

PATENT SPECIFICATION

(11) 1457950

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(54) ANTIBACTERIAL COMPOSITIONS

(71) We, MERCK & CO INC, a corporation duly organised and existing under the laws of the State of New Jersey, United States of America, of Rahway, New Jersey, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention is concerned generally with compositions having antibacterial activity and containing a mixture of a certain type of 3-fluoro - D - alanine compound with a certain type of cycloserine compound, which acts as a 3 - fluoro - D - alanine autoantagonist-inhibitor.

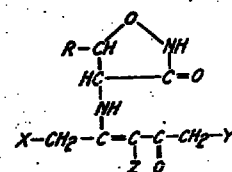
Certain 3 - fluoro - D - alanine compounds, viz. 3 - fluoro - D - alanine, 2 - deuterio - 3 - fluoro - D - alanine, 2,3 - dideutero - 3 - fluoro - D - alanine, 2,3,3 - trideutero - 3 - fluoro - D - alanine and their C₁₋₃ alkyl esters and pharmaceutically acceptable salts, (herein led FDA), are known to be valuable antibacterial agents showing effective activity in inhibiting the growth of pathogenic bacteria of both gram-positive and gram-negative genera, including *Streptococcus*, *Escherichia*, *Staphylococcus*, *Pseudomonas*, *Diplococcus*, *Klebsiella*, *Proteus*, *Mycobacterium*, *Serratia*, *Vibrio* and *Pasteurella*, and in particular, bacteria belonging to the species *Escherichia coli*, *Proteus vulgaris*, *Proteus mirabilis*, *Proteus morganii*, *Streptomyces pyogenes*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Aerobacter aerogenes*.

These FDA compounds do, however, possess the unusual characteristic of being autoantagonists at high concentration, which characteristic is disadvantageous where it is desired to use them at elevated dosages, or where high autoantagonist concentrations of the FDA compound could result during therapy as, for example, in patients with diminished renal function. Indeed, since ele-

vated dosages of FDA compounds may be used with safety, it is advantageous in treatment of infections to utilize the FDA compound at a sufficiently high dosage to provide effective antibacterial action against the most resistant organism by which the infection may be caused, at which high dosage the FDA compounds is an autoantagonist with respect to more sensitive microorganisms that may be present.

The present invention is based on the discovery that this autoantagonist action shown by the said FDA compounds at elevated concentration can be suppressed by certain substances, which act as FDA-autoantagonist-inhibitors.

In accordance with the present invention, there is provided an antibacterial composition comprising as active ingredients a mixture of 3 - fluoro - D - alanine, 2 - deuterio - 3 - fluoro - D - alanine, 2,3 - dideutero - 3 - fluoro - D - alanine, 2,3,3 - trideutero - 3 - fluoro - D - alanine, a C₁₋₃ alkyl ester thereof or a pharmaceutically acceptable salt thereof, with an isoxazolidinone compound of formula



in which R is hydrogen or methyl, and each of X, Y and Z is hydrogen or alkyl, or a pharmaceutically acceptable salt thereof, preferably in admixture with a pharmaceutically acceptable carrier.

Compositions of the present invention have been found to demonstrate, even at greatly elevated concentrations, the highly effective antibacterial action of the FDA compounds, and are further characterized as showing a remarkable synergistic action, the antibacterial

potency of the combination being substantially in excess of that contributed by the individual components.

The isoxazolidinone compounds used in the compositions of the present invention may be referred to as D - 4 - (1' - methyl - 3' - oxo - 1' - butenyl or alkyl - substituted - 1' - methyl - 3' - oxo - 1' - butenyl)amino - 3 - isoxazolidinones, and their 5 - methyl derivatives, for example D - 4 - (1' - methyl - 3' - oxo - 1' - butenyl)amino - 3 - isoxazolidinone, D - 4 - (1' - methyl - 3' - oxo - 1' - butenyl)amino - 5 - methyl - 3 - isoxazolidinone, D - 4 - (1',2' - dimethyl - 3' - oxo - 1' - butenyl)amino - 3 - isoxazolidinone, D - 4 - 1',2' - dimethyl - 3' - oxo - 1' - butenyl)amino - 5 - methyl - 3 - isoxazolidinone, D - 4 - (1' - ethyl - 3' - oxo - 1' - butenyl)amino - 3 - isoxazolidinone, D - 4 - (1' - ethyl - 3' - oxo - 1' - butenyl)amino - 5 - methyl - 3 - isoxazolidinone, D - 4 - (1' - ethyl - 2' - methyl - 3' - oxo - 1' - butenyl)amino - 3 - isoxazolidinone, D - 4 - (1' - ethyl - 2' - methyl - 3' - oxo - 1' - butenyl)amino - 5 - methyl - 3 - isoxazolidinone, D - 4 - (1' - ethyl - 3' - oxo - 1' - pentenyl)amino - 3 - isoxazolidinone, D - 4 - (1' - ethyl - 3' - oxo - 1' - pentenyl)amino - 5 - methyl - 3 - isoxazolidinone, D - 4 - (1' - ethyl - 2' - methyl - 3' - oxo - 1' - pentenyl)amino - 3 - isoxazolidinone, and D - 4 - (1' - ethyl - 2' - methyl - 3' - oxo - 1' - pentenyl)amino - 5 - methyl - 3 - isoxazolidinone; pharmaceutically acceptable salts of the foregoing such as alkali metal salts, preferably the sodium and potassium salts; alkaline-earth metal salts preferably the calcium and magnesium salts; ammonium salts; and amine salts, preferably salts with triethylamine, diethylamine, N - methyl - glucamine, diethanolamine, triethanolamine or 2 - amino - 2 - hydroxymethyl - 1,3 - propanediol; for example, the sodium salt of D - 4 - (1' - methyl - 3' - oxo - 1' - butenyl)amino - 3 - isoxazolidinone, the calcium salt of D - 4 - (1' - methyl - 3' - oxo - 1' - butenyl)amino - 3 - isoxazolidinone, and the sodium salt of D - 4 - (1' - methyl - 3' - oxo - 1' - butenyl)amino - 5 - methyl - 3 - isoxazolidinone.

These compounds, and more particularly D - 4 - (1' - methyl - 3' - oxo - 1' - butenyl)amino - 3 - isoxazolidinone and D - 4 - (1',2' - dimethyl - 3' - oxo - 1' - butenyl)amino - 3 - isoxazolidinone, and their sodium and potassium salts such as D - 4 - (1' - methyl - 3' - oxo - 1' - butenyl)amino - 3 - isoxazolidinone sodium salt-hemihydrate, are remarkably stable in aqueous solution; when administered clinically, they are extremely effective in releasing cycloserine or methyl - cycloserine *in vivo* and, at the same time, substantially avoiding the unwanted dimerization of the cycloserine or methyl-cycloserine. The D - 4 - (1' - methyl - 3' - oxo - 1' - butenyl)amino - 3 - isoxazolidinone and its pharmaceutically acceptable salts are of particular effectiveness

in releasing cycloserine in the blood stream and in the bladder, and are thus of value in providing FDA - autoantagonist - inhibitor action throughout the vascular system as well as in the urinary tract.

The preferred FDA compounds for use in the compositions of the present invention are 3 - fluoro - D - alanine itself, 2 - deuterio - 3 - fluoro - D - alanine, and their pharmaceutically acceptable salts. Particularly preferred pairs of compounds are (a) 3 - fluoro - D - alanine and D - 4 - (1' - methyl - 3' - oxo - 1' - butenyl)amino - 3 - isoxazolidinone, (b) 2 - deuterio - 3 - fluoro - D - alanine and D - 4 - (1' - methyl - 3' - oxo - 1' - butenyl)amino - 3 - isoxazolidinone or the sodium salt of the latter, and (c) 2 - deuterio - 3 - fluoro - D - alanine and D - 4 - (1',2' - dimethyl - 3' - oxo - 1' - butenyl)amino - 3 - isoxazolidinone. A pharmaceutically acceptable salt can be substituted for either or both of the free compounds.

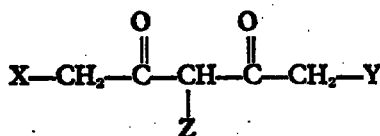
The critical effectiveness of FDA - autoantagonist - inhibitors is exemplified as follows: 3 - fluoro - D - alanine at a concentration of 3.2 $\mu\text{g/ml}$, completely represses the growth of a virulent culture of *Escherichia coli*, (Strain 2017); however, at higher concentrations (i.e. at 50 $\mu\text{g/ml}$) extensive growth of the organism occurs characteristic of the autoantagonist action of the 3 - fluoro - D - alanine. When the 3 - fluoro - D - alanine, at this concentration of 50 $\mu\text{g/ml}$, is combined with an FDA - autoantagonist - inhibitor such as D - 4 - (1' - methyl - 3' - oxo - 1' - butenyl)amino - 3 - isoxazolidinone or its sodium salt at 0.72 $\mu\text{g/ml}$, growth of the *Escherichia coli* is completely repressed. Surprisingly, the 0.72 $\mu\text{g/ml}$ D - 4 - (1' - methyl - 3' - oxo - 1' - butenyl)amino - 3 - isoxazolidinone concentration, completely effective as FDA - autoantagonist - inhibitor, is less than 2% of the minimum concentration which is itself able to completely repress the growth of the *Escherichia coli* organisms.

This novel admixture is conveniently administered for antibacterial effectiveness in the form of a pharmaceutical composition containing the FDA compound in admixture with the FDA - autoantagonist - inhibitor and a pharmacologically acceptable carrier. When a FDA compound is combined with the isoxazolidinone compound of Formula I, the ratio of the FDA compound to the said isoxazolidinone compound of the formula given above in the combination ordinarily varies from 1:120 to 200:1 with ratios within the range of 1:4 to 4:1 being preferred. As already stated, the FDA compound is 3 - fluoro - D - alanine, 2 - deuterio - 3 - fluoro - D - alanine, 2,3 - di-deuterio - 3 - fluoro - D - alanine, 2,3,3 - tri-deuterio - 3 - fluoro - D - alanine, a C₁₋₅ alkyl ester thereof such as the methyl ester, or a pharmaceutically acceptable salt such as the sodium, calcium, or hydrochloride salt.

Pharmaceutically acceptable carriers for the aforesaid compositions include convenient vehicles suitable for oral administration such as capsules, tablets, or liquid solutions or suspensions, or the admixture may be dissolved in a vehicle adapted for administration by injection. Suitable formulations for oral use may include diluents, granulating agents, preservatives, binders, flavouring agents and coating agents, which are well known, and the dosage of the products may be varied over a wide range. A composition for oral use comprising the combination of FDA compound and the isoxazolidinone compound of Formula I is conveniently prepared by intermixing the individual components in a dry, pulverulent state with gelatin, starch, magnesium stearate and alginic acid, compressed into a tablet. The FDA compound and the said isoxazolidinone compound may also be incorporated together in aqueous solution, and the solution evaporated to provide the combination in the form of an intimate mixture of the components.

Alternatively, in accordance with a further embodiment of this invention, the components of the admixture may be separately administered, particularly where it is desired for maintenance of blood levels of the more rapidly excreted FDA component.

The isoxazolidinone FDA - antoantagonist inhibitors may be prepared by stirring together, at room temperature for a period of about two days, cycloserine or methyl - cycloserine with an excess of a 2,4 - pentanedione compound having the following formula:



wherein X, Y and Z are as defined above, such as 2,4 - pentanedione, under which conditions, the cycloserine compound gradually goes into solution, with formation of the corresponding compound of formula I above.

As the reaction proceeds, this compound crystallizes from the resulting reaction solution, and is recovered by filtration, washed with ether and dried. For example, D - 4 - (1' - methyl - 3' - oxo - 1' - butenyl)amino - 3 - isoxazolidinone is conveniently prepared by stirring a mixture of about 3.0 g. of D - 4 - amino - 3 - isoxazolidinone and 30 ml. of 2,4 - pentanedione in a dry atmosphere at approximately room temperature for about two days. The D - 4 - amino - 3 - isoxazolidinone gradually goes into solution, and the reaction product, which crystallizes from the reaction solution, is recovered by filtration, washed with three 20-ml. portions of ether, and dried at room temperature *in vacuo* to give about 3.5

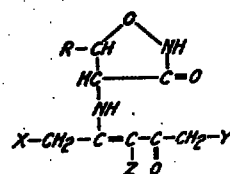
g. of D - 4 - (1' - methyl - 3' - oxo - 1' - butenyl)amino - 3 - isoxazolidinone.

The latter is conveniently converted to its pharmaceutically acceptable salts, e.g. the sodium or calcium salts, as follows: 0.143 g. of D - 4 - (1' - methyl - 3' - oxo - 1' - butenyl)amino - 3 - isoxazolidinone is dissolved in 0.8 ml. of methanol, and 1.56 ml. of an 0.05 molar solution of sodium hydroxide in methanol is added to give a pH of approximately 7.0. The methanol is evaporated from the resulting solution under a stream of nitrogen until crystals form; the crystalline slurry is further evaporated to dryness *in vacuo*. The residual material is washed with 2.5 ml. of acetone, and redissolved in 0.55 ml. of methanol; the solution is filtered and the filtrate diluted with 0.45 ml. of methanol. To the resulting solution is added 4 ml. of ether, and the crystalline precipitate which forms is recovered by filtration, washed with ether and dried to give substantially pure D - 4 - (1' - methyl - 3' - oxo - 1' - butenyl)amino - 3 - isoxazolidinone sodium salt-hemihydrate.

3 g. of calcium oxide is slurried in 40 ml. of water, and the slurry cooled to 0—5°C. 5 g. of D - 4 - (1' - methyl - 3' - oxo - 1' - butenyl)amino - 3 - isoxazolidinone is added to the cooled slurry, the resulting mixture is heated with slurry to room temperature and stirred at that temperature for 5 minutes. The mixture is filtered, and the insoluble material is washed with 10 ml. of water. To the combined solution and washings (having a pH of 11.0—11.5) is added sufficient D - 4 - (1' - methyl - 3' - oxo - 1' - butenyl)amino - 3 - isoxazolidinone to give a final pH of about 9.5. Activated carbon (0.5 g.) is added to the resulting solution, and the mixture is stirred for about 15 minutes and filtered. The filtered solution is diluted with six times its volume of ethanol, and the crystalline precipitate which forms is recovered by filtration, washed with ethanol and dried *in vacuo* to give, in substantially pure form, the calcium salt of D - 4 - (1' - methyl - 3' - oxo - 1' - butenyl)amino - 3 - isoxazolidinone.

WHAT WE CLAIM IS:—

1. An antibacterial composition comprising as active ingredients a mixture of an FDA, viz. 3 - fluoro - D - alanine, 2 - deutero - 3 - fluoro - D - alanine, 2,3 - dideutero - 3 - fluoro - D - alanine, 2,3,3 - trideutero - 3 - fluoro - D - alanine, a C₁₋₃ alkyl ester thereof or a pharmaceutically acceptable salt thereof, with an isoxazolidinone compound of formula





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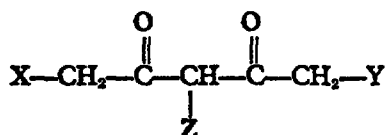
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Pharmaceutically acceptable carriers for the aforesaid compositions include convenient vehicles suitable for oral administration such as capsules, tablets, or liquid solutions or suspensions, or the admixture may be dissolved in a vehicle adapted for administration by injection. Suitable formulations for oral use may include diluents, granulating agents, preservatives, binders, flavouring agents and coating agents, which are well known, and the dosage of the products may be varied over a wide range. A composition for oral use comprising the combination of FDA compound and the isoxazolidinone compound of Formula I is conveniently prepared by intermixing the individual components in a dry, pulverulent state with gelatin, starch, magnesium stearate and alginic acid, compressed into a tablet. The FDA compound and the said isoxazolidinone compound may also be incorporated together in aqueous solution, and the solution evaporated to provide the combination in the form of an intimate mixture of the components.

Alternatively, in accordance with a further embodiment of this invention, the components of the admixture may be separately administered, particularly where it is desired for maintenance of blood levels of the more rapidly excreted FDA component.

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As the reaction proceeds, this compound crystallizes from the resulting reaction solution, and is recovered by filtration, washed with ether and dried. For example, D - 4 - (1' - methyl - 3' - oxo - 1' - butenyl)amino - 3 - isoxazolidinone is conveniently prepared by stirring a mixture of about 3.0 g. of D - 4 - amino - 3 - isoxazolidinone and 30 ml. of 2,4 - pentanedione in a dry atmosphere at approximately room temperature for about two days. The D - 4 - amino - 3 - isoxazolidinone gradually goes into solution, and the reaction product, which crystallizes from the reaction solution, is recovered by filtration, washed with three 20-ml. portions of ether, and dried at room temperature *in vacuo* to give about 3.5

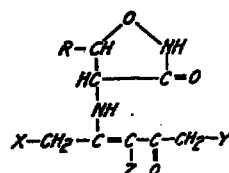
g. of D - 4 - (1' - methyl - 3' - oxo - 1' - butenyl)amino - 3 - isoxazolidinone.

The latter is conveniently converted to its pharmaceutically acceptable salts, e.g. the sodium or calcium salts, as follows: 0.143 g. of D - 4 - (1' - methyl - 3' - oxo - 1' - butenyl)amino - 3 - isoxazolidinone is dissolved in 0.8 ml. of methanol, and 1.56 ml. of an 0.05 molar solution of sodium hydroxide in methanol is added to give a pH of approximately 7.0. The methanol is evaporated from the resulting solution under a stream of nitrogen until crystals form; the crystalline slurry is further evaporated to dryness *in vacuo*. The residual material is washed with 2.5 ml. of acetone, and redissolved in 0.55 ml. of methanol; the solution is filtered and the filtrate diluted with 0.45 ml. of methanol. To the resulting solution is added 4 ml. of ether, and the crystalline precipitate which forms is recovered by filtration, washed with ether and dried to give substantially pure D - 4 - (1' - methyl - 3' - oxo - 1' - butenyl)amino - 3 - isoxazolidinone sodium salt-hemihydrate.

3 g. of calcium oxide is slurried in 40 ml. of water, and the slurry cooled to 0—5°C. 5 g. of D - 4 - (1' - methyl - 3' - oxo - 1' - butenyl)amino - 3 - isoxazolidinone is added to the cooled slurry, the resulting mixture is heated with slurry to room temperature and stirred at that temperature for 5 minutes. The mixture is filtered, and the insoluble material is washed with 10 ml. of water. To the combined solution and washings (having a pH of 11.0—11.5) is added sufficient D - 4 - (1' - methyl - 3' - oxo - 1' - butenyl)amino - 3 - isoxazolidinone to give a final pH of about 9.5. Activated carbon (0.5 g.) is added to the resulting solution, and the mixture is stirred for about 15 minutes and filtered. The filtered solution is diluted with six times its volume of ethanol, and the crystalline precipitate which forms is recovered by filtration, washed with ethanol and dried *in vacuo* to give, in substantially pure form, the calcium salt of D - 4 - (1' - methyl - 3' - oxo - 1' - butenyl)amino - 3 - isoxazolidinone.

WHAT WE CLAIM IS:—

1. An antibacterial composition comprising as active ingredients a mixture of an FDA, viz. 3 - fluoro - D - alanine, 2 - deutero - 3 - fluoro - D - alanine, 2,3 - dideutero - 3 - fluoro - D - alanine, 2,3,3 - trideutero - 3 - fluoro - D - alanine, a C₁₋₃ alkyl ester thereof or a pharmaceutically acceptable salt thereof, with an isoxazolidinone compound of formula



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- 4 in which R is hydrogen or methyl, and each of X, Y and Z is hydrogen or alkyl, or a pharmaceutically acceptable salt thereof.
- 5 2. A composition as claimed in Claim 1 and also including a pharmaceutical carrier.
3. A composition as claimed in Claim 2 in which the pharmaceutical carrier is orally administrable.
- 10 4. A composition as claimed in Claim 2 in which the pharmaceutical carrier is injectable.
5. A composition as claimed in Claim 3 in the form of a capsule, tablet, or liquid solution or suspension.
- 15 6. A composition as claimed in any one of Claims 1 to 5 in which the FDA is 3 - fluoro-D - alanine or its 2 - deuterio analog, or a pharmaceutically acceptable salt thereof.
- 20 7. A composition as claimed in Claim 6 in which the FDA is 3 - fluoro D - alanine or a pharmaceutically acceptable salt thereof, and the isoxazolidinone compound is D - 4 - (1' - methyl - 3' - oxo - 1' - butenyl)amino - 3 - isoxazolidinone or a pharmaceutically acceptable salt thereof.
- 25 8. A composition as claimed in Claim 6 in which the FDA is 3 - fluoro - D - alanine and the isoxazolidinone compound is D - 4 - (1' - methyl - 3' - oxo - 1' - butenyl)amino - 3 - isoxazolidinone.
- 30 9. A composition as claimed in Claim 6 in which the FDA is 2 - deuterio - 3 - fluoro - D - alanine or a pharmaceutically acceptable salt thereof, and the isoxazolidinone compound is D - 4 - (1' - methyl - 3' - oxo - 1' - butenyl)amino - 3 - isoxazolidinone or a pharmaceutically acceptable salt thereof.
- 35 10. A composition as claimed in Claim 6 in which the FDA is 2 - deuterio - 3 - fluoro - D - alanine, and the isoxazolidinone compound is D - 4 - (1' - methyl - 3' - oxo - 1' - butenyl)amino - 3 - isoxazolidinone.
- 40 11. A composition as claimed in Claim 6 in which the FDA is 2 - deuterio - 3 - fluoro - D - alanine and the isoxazolidinone compound is the sodium salt of D - 4 - (1' - methyl - 1 - 3' - oxo - 1' - butenyl)amino - 3 - isoxazolidinone.
12. A composition as claimed in Claim 6 in which the FDA is 2 - deuterio - 3 - fluoro - D - alanine or a pharmaceutically acceptable salt thereof, and the isoxazolidinone compound is D - 4 - (1',2' - dimethyl - 3' - oxo - 1' - butenyl)amino - 3 - isoxazolidinone or a pharmaceutically acceptable salt thereof.
- 50 13. A composition as claimed in Claim 6 in which the FDA is 2 - deuterio - 3 - fluoro - D - alanine and the isoxazolidinone compound is D - 4 - (1',2' - dimethyl - 3' - oxo - 1' - butenyl)amino - 3 - isoxazolidinone.
- 55 14. A method of treating a bacterial infection in a non-human host which comprises administering to said host an FDA, viz. 3 - fluoro - D - alanine, 2 - deuterio - 3 - fluoro - D - alanine, 2,3 - dideuterio - 3 - fluoro - D - alanine, 2,3,3 - trideuterio - 3 - fluoro - D - alanine, a C₁₋₃ alkyl ester thereof or a pharmaceutically acceptable salt thereof and, as 3 - fluoro - D - alanine - autoantagonist - inhibitor, an isoxazolidinone compound of the formula set forth in Claim 1.
- 60 15. A method as claimed in Claim 12 in which the FDA and the isoxazolidinone compound are separately administered.
- 65 16. A method as claimed in Claim 14 or 15 in which the FDA is 3 - fluoro - D - alanine or its 2 - deuterio analog, or a pharmaceutically acceptable salt thereof.
- 70 17. A method as claimed in Claim 16 in which the FDA and the isoxazolidinone compound are as defined in any one of Claims 7 to 13.
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