EUROPEAN PATENT SPECIFICATION

Date of publication of patent specification: 12.06.91
Int. Cl. C07G 11/00, C12P 1/06,
//C12P1/06,C12R1:045

Application number: 84112230.2

Date of filing: 11.10.84

The file contains technical information submitted after the application was filed and not included in this specification

Extraction of teichmonycin a2 from whole culture fermentation broth.

Priority: 11.10.83 US 540266
Date of publication of application: 17.04.85 Bulletin 85/16
Publication of the grant of the patent: 12.06.91 Bulletin 91/24
Designated Contracting States:
DE FR GB IT NL

References cited:
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US-A- 4 169 887
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The present invention relates to a method of extracting Teichomycin A₂ from a whole culture fermentation broth with a suitable water miscible solvent. Under the nomenclature established in the World Health Organization Handbook for International Non-Proprietary Names (INN), the name "Teichomycin A₂" has been changed to "Teicoplanin A₂" as the most recent name for this antibiotic factor.

United States Patent 4,239,751 discloses the antibiotic Teichomycin A₂ which is obtained by cultivation of strain Actinoplanes teichomyceticus nov. sp. ATCC 31121. Teichomycin A₂ is one of a mixture of antibiotic factors Teichomycin A₁, Teichomycin A₂, Teichomycin A₃, and Antibiotic 8327 Factor B and Antibiotic 8327 Factor C produced by this strain. U.S. patent 4,239,751 teaches a method of isolating the antibiotic factors. In the method described in U.S. Patent 4,239,751, the fermentation broth is filtered in order to remove the mycelial cell mass leaving a mycelial cell cake. The filtered fermentation broth is then mixed with a water immiscible organic solvent, such as, halogenated C₁₋₄ hydrocarbons or C₄₋₆ alkanols, in which the antibiotic mixture is soluble. The water immiscible organic solvent is then separated from the filtered fermentation broth by high-speed centrifugation, concentrated to about 1/10 to 1/20 of its original volume, cooled and allowed to stand until a precipitate (the antibiotic) forms which is recovered by filtration. Additional product may be recovered by extracting the mycelial cake with aqueous acetone. After distillation of the acetone, the aqueous phase is submitted to the same treatment described above for the filtered fermentation broth.

Briefly, in accordance with the present invention, Teichomycin A₂ is extracted from whole culture fermentation broth of Actinoplanes teichomyceticus or any similar microorganism producing Teichomycin A₂ containing a mycelial mass by a process comprising mixing the broth containing the mycelial mass with an effective amount of a water miscible solvent to accumulate Teichomycin A₂ in the broth/solvent liquid; separating the broth/solvent liquid containing accumulated Teichomycin A₂ from the mycelial mass; and precipitating and separating Teichomycin A₂ from the broth/solvent liquid. The useful water miscible solvents include acetone, acetonitrile, dimethylsulfoxide, n-propanol, methyl ethyl ketone or mixtures of these solvents.

The present extraction process is carried out by mixing an effective amount of a solvent, described above, with whole culture fermentation broth of Actinoplanes teichomyceticus which contains Teichomycin A₂. The solvent and broth are thoroughly contacted for an effective time period whereby the Teichomycin A₂ is accumulated in the liquid solvent/broth mixture. The Teichomycin A₂ is then recovered from this liquid employing separation and purification techniques.

The present invention employs a whole culture fermentation broth of Actinoplanes teichomyceticus containing Teichomycin A₂. Water miscible solvents used to accumulate Teichomycin A₂ in the broth/solvent liquid include acetone, acetonitrile, dimethylsulfoxide (DMSO), n-propanol, methyl ethyl ketone (MEK) and mixtures of these solvents.

The preparation of Teichomycin A₂ by the fermentation of Actinoplanes teichomyceticus is described in U.S. Patent 4,239,751 which is incorporated herein by reference. The whole culture fermentation broth containing the cell mass, aqueous nutrient medium and the Teichomycin A₂, is treated according to the present invention after the fermentation is complete.

The solvents employed in the practice of the present invention include acetone, acetonitrile, DMSO, n-propanol or MEK. Mixtures of these solvents can also be used. The solvents are employed in amounts between 5 to 80 percent by volume based on the total volume of solvent and broth, i.e., solvent/broth ratio of from 1/10 to 4/1. Preferably, from 10 to 60 percent by volume of solvent is employed. Preferred solvents are acetone and n-propanol.

The present invention is advantageously conducted at ambient temperatures and ambient pressures. The mixing is typically conducted with mild agitation sufficient to maintain a thorough contacting of fermentation broth and solvent. The pH of the fermentation broth is not critical to the present process. An acidic pH is preferred and it is especially preferred that the pH of the fermentation broth be in the range of from 3 to 4. The pH of the fermentation broth can be adjusted by the addition of an acid or base. Suitable acids include H₂SO₄, HCl, acetic acid and formic acid. Suitable bases include alkali metal bases, such as, NaOH or KOH.

In conducting the extraction process, an effective amount of the herein described solvents is mixed with whole culture fermentation broth of Actinoplanes teichomyceticus, which contains Teichomycin A₂, for an effective time period to accumulate the Teichomycin A in the mixed fermentation broth/solvent liquid. The rate of mixing the solvent and the fermentation broth is not critical. Preferably the solvent is added to the fermentation broth. An effective mixing time period is usually achieved in from 2 to 60 minutes.

After completion of the mixing period, the fermentation broth/solvent liquid is separated from the
mycelial mass by centrifugation or filtration. The fermentation broth/solvent liquid, separated from the mycelial cake, hereinafter referred to as "supernatant", is then concentrated, for example, to about 1/2 to 1/10 of its original volume, cooled and allowed to stand until a precipitate forms. The precipitate, containing Teichomycin A₂, is then recovered from the supernatant by filtration. Alternatively, Teichomycin A₂ can be recovered from the supernatant by acid precipitation.

In a preferred embodiment of the present invention, acetone or n-propanol is added to whole culture fermentation broth of Actinoplanes teichomyceticus which contains Teichomycin A₂ whereby the acetone or n-propanol represents from 20 to 50 percent by volume of the mixture. The mixture is agitated, from 2 to 60 minutes, such as by stirring or shaking to cause a thorough mixing of the acetone or n-propanol with the fermentation broth. The fermentation broth/solvent liquid is separated from the mycelial mass by centrifuging or filtering. Teichomycin A₂ is isolated from the supernatant by concentrating the supernatant to 1/2 - 1/10 its original volume, cooling the concentrate and allowing it to stand until a precipitate forms. The precipitate is Teichomycin A₂ which is collected by standard separation techniques such as filtration.

The following examples illustrate the practice of the present invention but should not be construed as limiting its scope.

Example 1

Samples (3 ml each) from whole culture fermentation broth (pH = 6.8-7) of Actinoplanes teichomyceticus were mixed with volumes of acetonitrile to give from 20 to 60 percent by total volume mixtures of the acetonitrile in fermentation broth. After a 5 minute mixing period with continuous agitation, the samples were centrifuged and the supernatant was assayed for Teichomycin A₂ employing standard high pressure liquid chromatography (HPLC) methods employing:

Column: DuPont ZORBAX® 4.6 mm x 25 cm ODS
Flow: 1.8 ml/min.
Solvent: 24.5/75.5 acetonitrile/0.03 M ammonium formate at pH 6.0
Absorbance: 254 mm

The results are listed below:

<table>
<thead>
<tr>
<th>% Acetonitrile (by volume)</th>
<th>Teichomycin A₂ (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>235</td>
</tr>
<tr>
<td>40</td>
<td>263</td>
</tr>
<tr>
<td>30</td>
<td>269</td>
</tr>
<tr>
<td>20</td>
<td>254</td>
</tr>
<tr>
<td>Control (no solvent)</td>
<td>98</td>
</tr>
</tbody>
</table>

Example 2

Employing substantially the same procedures of Example 1, various water miscible solvents contemplated by the present invention were used to extract Teichomycin A₂ from samples of whole culture fermentation broth of Actinoplanes teichomyceticus. The fermentation broth had a pH of 6.0. The results are listed below:
### Solvent Concentration and Teichomycin A2 Concentration

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Teichomycin A2 (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no solvent)</td>
<td>124</td>
</tr>
<tr>
<td>20% acetonitrile</td>
<td>383</td>
</tr>
<tr>
<td>20% acetone</td>
<td>317</td>
</tr>
<tr>
<td>30% acetone</td>
<td>366</td>
</tr>
<tr>
<td>40% acetone</td>
<td>392</td>
</tr>
<tr>
<td>50% acetone</td>
<td>303</td>
</tr>
<tr>
<td>20% n-propanol</td>
<td>328</td>
</tr>
<tr>
<td>30% n-propanol</td>
<td>445</td>
</tr>
<tr>
<td>40% n-propanol</td>
<td>487</td>
</tr>
<tr>
<td>50% n-propanol</td>
<td>318</td>
</tr>
<tr>
<td>10% MEK*</td>
<td>215</td>
</tr>
<tr>
<td>15% MEK*</td>
<td>256</td>
</tr>
<tr>
<td>20% MEK*</td>
<td>325</td>
</tr>
<tr>
<td>25% MEK*</td>
<td>379</td>
</tr>
</tbody>
</table>

*MEK represents methyl ethyl ketone*

The supernatant fluids containing high levels of Teichomycin A2 from Examples 1 and 2 above are then treated to isolate the Teichomycin A2. The supernatant fluids are concentrated from 1/2 to 1/10 their original volume, cooled and allowed to stand until a precipitate forms. The precipitate, containing Teichomycin A2, is recovered by filtration from the supernatant.

The Teichomycin A2 recovered employing the procedures of the present invention is then used for known uses, such as, an antibiotic.

### Claims

1. A method of extracting Teichomycin A2 from a whole culture fermentation broth containing a mycelial mass comprising
   (a) mixing the broth containing the mycelial cake with an effective amount of a water miscible solvent to accumulate Teichomycin A2 in the broth/solvent liquid;
   (b) separating the broth/solvent liquid containing accumulated Teichomycin A2 from the mycelial mass; and
   (c) precipitating and separating Teichomycin A2 from the broth/solvent liquid.

2. The method of Claim 1 wherein the water miscible solvent is acetone, n-propanol, acetonitrile, methyl ethyl ketone, dimethylsulfoxide, or mixtures thereof.

3. The method of Claim 1 wherein the solvent is between 5 to 80 percent of the volume of the broth/solvent liquid.

4. The method of Claim 1 wherein the water miscible solvent is mixed with an acidic fermentation broth.

5. The method of Claim 4 wherein the acidity of the fermentation broth is set to a pH of 3 to 4.

6. The method of Claim 4 wherein the acidity of the fermentation broth is adjusted to a desired pH using sulfuric acid, hydrochloric acid, acetic acid, or formic acid.

7. The method of Claim 1 wherein the mixing of the fermentation broth and the water miscible solvent is carried out for a time of at least 2 minutes.

8. The method of Claim 1 wherein the broth/solvent liquid containing the accumulated Teichomycin A2 is separated from the mycelial mass by centrifugation.
9. The method of Claim 1 wherein the broth/ solvent liquid containing the accumulated Teichomycin A₂ is separated from the mycelial mass by filtration.

10. The method of Claim 1 wherein the precipitated Teichomycin A₂ is separated from the broth/ solvent liquid by filtration.

Revendications

1. Un procédé d'extraction de teichomycine A₂ à partir d'un bouillon de fermentation complet contenant une masse mycélienne, comprenant les étapes consistant à
   (a) mélanger le bouillon contenant le gâteau mycélien avec une quantité efficace d'un solvant miscible à l'eau, pour accumuler la teichomycine A₂ dans le liquide bouillon/solvant;
   (b) séparer le liquide bouillon/solvant, contenant la teichomycine A₂ accumulée, de la masse mycélienne; et
   (c) faire précipiter la teichomycine A₂ et la séparer du liquide bouillon/solvant.

2. Le procédé de la revendication 1, dans lequel le solvant miscible à l'eau est l'acétone, le n-propanol, l'acétonitrile, la méthyléthylcétoné, le diméthylsulfoxide ou des mélanges de ceux-ci.

3. Le procédé de la revendication 1, dans lequel le solvent représente entre 5 et 80 pourcent du volume du liquide bouillon/solvant.

4. Le procédé de la revendication 1, dans lequel le solvant miscible à l'eau est mélange à un bouillon de fermentation acide.

5. Le procédé de la revendication 4, dans lequel l'acidité du bouillon de fermentation est fixée à un pH de 3 à 4.

6. Le procédé de la revendication 4, dans lequel on ajuste l'acidité du bouillon de fermentation au pH souhaité en utilisant l'acide sulfurique, l'acide chlorhydrique, l'acide acétique ou l'acide formique.

7. Le procédé de la revendication 1, dans lequel le mélange du bouillon de fermentation et du solvant miscible à l'eau est effectué en une durée d'au moins 2 minutes.

8. Le procédé de la revendication 1, dans lequel le liquide bouillon/solvant contenant la teichomycine A₂ accumulée est séparé de la masse mycélienne par centrifugation.

9. Le procédé de la revendication 1, dans lequel le liquide bouillon/solvant contenant la teichomycine A₂ accumulée est séparé de la masse mycélienne par filtration.

10. Le procédé de la revendication 1, dans lequel la teichomycine A₂ précipitée est séparée du liquide bouillon/solvant par filtration.

Ansprüche

1. Verfahren zur Extraktion von Teichomycin A₂ aus einer Vollkultur-Fermentationsbrühe, enthaltend eine mycelische Masse, umfassend:
   (a) Mischen der Brühe, enthaltend den mycelischen Kuchen, mit einer wirksamen Menge eines Wasser-mischbaren Lösungsmittels, um Teichomycin A₂ in der Brühe/Lösungsmittel-Flüssigkeit zu akkumulieren;
   (b) Trennen der Brühe/Lösungsmittel-Flüssigkeit, enthaltend akkumuliertes Teichomycin A₂, von der mycelischen Masse; und
   (c) Füllen und Trennen von Teichomycin A₂ aus der Brühe/Lösungsmittel-Flüssigkeit.

2. Verfahren nach Anspruch 1, worin das Wasser-mischbare Lösungsmittel Aceton, n-Propanol, Acetonitril, Methyléthylketon, Dimethylsulfoxid oder Mischungen davon ist.
3. Verfahren nach Anspruch 1, worin das Lösungsmittel 5 bis 80 % des Volumens der Brühe/Lösungsmittel-Flüssigkeit darstellt.

4. Verfahren nach Anspruch 1, worin das Wasser-mischbare Lösungsmittel mit einer sauren Fermentationsbrühe gemischt wird.

5. Verfahren nach Anspruch 4, worin die Azidität der Fermentationsbrühe auf einen pH von 3 bis 4 eingestellt wird.


7. Verfahren nach Anspruch 1, worin das Mischen der Fermentationsbrühe und des Wasser-mischbaren Lösungsmittels über einen Zeitraum von mindestens 2 Minuten ausgeführt wird.

8. Verfahren nach Anspruch 1, worin die Brühe/Lösungsmittel-Flüssigkeit, enthaltend das akkumulierte Teichomycin A₂, von der mycelischen Masse durch Zentrifugieren getrennt wird.

9. Verfahren nach Anspruch 1, worin die Brühe/Lösungsmittel-Flüssigkeit, enthaltend das akkumulierte Teichomycin A₂, von der mycelischen Masse durch Filtration getrennt wird.

10. Verfahren nach Anspruch 1, worin das präzipitierte Teichomycin A₂ von der Brühe/Lösungsmittel-Flüssigkeit durch Filtration abgetrennt wird.