hultmark et al articles cited thereat). The results of the assays of these novel peptides are shown in Table 3.

As seen from Table 3, the hybrid peptides are less toxic than melittin; and, are more active than at least one of the naturally occurring peptides from which it is derived.

TABLE 1

Cecropin A:

5 H-Lys-Trp-Lys-Leu-Phe-Lys-Lys-Ile-Glu-Lys-Val-Gly-Gln-Asn-
Ile-Arg-Asp-Gly-Ile-Ile-Lys-Ala-Gly-Pro-Ala-Val-Ala-Val-Val-Gly
Gln-Ala-Thr-Gln-Ile-Ala-Lys-NH₂

Cecropin B:

10 H-Lys-Trp-Lys-Val-Phe-Lys-Lys-Ile-Glu-Lys-Met-Gly-Arg-Asn-
Ile-Arg-Asn-Gly-Ile-Val-Lys-Ala-Gly-Pro-Ala-Ile-Ala-Val-Leu-Gly-
Glu-Ala-Lys-Ala-Leu-NH₂

15 Cecropin D:

H-Trp-Asn-Pro-Phe-Lys-Glu-Leu-Glu-Lys-Val-Gly-Gln-Arg-Val-
Arg-Asp-Ala-Val-Ile-Ser-Ala-Gly-Pro-Ala-Val-Ala-Thr-Val-Ala-Gln-
Ala-Thr-Ala-Leu-Ala-Lys-NH₂

20 Melittin:

H-Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-
Ala-Leu-Ile-Ser-Trp-Ile-Lys-Arg-Lys-Arg-Gln-Gln(NH₂)

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TABLE 2
LETHAL CONCENTRATION IN uM

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
PEPTIDE														
CA(1-37)	0.2 ^a	0.5 ^c	1.6 ^c /2 ^b	1 ^c	1 ^c	4 ^a	200 ^c	2 ^c	>200 ^a	3 ^c	6 ^c /4 ^b	>200 ^c	60 ^c	>200 ^a
CD(2-37)*	3.6 ^c	0.6 ^c	90 ^c	30 ^c	12 ^c	100 ^c								
M(1-26)	0.8 ^a		3 ^a			0.2 ^a	0.3	9-50 ^c	0.2 ^a	0.3 ^c	0.5 ^a	150 ^c	10 ^c	4-6 ^c / 4-8 ^b
CA(1-13)M(1-13)	0.5 ^a		1 ^a			0.7 ^a	0.9	4 ^c	2 ^a	0.6 ^c	1 ^a	160 ^c	20 ^c	>200 ^a
CA(1-24)M(1-13)	0.3 ^a		1 ^a			0.5 ^a	1 ^c	2 ^c	6 ^a	0.9 ^c	2 ^a	>200 ^c	50 ^c	>200 ^a
M(16-26)M(1-13)	0.7 ^a		8 ^a			0.7 ^a	1 ^c	30 ^c	10 ^a	0.8 ^c	1 ^a	180 ^c	25 ^c	>200 ^a
CA(1-11)CD(12-37)*	0.5 ^c	0.3 ^c	1 ^c	0.8 ^c	0.7 ^c	6 ^c								-22 ⁻
CA(25-37)CA(1-13)	200 ^b		300 ^b			20 ^b			>300 ^b		20 ^b			>300 ^b
M(1-13)CA(1-13)	1 ^b		5 ^b			0.3 ^b			5 ^b ***		1 ^b			80?

15	1. <u>Escherichia coli</u> D21	8. <u>Xenorhabdus nematophilis</u> Xn21
	2. <u>Escherichia coli</u> D22	9. <u>Staphylococcus aureus</u> Cowan I
	3. <u>Pseudomonas aeruginosa</u> OT97	10. <u>Staphylococcus haemolyticus</u>
	4. <u>Bacillus megaterium</u> BM11	11. <u>Streptococcus pyogenes</u>
	5. <u>Micrococcus luteus</u> M111	12. <u>Candida albicans</u>
	6. <u>Bacillus subtilis</u> Bs11	13. <u>Saccharomyces cerevisiae</u>
	7. <u>Bacillus subtilis</u> Bs11 (Medium E)**	14. Sheep Red Cells

20 *Using cecropin A numbering.

-23.-

Values from plates with agar are underlined. In all other cases, the values were determined on agarose plates.

5 **Vogel and Bonner, J. Biol. Chem. 218:97-106, 1956

*** Indicates flat concentration dependence

^a Values obtained after incubation overnight at 30°C as well as

after incubation overnight at 37°C

10 ^b Values obtained after incubation overnight at 30°C

^c Values obtained after incubation overnight at 37°C

? Indicates turbid zones

TABLE 3

LETHAL CONCENTRATION IN μ M

	Compound	Size (aa)	D21	OT97	Bs11	Bs11 + ME	Staph	Strep	SRC
5	CA(1-37)	37	0.2	2	4	200	>200	4	>200
	CB(1-35)	35	0.3	1	nd	nd	>200	12	nd
	M(1-26)	26	0.8	3	0.2	0.3	0.2	0.5	4-8
	CA(1-8)M(1-18)	26	0.3	0.7	0.4	0.5	1	2	>600
	CA(1-9)M(1-17)	26	0.3	1	0.7	0.9	6	0.3	>600
10	CB(1-13)M(1-13)	26	0.3	1	0.4	0.4	4	1	>400
	CA(1-17)M(1-9)	26	0.5	5	0.5	1	4	0.2	>600
	CA(1-18)M(1-8)	26	1	15	4	20	>600	3	>600
	M(1-13)CA(1-22)	35	0.7	nd	nd	0.2	5*	1	40?
	M(1-13)CA(1-13)	26	1	5	0.3	0.1	5*	1	80?

- 15 D21 = E. coli
OT97 = P. aeruginosa
Bs11 = B. subtilis
Bs11 + ME = B. subtilis, plates prepared with medium E
Staph = S. aureus Cowan I
- 20 Strep = S. pyogenes
SRC = Sheep Red Cells
? indicates turbid zone (melittin gives clear zone)
* indicates flat concentration dependence
nd = not determined

CLAIMS:

1. Antibiotically active, synthetic hybrid peptides containing from 20 to 40 amino acid residues and comprising at least one region containing from 5 to 20 amino acid residues in a sequence corresponding to a sequence on a naturally occurring antibiotically active peptide selected from cecropin (C), cecropin A (CA), cecropin B (CB), cecropin D (CD), melittin (M), magainin (Mag), or attacin, said region being combined with one or more other regions of the same or another naturally occurring antibiotically active peptide.
2. Synthetic hybrid peptides according to claim 1 which are antimalarially active, wherein said selected naturally occurring antibiotically active peptides are antimalarially active.
3. A peptide of claim 1 or claim 2 containing two regions each containing from 10 to 15 amino acid residues in a sequence corresponding to a sequence on said naturally occurring antibiotically active peptide selected from cecropin (C), cecropin A (CA), cecropin B (CB), cecropin D (CD), melittin (M), magainin (Mag), or attacin.
4. A peptide of claim 3, wherein both of the regions are from the same naturally occurring antibiotically active peptide.
5. A peptide of claim 1 or claim 2, comprising CA(1-13)M(1-13), CA(1-24)M(1-13), CA (1-11)CD(12-37) or M(16-26)M(1-13).
6. A peptide of claim 1 or claim 2 selected from the group consisting of CA(1-13)Mag(13-23), M(15-26)Mag(13-23), Mag(13-23)CA(1-13), Mag(13-23)M(15-26), M(1-13)CB(1-13), M(1-12)ProCA(1-13), M(1-15)C(1-11), CA(25-36)ProCA(1-13), CA (25-37)CA(1-13), CA(1-13)M(1-13), M(16-26)M(1-13), M(16-26)CA(23-37), CB(25-35)M(14-26), CB(1-13)M(1-13),

M(1-13)CA(1-13), M(16-26)CA(14-37), CA(1-24)M(1-13), CA(1-24)M(16-26), CA (1-11)CD(12-37), CA(1-8)M(1-18), CA(1-9)M(1-17), CA(1-17)M(1-9), CA(1-18)M(1-8), and M(1-13)CA(1-22).

7. A pharmaceutical composition containing a pharmaceutically acceptable carrier together with one or more antibiotically active, synthetic hybrid peptides containing from 20 to 40 amino acid residues and comprising at least one region containing from 5 to 20 amino acid residues in a sequence corresponding to a sequence on a naturally occurring antibiotically active peptide selected from cecropin (C), cecropin A (CA), cecropin B (CB), cecropin D (CD), melittin (M), magainin (Mag), or attacin, said region being combined with one or more other regions of the same or another naturally occurring antibiotically active peptide.

8. A pharmaceutical composition according to claim 7, wherein said antibiotically active, synthetic hybrid peptides and said naturally occurring antibiotically active peptides are antimalarially active.

9. A composition of claim 7 or claim 8, wherein the peptide is one which contains two regions each containing from 10 to 15 amino acid residues in a sequence corresponding to a sequence on said naturally occurring antibiotically active peptide selected from cecropin (C), cecropin A (CA), cecropin B (CB), cecropin D (CD), melittin (M), magainin (Mag), or attacin.

10. A composition of claim 9, wherein the peptide is one in which both of the regions are from the same naturally occurring antibiotically active peptide.

11. A composition of claim 6, wherein the peptide is selected from one or more of CA(1-24)M(1-13), CA(1-11)CD(12-37), M(16-26)M(1-13), M(16-26)CA(14-37), CA(1-24)M(1-13), CA(1-24)M(16-26), CA (1-11)CD(12-37), CA(1-8)M(1-18), CA(1-9)M(1-17), CA(1-17)M(1-9),

CA(1-18)M(1-8), M(1-13)CA(1-22), CA(1-13)Mag(13-23), M(15-26)Mag(13-23),
Mag(13-23)CA(1-13), Mag(13-23)M(15-26), M(1-13)CB(1-13),
M(1-12)ProCA(1-13), M(1-15)C(1-11), CA(25-36)ProCA(1-13),
5 CA(25-37)CA(1-13), CA(1-13)M(1-13), M(16-26)CA(23-37),
CB(25-35)M(14-26), CB(1-13)M(1-13), and M(1-13)CA(1-13).

12. A process for preparing the peptides according to any one of claims 1 to
6, wherein the peptides are synthesized by solid phase technique.

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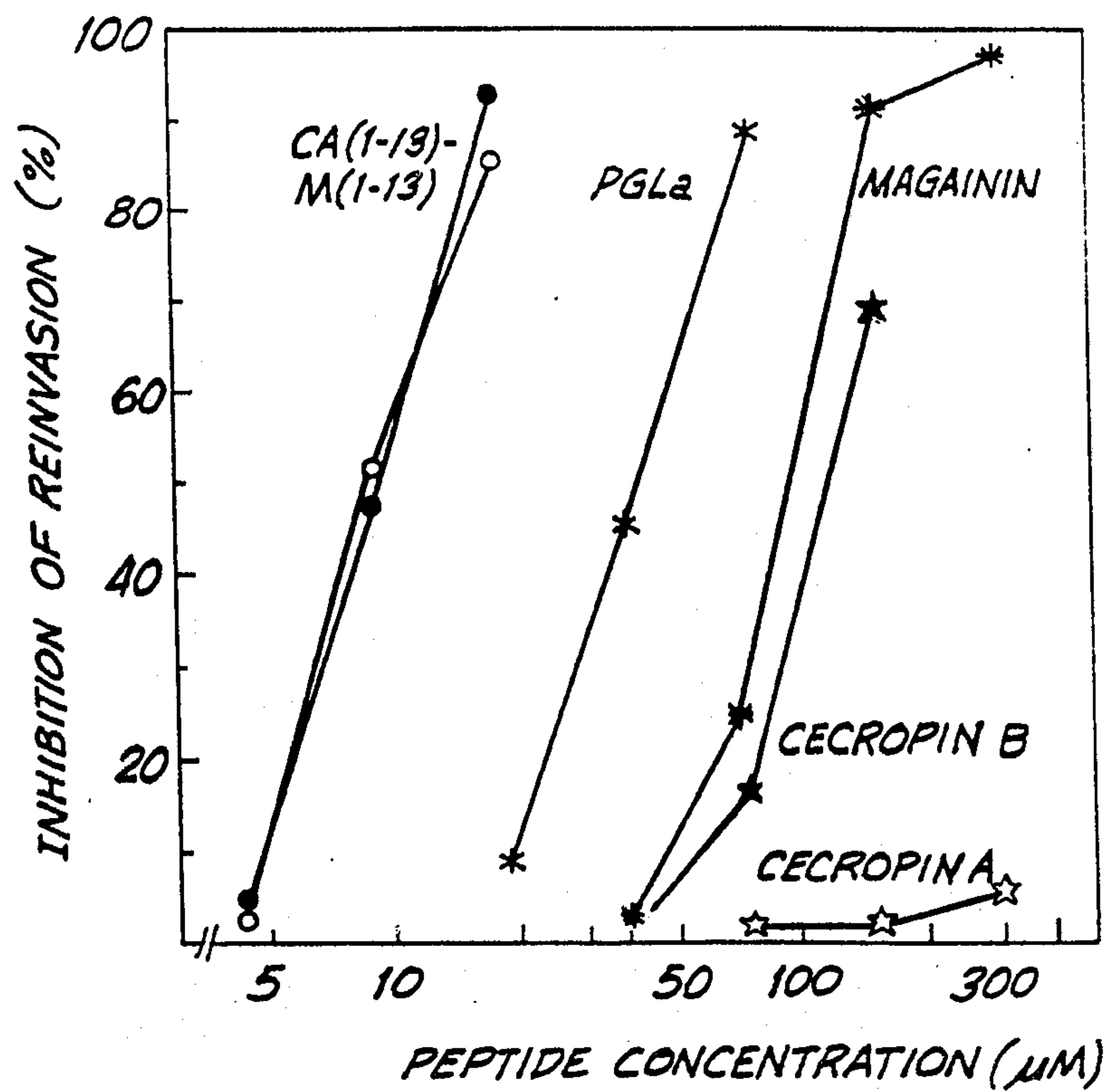


FIG.1

SUBSTITUTE SHEET