IN THE MATTER OF a Singapore Application corresponding to PCT Application PCT/FR96/00575

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c/o Europa House, Marsham Way, Gerrards Cross, Buckinghamshire, England, do solemnly and sincerely declare that I am conversant with the English and French languages and am a competent translator thereof, and that to the best of my knowledge and belief the following is a true and correct translation of the PCT Application filed under No. PCT/FR96/00575.

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# (54) Title: METHOD FOR PREPARING STREPTOGRAMINES

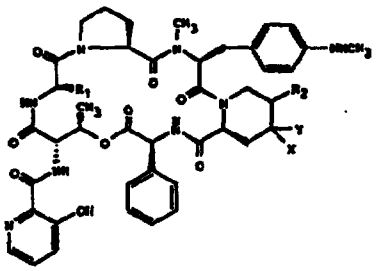
# (54) Titre: PROCEDE DE PREPARATION DE STREPTOGRAMINES

**(57) Abstract**

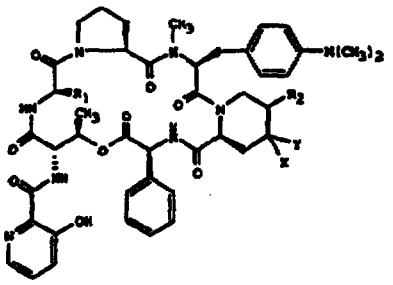
A method for preparing streptogramines of general formula (I), wherein $R_1$ is methyl or ethyl, $R_2$ is H and X and Y together form an oxo radical, or $R_1$ is ethyl, $R_2$ and X are H and Y is H or OH, or else $R_1$ is ethyl, $R_2$ is OH and X and Y together form an oxo radical, by demethylating a synergistin derivative of general formula (II), wherein $R_1$, $R_2$, X and Y are as defined above, by means of a treatment with a periodate in an acetic medium, followed by a treatment in an aqueous medium.

**(57) Abrégé**

Procédé de préparation de streptogramines de formule générale (I) dans laquelle soit $R_1$ est méthyle ou éthyle, $R_2$ est H et X et Y forment ensemble un radical oxo, soit $R_1$ est éthyle, $R_2$ et X représentent H et Y est H ou OH, soit $R_1$ est éthyle $R_2$ est OH et X et Y forment ensemble un radical oxo, par déméthylation d'un dérivé de synergistine de formule générale (II) dans laquelle $R_1$, $R_2$, X et Y sont définis comme ci-dessus, par traitement par un periodate en milieu acétique suivi d'un traitement en milieux aqueux.



(I)



(II)

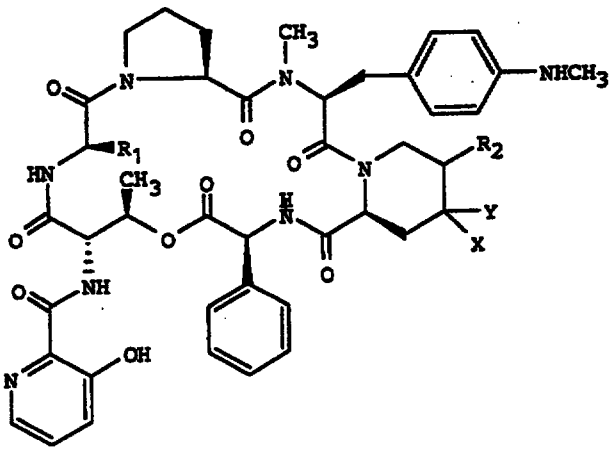
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# PROCESS FOR THE PREPARATION OF STREPTOGRAMINS

The present invention relates to a novel process for the preparation of streptogramins of general formula:

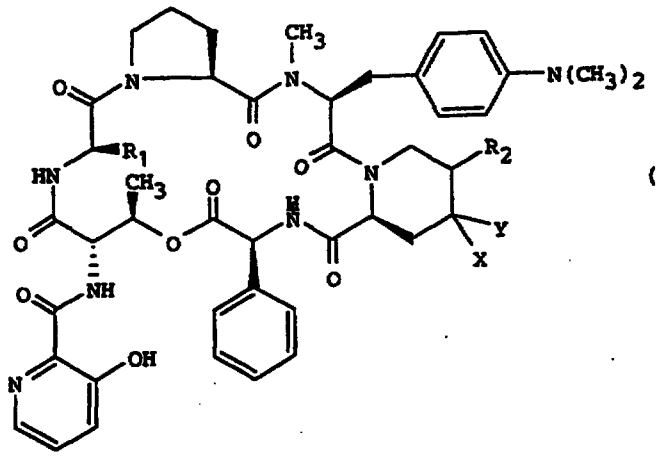


(I)

in which:

- either the radical $R_1$ represents a methyl or ethyl group, the radical $R_2$ represents a hydrogen atom and X and Y together form an oxo radical,
- or $R_1$ represents an ethyl radical, $R_2$ and X represent a hydrogen atom and Y represents a hydrogen atom or a hydroxyl radical,
- or $R_1$ represents an ethyl radical, $R_2$ represents a hydroxyl radical and X and Y together form an oxo radical,

from a synergistin derivative of general formula:



(II)

in which the radicals $R_1$, $R_2$, X and Y are as defined above.

Streptogramins are a known class of compounds comprising components of group B [to which the products of general formula (I) belong] which, when combined with components of group A, bring about a synergism of the antimicrobial action.

The product of general formula (I) for which $R_1$ is ethyl and $R_2$ is hydrogen is known under the name of pristinamycin IB. The product of general formula (I) for which $R_1$ is methyl and $R_2$ is hydrogen is known under the name of vernamycin B$\delta$. The product of general formula (II) for which $R_1$ is ethyl and $R_2$ is hydrogen is known under the name of pristinamycin IA. The product of general formula (II) for which $R_1$ is methyl and $R_2$ is hydrogen is known under the name of pristinamycin IC or vernamycin B$\gamma$. The product of general formula (II) for which $R_1$ is ethyl and $R_2$ is hydroxyl is known under the name of pristinamycin ID.

General methods of demethylation were already

known, for example such as the methods described in Tet. Lett., 18, 1567 (1977); J. Org. Chem., 49, 2795 (1984); J. C. S. Chem. Comm., 905 (1989), Tet. Lett., 33, 6991 (1992); however, these methods could not be adapted to fragile products such as streptogramins, either because the reaction did not take place or because the operating conditions were degrading towards these products. Yet other methods involved toxic reactants which were not totally removable from the final product, this being unacceptable from the pharmaceutical point of view.

It has now been found, and this forms the subject of the present invention, that the streptogramins of general formula (I) could be obtained by demethylation of the corresponding derivative of general formula (II) by treatment with a periodate in acetic medium followed by a treatment in aqueous acidic medium or by a treatment with an agent capable of consuming the formaldehyde in situ.

The periodate used is advantageously tetra-n-butylammonium periodate or an alkaline periodate (sodium periodate). The reaction is carried out in a solvent such as a chlorinated solvent (for example dichloromethane, chloroform, dichloroethane or trichloroethane), an ester (for example ethyl acetate), a nitrile (for example acetonitrile) or in tetrahydrofuran, N-methylpyrrolidone or optionally a mixture of these solvents, in the presence or absence

of ethylene glycol. The reaction takes place at a temperature of between 20 and 40°C.

The subsequent treatment is a hydrolysis, in aqueous medium, which releases formaldehyde. It is possible to perform the process by treatment of the product obtained in a homogeneous aqueous medium to which is added a strong acid, or directly in an acidic or non-acidic two-phase medium; in particular, the process may be performed in a dichloromethane/water mixture. In this case, preferably, the pH of the aqueous medium will be weakly acidic; it is understood that the acidity of the medium will be provided, indiscriminately, by addition of a strong or weak acid.

The acids used may be chosen in particular from trifluoroacetic acid, sulphuric acid, hydrochloric acid, methanesulphonic acid, p-toluenesulphonic acid or formic acid. The treatment in acidic medium is carried out at a temperature of between 0 and 40°C.

When the subsequent treatment is carried out, it is also possible further to add an agent capable of consuming the formaldehyde in situ, this agent is advantageously chosen from hydroxylamine, a bisulphite (for example sodium bisulphite) or hydrogen peroxide in aqueous medium. The process is preferably performed in a two-phase medium at a temperature of between 0 and 40°C, at a pH of between 1 and 7.

The products of general formula (I) thus obtained may be purified, where appropriate, by the

usual methods such as crystallization, precipitation, flash chromatography or HPLC.

The products of general formula (I) in which $R_1$ represents an ethyl radical, $R_2$ and X represent a hydrogen atom and Y represents a hydrogen atom or a hydroxyl radical are novel products of the streptogramin family.

The examples which follow, given without any limitation being implied, illustrate the process according to the invention.

Example 1

540 g of crude pristinamycin I [pristinamycin $I_A$ 72.2 % (433 g), pristinamycin $I_B$ 4.2 % (25 g), pristinamycin $I_C$ 2.67 % (16 g), pristinamycin $I_D$ 3.17 % (19 g)] are placed in solution in a mixture of 1460 $cm^3$ of dichloromethane, 500 $cm^3$ of acetic acid and 40 $cm^3$ of ethylene glycol, in a three-necked flask. 97.5 g of tetra-n-butylammonium periodate are added, while maintaining the temperature at 30°C. After stirring for 3 hours at 30°C, the reaction is stopped by addition, with stirring, of 2000 $cm^3$ of demineralized water. The aqueous phase is separated out by settling and the organic phase is washed again with 2000 $cm^3$ of demineralized water. The aqueous phase is separated out by settling and the organic phase is concentrated to a volume of 800 $cm^3$. 1000 $cm^3$ of methyl ethyl ketone are added to the concentrate and the mixture is concentrated under reduced pressure (1.5 kPa) to a

volume of 1300 $cm^3$. Methyl ethyl ketone is added up to a total volume of 2400 $cm^3$ and the mixture is cooled to 0°C. The precipitated solid is filtered off, washed with 3 times 250 $cm^3$ of methyl ethyl ketone and then dried at 40°C under reduced pressure (1.5 kPa). 441 g of a white solid are thus obtained, which product is dissolved in 8800 $cm^3$ of 0.25 N hydrochloric acid and stirred for 1 hour and then extracted with 3500 $cm^3$ of dichloromethane, adjusting the pH of the aqueous phase to 4 with 30 % sodium hydroxide. The organic phase is separated out by settling, washed with 3500 $cm^3$ of water and then concentrated to dryness under reduced pressure (50 kPa at 30°C) to a volume of about 1100 $cm^3$. 2200 $cm^3$ of ethanol are added to this solution and the evaporation under reduced pressure is continued down to 1800 $cm^3$. 3500 $cm^3$ of ethanol are then added. The crystals obtained are filtered off at 10°C, filtered off and then rinsed with 3 times 330 $cm^3$ of cold ethanol, then dried at 40°C under reduced pressure (1.5 kPa). 360 g of pristinamycin $I_B$ are thus obtained in the form of white, 80.7 % pure crystals, i.e. containing 290.4 g of pristinamycin $I_B$.

Moreover, 1.1 % of vernamycin Bδ was obtained, equivalent to a conversion yield of 41.2 %, and 2 % of pristinamycin of general formula (I) in which $R_1$ is ethyl and $R_2$ is hydroxyl, equivalent to a conversion yield of 63 % (HPLC assay).

Example 2

20 g of pristinamycin I [pristinamycin $I_A$ 76.5 % (15.3 g), pristinamycin $I_B$ 7 % (1.4 g)] are placed in solution, in a three-necked flask, in a mixture of 28 $cm^3$ of 1,2-dichloroethane, 70 $cm^3$ of acetic acid and 2 $cm^3$ of ethylene glycol. 4.9 g of sodium periodate are added, while maintaining the temperature at 25°C. After stirring for 6 hours, the reaction is stopped by addition, with stirring, of 100 $cm^3$ of demineralized water. The aqueous phase is separated out by settling and the organic phase is washed again with 50 $cm^3$ of demineralized water. The aqueous phase is separated out by settling and the organic phase is concentrated to dryness under reduced pressure. The solid is taken up in 400 $cm^3$ of methyl isobutyl ketone and the product is extracted with twice 320 $cm^3$ and then 80 $cm^3$ of 0.2 N sulphuric acid. The aqueous phases are combined and then extracted with 400 $cm^3$ of dichloromethane. The organic phase is separated out by settling, concentrated to dryness under reduced pressure (30 kPa) at 30°C and then dried under reduced pressure (150 Pa) at 40°C to give 12.5 g of a white solid containing 72 % (9 g) of pristinamycin $I_B$ and 5.6 % (0.7 g) of pristinamycin $I_A$. Conversion yield: 84.9 %.

Example 3

540 g of crude pristinamycin I (pristinamycin $I_A$ 433 g, pristinamycin $I_B$ 25 g, pristinamycin $I_C$ 16 g,

pristinamycin $I_D$ 19 g) are placed in solution, in a three-necked flask, in a mixture of 1460 $cm^3$ of dichloromethane, 500 $cm^3$ of acetic acid and 40 $cm^3$ of ethylene glycol. 97.5 g of tetra-n-butylammonium periodate are added, while maintaining the temperature at 30°C. After stirring for 3 hours at 30°C, the reaction is stopped by addition, with stirring, of 2000 $cm^3$ of demineralized water containing 34.7 g of hydroxylamine hydrochloride. The aqueous phase is settled and then separated out. The organic phase is washed with 2000 $cm^3$ of water. After settling and separation, the organic phase is concentrated to a syrup. 2500 $cm^3$ of ethyl acetate are poured onto this concentrate and the solution is then concentrated to a final volume of 1300 $cm^3$. The suspension is filtered at 5°C. The crystals are washed with 3 times 400 $cm^3$ of fresh ethyl acetate and dried at 40°C under 1500 Pa of residual pressure. 331 g of a white product giving a pristinamycin IB assay of 91 % are thus obtained.

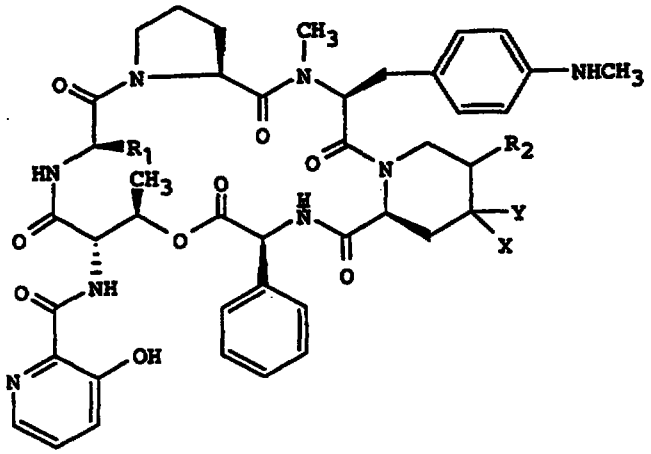
Example 4

180 g of crude pristinamycin I (containing 111.1 g of pristinamycin IA and 35.6 g of pristinamycin IB) are placed in solution, in a three-necked flask, in a mixture of 444 $cm^3$ of dichloromethane, 128 $cm^3$ of acetic acid and 10 $cm^3$ of ethylene glycol. 25.9 g of tetra-n-butylammonium periodate are added. After stirring for 4 hours at 32°C, the reaction is stopped by addition, with stirring, of 1100 $cm^3$ of tap water.

The two phases are settled and separated. The organic phase is washed again 4 times in succession with, on each occasion, 1400 $cm^3$ of tap water. The pH of these four washes is readjusted downwards with 5 ml of normal hydrochloric acid to facilitate the settlings. These four washes, settlings and separations are carried out at 35°C. The organic phase is concentrated by a factor of about two. 600 $cm^3$ of ethyl acetate are gradually poured onto this concentrate, the crystallization being initiated after about one-third has been added. After the addition, supplying with ethyl acetate is continued with concomitant distillation, so as to keep a constant volume in the flask, i.e. about 600 $cm^3$. After distillation to constant volume of about 800 $cm^3$, the suspension is cooled to 0°C and filtered. The filter cake is washed with twice 125 $cm^3$ of ethyl acetate at 0°C and dried under reduced pressure (1.5 kPa) at 40°C to constant weight. 120 g of a light beige product containing 110 g of pristinamycin IB are obtained.

## CLAIMS

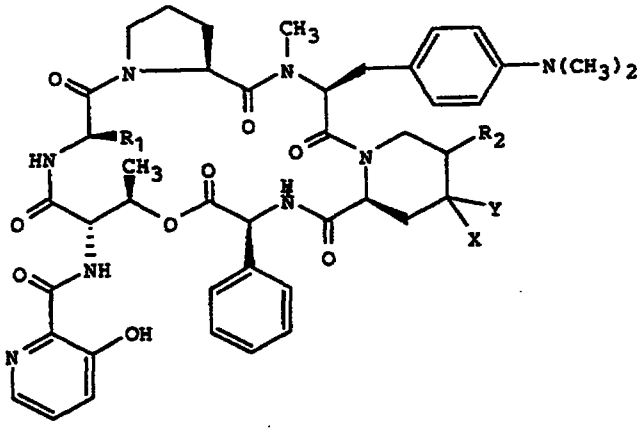
1. A process for the preparation of streptogramins of general formula:



in which:

- either the radical $R_1$ represents a methyl or ethyl group, the radical $R_2$ represents a hydrogen atom and X and Y together form an oxo radical,
- or $R_1$ represents an ethyl radical, $R_2$ and X represent a hydrogen atom and Y represents a hydrogen atom or a hydroxyl radical,
- or $R_1$ represents an ethyl radical, $R_2$ represents a hydroxyl radical and X and Y together form an oxo radical,

by demethylation of a synergistin derivative of general formula:



in which the radicals $R_1$, $R_2$, X and Y are as defined above, by treatment with a periodate in acetic medium followed by a treatment in aqueous medium.

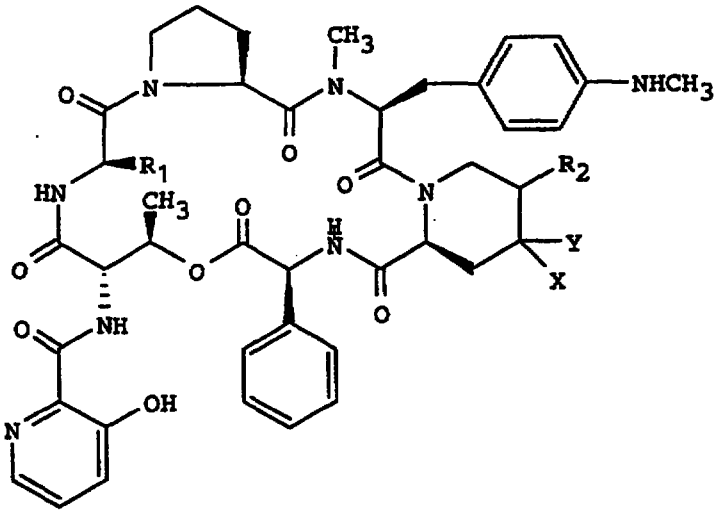
2. Process according to claim 1, characterized in that the periodate is chosen from tetra-n-butylammonium periodate or an alkaline periodate.

3. Process according to claim 2, characterized in that the alkaline periodate is sodium periodate.

4. Process according to claim 1, characterized in that the subsequent treatment is a hydrolysis, in aqueous medium, either in a homogeneous medium to which is added a strong acid, or in an acidic or non-acidic two-phase medium.

5. Process according to claim 1, characterized in that an agent capable of consuming the formaldehyde in situ is added, this agent being chosen from hydroxylamine, a bisulphite or hydrogen peroxide, during the subsequent treatment.

6. A streptogramin derivative, characterized in that it corresponds to the general formula:



in which $R_1$ represents an ethyl radical, $R_2$ and X represent a hydrogen atom and Y represents a hydrogen atom or a hydroxyl radical.

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(54) Title: METHOD FOR PREPARING STREPTOGRAMINES

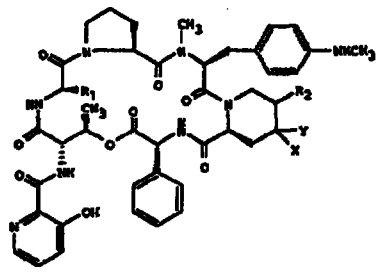
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(57) Abstract

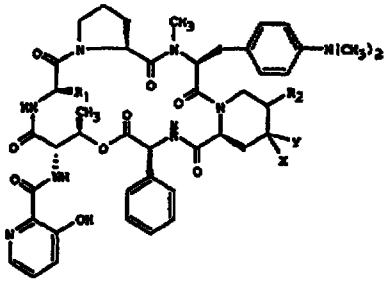
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(57) Abrégé

Procédé de préparation de streptogramines de formule générale (I) dans laquelle soit $R_1$ est méthyle ou éthyle, $R_2$ est H et X et Y forment ensemble un radical oxo, soit $R_1$ est éthyle, $R_2$ et X représentent H et Y est H ou OH, soit $R_1$ est éthyle $R_2$ est OH et X et Y forment ensemble un radical oxo, par déméthylation d'un dérivé de synergistine de formule générale (II) dans laquelle $R_1$, $R_2$, X et Y sont définis comme ci-dessus, par traitement par un periodate en milieu acétique suivi d'un traitement en milieux aqueux.



(I)



(II)