Summary of the Disclosure ~~of the Invention~~

The present ~~invention~~disclosure relates to the cloned, sequenced and elucidated structural and regulatory information relevant to the biosynthetic gene cluster for the type-B lantibiotic, actagardine, from *Actinoplanes garbadinensis* and *A. liguriae*.

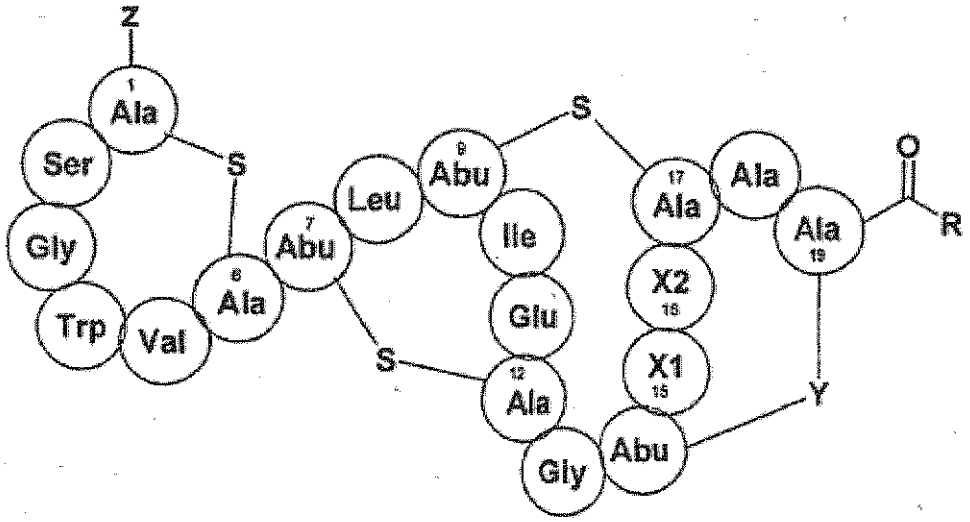
We have also surprisingly found that in an isolate of *A. liguriae*, designated herein as *A. liguriae* NCIMB 41362, a novel form of actagardine is produced which we have termed actagardine B or, in the non-oxidised form, deoxy-actagardine B. These forms have similar anti-microbial activity to actagardine and are generated from the primary polypeptide sequence of SEQ ID NO:1, which undergoes similar cross-linking to actagardine. The variants provide new and useful alternatives to actagardine. In addition, the identification of residues in actagardine B which are different from actagardine leads to the provision of further lantibiotics based on these differences.

We have also isolated gene clusters from both actagardine-producing *A. garbadinensis* and *A. liguriae* NCIMB 41362 which comprise the genes for the production of actagardine and actagardine B.

In one aspect, the present ~~invention~~disclosure provides the novel actagardine B and variants thereof, including variants based on the primary polypeptide sequences of SEQ ID NO:2 and SEQ ID NO:3, as well as variants thereof.

In a further aspect, the ~~invention~~disclosure provides nucleic acids encoding actagardine B and its variants, sets of nucleic acids and variants thereof derived from the above-mentioned gene clusters, methods of making actagardine B and its variants, and methods of generating novel variants of actagardine B.

In a first aspect, there is provided a compound of the formula:



wherein:

-X1-X2- represents -Leu-Val- or -Leu-Ile-;

-Y- is -S- or -S(O)-;

Z is either an amino or $-NH_2$ wherein the latter represents the N-terminus of the Ala at position 1;

R represents the group —OH or —$NR^1 R^2$, wherein R^1 and R^2 independently represent:

(i) hydrogen;

(ii) a group of formula $-(CH_2)_n$,$-NR^3R^4$, in which n represents an integer from 2 to 8 and R^3 and R^4 independently represent hydrogen or C_{1-4}alkyl, or R^3 and R^4 taken together represents a group $-(CH_2)_3-$, $-(CH_2)_4-$, $(CH_2)_2-O-(CH_2)_2$, $-(CH_2)_2-S-(CH_2)_2$ or $-(CH_2)_5-$; or R^1 and R^2 taken together with the adjacent nitrogen atom represent a piperazine moiety which may be substituted at position 4 with a substituent selected from:

(a) C_{1-4}alkyl;

(b) C_{5-7}cycloalkyl;

(c) pyridyl,

(d) $-(CH_2)_p-NR^5R^6$ in which p represents an integer from 1 to 8 and R_5 and R^6 independently represent hydrogen or C_{1-4}alkyl;

(e) piperidinyl;

(f) substitute piperidinyl, wherein the substituted piperindinyl bears a N-substituent which is C_{1-4}alkyl;

(g) benzyl; and

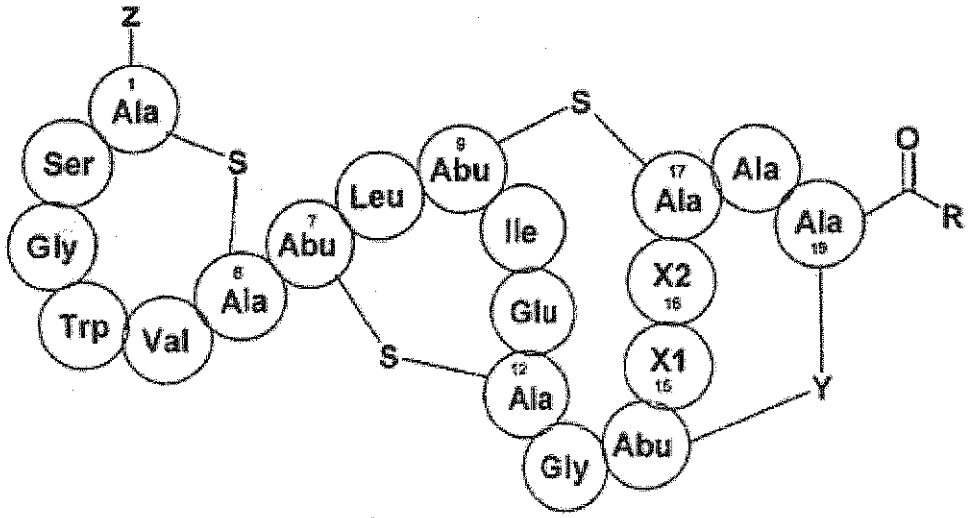
(h) substituted benzyl, wherein the phenyl moiety bears 1 or 2 substituents

selected from chloro, bromo, nitro, C_{1-4}alkyl and C_{1-4}alkoxy,

or a biologically active variant thereof, or a pharmaceutically acceptable salt of said compound or variant,

wherein the biologically active variant comprises 1, 2, 3 or 4 amino acids substituted at positions selected from positions 2, 3, 4, 5, 8, 10, 11, 13, 15, 16 and 18 of the compound, with the proviso that -X1-X2- is -Leu-Val- or -Leu-Ile-.

In another aspect, there is provided a compound of the formula



wherein:

-X1-X2- represents -Leu-Val- or -Leu-Ile-;

-Y- is -S- or -S(O)-;

Z is either an amino or $-NH_2$ wherein the latter represents the N-terminus of the Ala at position 1;

R represents the group —OH or $—NR^1R^2$, wherein R^1 and R^2 independently represent:

(i) hydrogen;

(ii) a group of formula $-(CH_2)_n-NR^3R^4$, in which n represents an integer from 2 to 8 and R^3 and R^4 independently represent hydrogen or C_{1-4}alkyl, or R^3 and R^4 taken together represents a group $-(CH_2)_3-$, $-(CH_2)_4-$, $(CH_2)_2-O-(CH_2)_2$, $-(CH_2)_2-S-(CH_2)_2$ or $-(CH_2)_5-$;

or R^1 and R^2 taken together with the adjacent nitrogen atom represent a piperazine moiety which may be substituted at position 4 with a substituent selected from:

(i) C_{1-4}alkyl;

(j) C_{5-7}cycloalkyl;

(k) pyridyl,

(l) $-(CH_2)_p-NR^5R^6$ in which p represents an integer from 1 to 8 and R^5 and R^6 independently represent hydrogen or C_{1-4}alkyl;

(m) piperidinyl;

(n) substitute piperidinyl, wherein the substituted piperindinyl bears a N-substituent which is C_{1-4}alkyl;

(o) benzyl; and

(p) substituted benzyl, wherein the phenyl moiety bears 1 or 2 substituents selected from chloro, bromo, nitro, C_{1-4}alkyl and C_{1-4}alkoxy,

or a pharmaceutically acceptable salt of said compound, with the proviso that -X1-X2- is -Leu-Val- or -Leu-Ile-.

In a second aspect, there is provided a pharmaceutical composition comprising a compound as defined above together with a pharmaceutically acceptable carrier or diluent.

In a third aspect, there is provided the use of a compound as defined above, or a composition as defined above in the manufacture of a medicament for the treatment of a bacterial infection.

In a fourth aspect, there is provided a method for producing a host cell suitable for expressing a type B lantibiotic peptide deoxyactagardine, actagardine or a variant thereof, the method comprising the step of transforming a cell by introducing:

a *lanA* gene, said gene encoding a LanA polypeptide comprising a precursor of actagardine, actagardine B, ala(0)-actagardine, ala(0)-actagardine B or an amino acid variant of any one of the same wherein:

1 to 5 amino acids in the sequence is/are substituted by another amino acid, or

the amino acid sequence is at least 90% identical thereto,

optionally together with one or more associated cluster genes suitable for conversion of a precursor peptide into said peptide,

thereby generating a host cell comprising a polynucleotide sequence encoding said peptide and suitable for expressing same, wherein the type B lantibiotic peptide has antimicrobial activity.

In a fifth aspect, there is provided a cell obtainable from the method as defined above, wherein said cell is selected from a lantibiotic producing host cell with a mutated endogenous *lanA* gene
and a non-producer capable of expressing a *lanA* gene, wherein the cell comprises a *lanA* gene encoding a LanA polypeptide comprising a precursor of actagardine, actagardine B, ala(0)-actagardine, ala(0)-actagardine B or an amino acid variant of any one of the same, wherein 1 to 5 amino acids in the LanA polypeptide sequence is/are substituted by another amino acid, or the amino acid sequence is at least 90% identical thereto.

In a sixth aspect, there is provided a vector suitable for transforming a host cell in the method defined above, said vector comprising an expression construct comprising a gene encoding a LanA peptide selected from actagardine, actagardine B, ala(0)-actagardine, ala(0)-actagardine B or a variant of any one of same wherein:

1 to 5 amino acids in the peptide are substituted by another amino acid, or
the amino acid sequence is at least 90% identical thereto,
optionally together with one or more associated cluster genes required for conversion of a precursor peptide into a product peptide, and wherein the LanA peptide has antimicrobial activity.

In a seventh aspect, there is provided an lantibiotic producing host cell suitable for expressing a type B lantibiotic peptide,
the host cell comprising a mutation in its endogenous *lanA* gene, said gene encoding a LanA polypeptide comprising a precursor of actagardine, actagardine B, ala(0)-actagardine, ala(0)-actagardine B or an amino acid variant of any one of the same, wherein 1 to 5 amino acids in the LanA polypeptide sequence is/are substituted by another amino acid, or the amino acid sequence is at least 90% identical thereto, such that the gene is not expressed or the gene product is inactive.

In an eighth aspect, there is provided the use of a host cell for the expression of actagardine, a variant thereof,
deoxyactagardine or a variant thereof, wherein the cell is a lantibiotic producing cell with a gene encoding a LanO polypeptide and said polypeptide is derived from the lantibiotic gene cluster and is a factor involved in the oxidation of deoxy-forms to -S(O)- analogue thereof and:

said gene is inactivated, or
the expression of said gene is increased.

In a ninth aspect, there is provided the use of a host cell comprising a gene encoding a LanA peptide, a precursor of a lantibiotic peptide for the production of actagardine, a variant thereof, deoxyactagardine or a variant thereof, with the proviso that wherein the host is a lantibiotic producing cell comprising an endogenous gene encoding a LanA peptide, the latter is mutated such that the endogenous gene is not expressed or the endogenous gene product is inactive or the endogenous gene is deleted.

Description Of The Drawings

Figure 1 provides a map of the actagardine encoding and regulatory gene cluster isolated from *A. garbadinensis.*

Figure 2 provides a map of the encoding and regulatory gene cluster isolated from *A. liguriae* which encodes a novel variant of actagardine, herein referred to as actagardine B.

instead a group -NHR^6 wherein R^6 represents C_{1-4}aalkyl.

13. A pharmaceutical composition comprising a compound according to any one of claims 1 to 12 together with a pharmaceutically acceptable carrier or diluent.

14. The use of a compound according to any one of claims 1 to 12, or a composition according to claim 13 in the manufacture of a medicament for the treatment of a bacterial infection.

15. The use according to claim 14, wherein said infection is a gut super-infection caused by *Clostridium difficile*.

16. A method for producing a host cell suitable for expressing a type B lantibiotic peptide deoxyactagardine, actagardine or a variant thereof, the method comprising the step of transforming a cell by introducing:

a *lanA* gene, said gene encoding a LanA polypeptide comprising a precursor of actagardine, actagardine B, ala(0)-actagardine, ala(0)-actagardine B or an amino acid variant of any one of the same wherein:

1 to 5 amino acids in the sequence is/are substituted by another amino acid, or

the amino acid sequence is at least 90% identical thereto,

optionally together with one or more associated cluster genes suitable for conversion of a precursor peptide into said peptide,

thereby generating a host cell comprising a polynucleotide sequence encoding said peptide and suitable for expressing same, wherein the type B lantibiotic peptide has antimicrobial activity.

17. A method according to claim 16, where the cell is transformed with a *lanA* gene and one or more cluster genes.

18. A method of according to claim 17, wherein the associated gene cluster comprises a gene encoding a LanR polypeptide or a variant thereof 90% identical thereto, wherein said polypeptide is a factor regulating the production of the LanA polypeptide.

wherein 1 to 5 amino acids in the LanA polypeptide sequence is/are substituted by another amino acid, or the amino acid sequence is at least 90% identical thereto, such that the gene is not expressed or the gene product is inactive.

35. Use of a host cell for the expression of actagardine, a variant thereof, deoxyactagardine or a variant thereof, wherein the cell is a lantibiotic producing cell with a gene encoding a LanO polypeptide and said polypeptide is derived from the lantibiotic gene cluster and is a factor involved in the oxidation of deoxy-forms to -S(O)- analogue thereof and:

said gene is inactivated, or

the expression of said gene is increased.

36. Use of a host cell according to claim 35, wherein the gene encoding a LanO polypeptide is inactivated by mutation or deletion.

37. Use of a host cell comprising a gene encoding a LanA peptide, a precursor of a lantibiotic peptide for the production of actagardine, a variant thereof, deoxyactagardine or a variant thereof, with the proviso that wherein the host is a lantibiotic producing cell comprising an endogenous gene encoding a LanA peptide, the latter is mutated such that the endogenous gene is not expressed or the endogenous gene product is inactive or the endogenous gene is deleted.