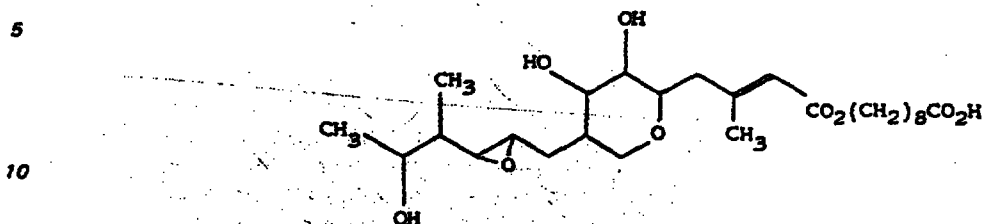


Lithium pseudomonate process for its isolation and its hydrolysis

This invention relates to a novel salt of an antibacterial compound and to a process for its preparation.

Pseudomonic acid is the compound of formula (I):



This compound is disclosed in British Patent No. 1,395,907 as having antibacterial activity and it is therefore of value in the treatment of bacterial infections in man and animals. The method described in Patent No. 1,395,907 for the preparation of pseudomonic acid comprises isolating a mixture containing the principle active acid components from a culture medium on or in which *Pseudomonas fluorescens* has been grown under aerobic conditions and thereafter separating the acid from the mixture of chromatography.

It has now been found that pseudomonic acid can be isolated directly from an extracted solution of the fermentation broth, in the form of its lithium salt.

The lithium salt of pseudomonic acid (referred to herein as lithium pseudomonate) is a novel compound and forms one aspect of the present invention. Although British Patent No. 1,395,907 discloses the sodium salt of pseudomonic acid, it does not disclose or foreshadow the use of the lithium salt. Whereas the sodium salt is an amorphous and hygroscopic compound, surprisingly lithium pseudomonate is stable, crystalline and non-hygroscopic. In addition, the lithium salt is very much less soluble than the sodium salt. These properties could not have been predicted from Patent No. 1,395,907 and make lithium pseudomonate a useful intermediate in the isolation or purification of pseudomonic acid.

Accordingly, in a second aspect the present invention provides a process for isolation of lithium pseudomonate from a crude preparation containing pseudomonic acid, which process comprises extracting the crude preparation into a polar, organic, water-immiscible solvent in which pseudomonic acid is soluble, treating the extract with a lithium salt which is at least slightly soluble in the water-immiscible solvent, and separating the resulting lithium pseudomonate.

The lithium pseudomonate may then be converted into the free acid, pseudomonic acid, or a different salt thereof, by conventional techniques, such as hydrolysis.

The process of this invention is particularly suitable for isolating pseudomonic acid, in the form of its lithium salt, from culture media on or in which pseudomonic acid producing strains of bacteria have been grown. In such a case, the process is most suitably carried out as follows.

A pseudomonic acid producing bacterium, in general a strain of bacterium of the family *Pseudomonas*, is cultured by a standard method under aerobic conditions in or on a suitable culture medium. Such culture media are generally known, and contain inorganic salts and sources of assimilable nitrogen and carbon. Most suitably the bacterium used is *Pseudomonas fluorescens*. One suitable publicly available strain is *Pseudomonas fluorescens* NCIB 10586. The microorganism is allowed to grow until a suitable quantity of pseudomonic acid is present in the culture medium. Solid particles may then be removed from the medium by filtration or centrifugation to produce a clear liquor. The pH of the clear liquor is adjusted to pH 3.0—5.0, suitably about pH 4.5.

This acidified aqueous solution is then extracted into a polar, organic, water-immiscible solvent. Aprotic solvents are preferred. The solvent should have sufficient polarity to adequately dissolve the pseudomonic acid. Suitable solvents include ethyl acetate, butanol, methyl isobutyl ketone (MIBK) and methylene dichloride. Alternatively, mixed solvents may be employed such as ether containing about 5% ethanol. A preferred solvent is MIBK.

The volume of solvent used for the extraction should be kept to a minimum whilst ensuring a good physical separation between the two phases. When extracting a culture liquor a suitable volume is 1/2 to 1/6, preferably 1/4 of the volume of the liquor to be extracted.

It is often advantageous to wash the extract with brine, for example using a volume of 1/10 that of the extract, in order to reduce the water content of the extract.

The next step of the process is to treat the extract with a lithium salt. The lithium salt employed should ideally be more soluble in the solvent than lithium pseudomonate. It is preferably the lithium salt of an organic carboxylic acid, for example a salt of an alkanic acid of formula (II):



wherein R is an alkyl group, containing for example from 1 to 20 carbon atoms, preferably from 1 to 8 carbon atoms.

Examples of suitable lithium salts include the acetate, propionate or hexanoate, a preferred salt being lithium 2-ethylhexanoate.

- 5 The lithium salt may be added to the extract in solid form, but it is preferable to add it as a concentrated solution in a co-solvent which dissolves the lithium salt but which does not substantially solubilise lithium pseudomonate. Such co-solvents include ethanol and, preferably, methanol.

In general it is preferred to use approximately a 10% excess of lithium salt and to add a solution of the salt slowly to the extract. In most cases, the lithium pseudomonate will crystallise out, a process
10 which is facilitated by cooling to about 0—5°C. The lithium pseudomonate may be separated by conventional techniques. It may be advantageous, after separating a first crop of lithium pseudomonate, to concentrate and separate a second crop.

Because lithium pseudomonate is not hygroscopic, it can be dried in an air oven. This is not possible with sodium pseudomonate, or even with pseudomonic acid itself which is difficult to dry in
15 other than a vacuum oven, or very carefully at low temperature.

The lithium pseudomonate can be converted into other salts or the free acid itself by conventional techniques, for example by acidification and extraction into organic solvent.

The invention is illustrated with reference to the following Examples.

Example 1

Isolation of Lithium Pseudomonate

A culture medium containing pseudomonic acid was acidified to pH 4.5 using 20% hydrochloric acid and extracted with 1/4 of its volume of methyl isobutyl ketone (MIBK). The MIBK extract was washed with 1/10 of its volume of saturated brine. The pseudomonic acid content at this state was
25 2,500 µg/ml, and the water content was 1.0%.

To 10 l of the MIBK extract was added dropwise a solution of 9.0 g of lithium ethyl hexanoate in 35 ml methanol over a period of 0.5 hours at ambient temperature and the mixture was stirred for a further one hour at 20°C and then one hour at 5°C. The precipitated product was isolated by filtration, washed with MIBK and air dried at 50°C to give lithium pseudomonate, mp 169°C, yield 26.5 g (90%),
30 purity 87%.

Example 2

Conversion to Pseudomonic Acid

10 g of lithium pseudomonate from Example 1 were dissolved in 200 ml of water. MIBK (85 ml)
35 was added and the pH adjusted to 4.0 with 4N hydrochloric acid. The upper MIBK layer was separated, dried over anhydrous sodium sulphate, and the free acid was precipitated by the addition of 130 ml of heptane. After cooling to 5°C, pseudomonic acid was isolated by filtration. Mp 77—78°C, yield 8.0 g (85%), purity 90%.

40 Claims

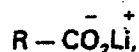
1. Lithium pseudomonate.

2. A process for isolation of lithium pseudomonate from a crude preparation containing pseudomonic acid, which process comprises extracting the crude preparation into a polar, organic,
45 water-immiscible solvent in which pseudomonic acid is soluble, treating the extract with a lithium salt which is at least slightly soluble in the water-immiscible solvent, and separating the resulting lithium pseudomonate.

3. A process as claimed in claim 2 wherein the crude preparation containing pseudomonic acid is a culture medium on or in which pseudomonic acid producing strains of bacteria have been grown.

50 4. A process as claimed in claim 3 wherein the bacterium is *Pseudomonas fluorescens*.

5. A process as claimed in any one of claims 2 to 4 wherein the lithium salt with which the extract is treated is an alkanonic acid of formula



55 wherein R is an alkyl group containing from 1 to 20 carbon atoms.

6. A process as claimed in claim 5 wherein the lithium salt is lithium 2-ethylhexanoate.

7. A process for the preparation of pseudomonic acid which process comprises the hydrolysis of lithium pseudomonate.

60 8. A process as claimed in claim 7 wherein the lithium pseudomonate is prepared by a process as claimed in any one of claims 2 to 6.



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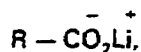
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Revendications

1. Psèudomonate de lithium.
2. Procédé pour isoler le pseudomonate de lithium à partir d'une préparation brute contenant de l'acide pseudomonique, caractérisé en ce qu'on extrait la préparation brute dans un solvant organique polaire non miscible à l'eau dans lequel l'acide pseudomonique est soluble, on traite l'extraite avec un sel de lithium qui est au moins légèrement soluble dans le solvant non miscible à l'eau, et on sépare le pseudomonate de lithium résultant.
3. Procédé suivant la revendication 2, caractérisé en ce que la préparation brute contenant de l'acide pseudomonique est un milieu de culture sur ou dans lequel des souches de bactéries produisant de l'acide pseudomonique ont été développées.
4. Procédé suivant la revendication 3, caractérisé en ce que la bactérie est *Pseudomonas fluorescens*.
5. Procédé suivant la revendication 2 à 4, caractérisé en ce que le sel de lithium avec lequel l'extrait est traité est un acide alcanoïque de formule



dans laquelle R est un groupe alcoyle contenant de 1 à 20 atomes de carbone.

6. Procédé suivant la revendication 5, caractérisé en ce que le sel de lithium est de la 2-éthylhexanoate de lithium.
7. Procédé pour la préparation d'acide pseudomonique, caractérisé en ce qu'on effectue l'hydrolyse de pseudomonate de lithium.
8. Procédé suivant la revendication 7, caractérisé en ce qu'on prépare le pseudomonate de lithium par un procédé suivant l'une quelconque des revendications 2 à 6.

Patentansprüche

1. Lithium-pseudomonat.
2. Verfahren zur Isolierung von Lithium-pseudomonat aus einem Pseudomonsäure enthaltenden Rohpräparat, die Maßnahme umfassend, daß das Rohpräparat in ein polares, organisches, mit Wasser nicht mischbares Lösungsmittel, in dem die Pseudomonsäure löslich ist, extrahiert wird, der Extrakt mit einem Lithiumsalz, das in dem mit Wasser nicht mischbaren Lösungsmittel zumindest schwach löslich ist, behandelt wird und das entstandene Lithium-pseudomonat abgetrennt wird.
3. Verfahren gemäß Anspruch 2, wobei das die Pseudomonsäure enthaltende Rohpräparat ein Kulturmedium ist, in oder auf dem Pseudomonsäure-produzierende Bakterienstämme kultiviert worden sind.
4. Verfahren gemäß Anspruch 3, wobei das Bakterium *Pseudomonas fluorescens* ist.
5. Verfahren gemäß der Ansprüche 2 bis 4, wobei das Lithiumsalz, mit dem der Extrakt behandelt wird, eine Alkancarbonsäure der Formel $R-CO_2Li^+$ ist, in der R ein Alkylrest mit 1 bis 20 Kohlenstoffatomen ist.
6. Verfahren gemäß Anspruch 5, wobei das Lithiumsalz Lithium-2-äthylhexanoat ist.
7. Verfahren zur Herstellung von Pseudomonsäure, die Maßnahme der Hydrolyse des Lithium-pseudomonats umfassend.
8. Verfahren gemäß Anspruch 7, wobei das Lithium-pseudomonat in einem Verfahren gemäß einem der Ansprüche 2 bis 6 hergestellt wird.



* C L M S *