PATENT SPECIFICATION

734,232

Date of Application and filing Complete Specification: April 22, 1953.

No. 11122/53.

Application made in Austria on April 22, 1952.

Application made in Austria on April 29, 1952.

Complete Specification Published: July 27, 1955.

Index at acceptance:—**Class 2(3)**, AA1B, AA1C(1B:2B), AA2C1.

COMPLETE SPECIFICATION

Production of Penicillins

We, BIOCHEMIE GESELLSCHAFT MIT BESCHRÄNKTER HAFTUNG, of 11, Ing. Etzelstrasse, Innsbruck, Tyrol, Austria, a Body Corporate organised under the Laws of Austria, 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 relates to a new penicillin compound and its production.

It has long been known that the various penicillins produced by mould-growth procedures are quite unstable in the presence of acids, and that when such penicillins are exposed even to mild acids they rapidly undergo degradation and loss of antibiotic activity. This instability towards acids has serious disadvantages, for not only does it increase the difficulty of penicillin purification, but it is an obstacle in the provision of stable pharmaceutical preparations of penicillin, and limits the usefulness of oral administration of penicillin.

The novel penicillin compound of the invention is solid crystalline phenoxymethyl penicillin acid, which displays a remarkable stability against acids. The most surprising acid stability of this new acid is well illustrated by the experimental observation that whereas penicillin G has a half-life potency value of only 18 minutes at a pH of 2.1 and a temperature of 24° C., phenoxymethyl penicillin acid shows only very slight loss of potency upon exposure to acid conditions of pH 1 to pH 2 at 24° C. for as long as 48 hours.

The surprising acid stability of the new penicillin acid makes the compound of especial utility for oral administration, for unlike the ordinary penicillins, the new penicillin acid is not destroyed by the acid content of the stomach, but can pass through the stomach without appreciable loss of potency into the intestine where it can be absorbed. The suitability of the penicillin acid of this invention for oral administration has been demonstrated by numerous tests carried out in both experimental animals and human subjects. In the tests, the penicillin acid was usually administered as a compressed tablet, but other suitable forms of administration can be used, such including filled capsules, and aqueous and oleginous suspensions.

Specification No. 643,514 describes a method of producing a novel penicillin which comprises growing a penicillin - producing mould in association with a culture medium containing nutrient material and an effective amount less than about one per cent. of a precursor compound represented by the formula:

$$R(CH_2)_n—R^1$$

wherein n represents an odd integer from 1 to 5 inclusive; R represents an organic radical other than a butene-1-yl, butene-2-yl, *n*-butyl, *n*-hexyl, phenyl or a *p*-hydroxyphenyl radical, and R^1 represents a carboxylic acid group or functional derivative thereof, such as, a carboxy, carboxy-ester, carboxy-salt or carboxyamide group; a carbinol; carbinylamine; acetal; or aldehyde group, R and R^1 not being the same in any one compound. It is stated in the said Specification that R may represent the phenoxy radical attached to the

$$—(CH_2)_nR^1$$

portion of the precursor compound by oxygen. According to Example 18 of the said Specification the sodium salt of phenoxymethyl penicillin (therein named phenoxypenicillin) is prepared by growing a penicillin mould of the notatum-chrysogenum group in the presence of N-(2-hydroxyethyl) - phenoxyacetamide, and then isolating as the sodium salt the phenoxymethyl penicillin which is produced.

According to the present invention phenoxymethyl penicillin acid is recovered from a solution of an alkali metal salt of phenoxymethyl penicillin acid, which may be a crude solution of the salt produced by the process of Specifi-

[*Price 3s. 0d.*]

cation No. 643,514, by adding a mineral acid, for example hydrochloric acid, to the solution to precipitate the desired acid in solid crystalline form. The precipitated crystalline acid is then filtered off and dried. The free acid form of phenoxymethyl penicillin, which is only slightly soluble in water, melts at about 120—128° C.

Alternatively the desired free acid may be prepared by making use of a process of the kind described in Specification No. 643,514, comprising growing a penicillin - producing mould in association with a culture medium and a precursor compound represented by the formula

$$C_6H_5—O—CH_2—R^1$$

where $C_6H_5—O—CH_2—$ is the phenoxy methyl radical and R^1 represents a carboxylic acid group or functional derivative thereof, such as, a carboxy, carboxy-ester, carboxy-salt or carboxy-amide group; a carbinol; carbinylamine; acetal; or aldehyde group, and then recovering the desired free acid by acidifying the clarified culture medium with a mineral acid to approximately pH 2.1, extracting the acidified material with a water-immiscible organic penicillin solvent, for example chloroform, butyl acetate or amyl acetate, and recovering solid crystalline phenoxymethyl penicillin acid from the organic solvent extract. Especially when chloroform is used, because of the favourable chloroform-water distribution ratio of the phenoxymethyl penicillin with respect to that of the precursor compound, the bulk of the phenoxymethyl penicillin is extracted into the chloroform phase whereas most of the precursor compound remains dissolved in the water phase.

Precursor compounds which may be employed include phenoxyacetic acid and β-phenoxyethanol.

The following example illustrates the production of the new penicillin acid.

A culture medium is prepared consisting of 4 gm. of phenoxyacetic acid, 200 cc. of brewers yeat autolysis product (containing 20 gm. of nitrogen per litre), and 1800 cc. of a stock culture medium having the following compositions:

$FeSO_4.7H_2O$	0.01	per cent.
H_3PO_4	0.4	„ „
$MnSO_4.H_2O$	0.025	„ „
$CaCl_2$	0.01	„ „
Lactose	3.0	„ „
Glucose	1.0	„ „

The medium is placed in a 5-litre flask, the medium is inoculated with the mould, air is supplied to the medium at a rate of 2 litres per minute, and the mixture is fermented at 24° C. for a period of 7 days, sterile conditions being maintained as is customary.

A total of 3 litres of culture medium obtained as described above, containing about 1200 units of penicillin activity per ml. as determined by iodometric assay, are clarified by filtration, are acidified to pH 2.1 and are extracted with 500 cc. of chloroform. The chloroform solution is decolorised with activated carbon and is extracted with buffer solution. The buffer is acidified to pH 2.1 and is extracted with 50 cc. of chloroform, thereby producing a chloroform solution containing 63,400 units of penicillin activity per cc. of solution. The chloroform is evaporated to dryness yielding 1.9 gm. of solid phenoxymethyl penicillin acid which assays about 1500 units of activity per milligram. The penicillin acid is further purified by taking it up in sufficient 1 per cent. aqueous sodium hydroxide solution to produce a neutral solution, and precipitating the penicillin acid by the addition of sufficient hydrochloric acid to bring the pH of the mixture to about pH 1.7. The precipitated penicillin acid is filtered off and washed with about 100 cc. of cold water. 1.5 Gm. of phenoxymethyl penicillin are obtained which upon iodometric assay give an assay value of 1690 units/mg.

The free acid of phenoxymethyl penicillin can also be obtained as follows:

The chloroform solution as described, containing 63400 units of penicillin activity per cc. of solution was dried with Na_2SO_4 and the penicillin was precipitated by an addition of 16 cc. of 0.5 molar butanolic potassium acetate solution. 2.07 Mg. of potassium-phenoxymethyl penicillin are obtained which upon iodometric assay give an assay value of 1310 units/mg. The potassium - phenoxymethyl penicillin was dissolved in 50 cc. of distilled water and the solution was acidified by the addition of sufficient hydrochloric acid to bring the pH of the mixture to about pH 1.5. The precipitated penicillin acid is filtered off and washed with water. 1.4 Gm. of phenoxymethyl penicillin are obtained which upon iodometric assay give an assay value of 1690 units/mg.

What we claim is:—

1. Solid crystalline phenoxymethyl penicillin acid.

2. A process for the production of solid crystalline phenoxymethyl penicillin acid, which comprises adding a mineral acid to a solution of an alkali metal salt of phenoxymethyl penicillin acid to precipitate the desired free acid in solid crystalline form.

3. A process for the production of solid crystalline phenoxymethyl penicillin acid, which comprises growing a penicillin-producing mould in association with a culture medium and a precursor compound represented by the formula:

$$C_6H_5—O—CH_2—R^1$$

where $C_6H_5—O—CH_2$ is the phenoxymethyl

cation No. 643,514, by adding a mineral acid, for example hydrochloric acid, to the solution to precipitate the desired acid in solid crystalline form. The precipitated crystalline acid is then filtered off and dried. The free acid form of phenoxymethyl penicillin, which is only slightly soluble in water, melts at about 120—128° C.

Alternatively the desired free acid may be prepared by making use of a process of the kind described in Specification No. 643,514, comprising growing a penicillin - producing mould in association with a culture medium and a precursor compound represented by the formula

$$C_6H_5—O—CH_2—R^1$$

where $C_6H_5—O—CH_2—$ is the phenoxy methyl radical and R^1 represents a carboxylic acid group or functional derivative thereof, such as, a carboxy, carboxy-ester, carboxy-salt or carboxy-amide group; a carbinol; carbinylamine; acetal; or aldehyde group, and then recovering the desired free acid by acidifying the clarified culture medium with a mineral acid to approximately pH 2.1, extracting the acidified material with a water-immiscible organic penicillin solvent, for example chloroform, butyl acetate or amyl acetate, and recovering solid crystalline phenoxymethyl penicillin acid from the organic solvent extract. Especially when chloroform is used, because of the favourable chloroform-water distribution ratio of the phenoxymethyl penicillin with respect to that of the precursor compound, the bulk of the phenoxymethyl penicillin is extracted into the chloroform phase whereas most of the precursor compound remains dissolved in the water phase.

Precursor compounds which may be employed include phenoxyacetic acid and β-phenoxyethanol.

The following example illustrates the production of the new penicillin acid.

A culture medium is prepared consisting of 4 gm. of phenoxyacetic acid, 200 cc. of brewers yeat autolysis product (containing 20 gm. of nitrogen per litre), and 1800 cc. of a stock culture medium having the following compositions:

$FeSO_4.7H_2O$	0.01	per cent.
H_3PO_4	0.4	" "
$MnSO_4.H_2O$	0.025	" "
$CaCl_2$	0.01	" "
Lactose	3.0	" "
Glucose	1.0	" "

The medium is placed in a 5-litre flask, the medium is inoculated with the mould, air is supplied to the medium at a rate of 2 litres per minute, and the mixture is fermented at 24° C. for a period of 7 days, sterile conditions being maintained as is customary.

A total of 3 litres of culture medium obtained as described above, containing about 1200 units of penicillin activity per ml. as determined by iodometric assay, are clarified by filtration, are acidified to pH 2.1 and are extracted with 500 cc. of chloroform. The chloroform solution is decolorised with activated carbon and is extracted with buffer solution. The buffer is acidified to pH 2.1 and is extracted with 50 cc. of chloroform, thereby producing a chloroform solution containing 63,400 units of penicillin activity per cc. of solution. The chloroform is evaporated to dryness yielding 1.9 gm. of solid phenoxymethyl penicillin acid which assays about 1500 units of activity per milligram. The penicillin acid is further purified by taking it up in sufficient 1 per cent. aqueous sodium hydroxide solution to produce a neutral solution, and precipitating the penicillin acid by the addition of sufficient hydrochloric acid to bring the pH of the mixture to about pH 1.7. The precipitated penicillin acid is filtered off and washed with about 100 cc. of cold water. 1.5 Gm. of phenoxymethyl penicillin are obtained which upon iodometric assay give an assay value of 1690 units/mg.

The free acid of phenoxymethyl penicillin can also be obtained as follows:

The chloroform solution as described, containing 63400 units of penicillin activity per cc. of solution was dried with Na_2SO_4 and the penicillin was precipitated by an addition of 16 cc. of 0.5 molar butanolic potassium acetate solution. 2.07 Mg. of potassium-phenoxymethyl penicillin are obtained which upon iodometric assay give an assay value of 1310 units/mg. The potassium - phenoxymethyl penicillin was dissolved in 50 cc. of distilled water and the solution was acidified by the addition of sufficient hydrochloric acid to bring the pH of the mixture to about pH 1.5. The precipitated penicillin acid is filtered off and washed with water. 1.4 Gm. of phenoxymethyl penicillin are obtained which upon iodometric assay give an assay value of 1690 units/mg.

What we claim is:—

1. Solid crystalline phenoxymethyl penicillin acid.

2. A process for the production of solid crystalline phenoxymethyl penicillin acid, which comprises adding a mineral acid to a solution of an alkali metal salt of phenoxymethyl penicillin acid to precipitate the desired free acid in solid crystalline form.

3. A process for the production of solid crystalline phenoxymethyl penicillin acid, which comprises growing a penicillin-producing mould in association with a culture medium and a precursor compound represented by the formula:

$$C_6H_5—O—CH_2—R^1$$

where $C_6H_5—O—CH_2$ is the phenoxymethyl

radical and R' represents a carboxylic acid group or functional derivative thereof, such as a carboxy, carboxy-ester, carboxy-salt or carboxy-amide group; a carbinol; carbinylamine; acetal; or aldehyde group, acidifying the clarified culture medium with a mineral acid to approximately pH 2.1, extracting the acidified material with a water-immiscible organic penicillin solvent, for example chloroform, butyl acetate, or amyl acetate, and recovering solid crystalline phenoxymethyl penicillin acid from the organic solvent extract.

4. A process for the production of solid crystalline phenoxymethyl penicillin acid substantially as described with reference to the Example.

ELKINGTON AND FIFE,
Consulting Chemists and Chartered Patent Agents,
Bank Chambers, 329, High Holborn,
London, W.C.1,
Agents for the Applicants.

PUBLISHED BY :-
THE PATENT OFFICE,
25, SOUTHAMPTON BUILDINGS,
LONDON, W.C.2.

* C L M S *