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PATENT SPECIFICATION

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International Classification:—A61k. C07g.

COMPLETE SPECIFICATION

A new Salt of Phenoxymethyl Penicillin and methods for its Production.

I, KNUD ABILDGAARD, formerly Knud Abildgaard-Elling, a Danish Subject, trading as LØVENS KEMISKE FABRIK VED. A. KONGSTED, of 19, Brønshøjvej, Copenhagen, Denmark, do hereby declare the invention, for which I pray that a patent may be granted to me, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to novel penicillin compounds which are sparingly soluble in water and, on account thereof, can be used for the production of pharmaceutical preparations of penicillin which form by parenteral injection, for instance subcutaneously or intramuscularly, a depot from which penicillin is slowly resorbed.

The novel salts of penicillin have other valuable properties which make them suitable for other purposes.

Sparingly soluble penicillin salts are widely used for producing pharmaceutical preparations having a retarded penicillin effect. The salt of penicillin most commonly employed for this purpose is procaine penicillinate which, however, suffers from certain drawbacks. Thus the basic component thereof is in itself an anæsthetic widely used in medical practice, and in some patients allergic effects are noted. In such cases fatal results may be caused by administering the penicillin salt thereof. Other examples of well known penicillin salts sparingly soluble in water are the penicillin salts of substituted alkylendiamines, such as N,N'-dibenzylethylenediamine. However, among other disadvantages, this salt also presents the disadvantage of containing an amine component, which may have undesirable physiological effects.

In the case of the afore-specified salts, it has generally been claimed that they can be produced from various kinds of penicillin, although the products in actual use have generally been the salts of benzyl penicillin.

[Price 3s. 6d.]

It has, however, been known for some time that a great variety of different penicillins may be produced by growing a penicillin-producing mould in association with a culture medium containing a nutrient material and a selected precursor compound, said penicillins being represented by the formula:



where R denotes a member of the class consisting of aliphatic radicals containing from 2 to 6 carbon atoms, phenyl and monosubstituted phenyl.

The free acid of the penicillin of the latter kind in which R is phenyl has recently acquired considerable interest, because it has been found that this free penicillin acid displays a remarkable stability against acids. Consequently this compound is of special utility for oral administration since, unlike the ordinary penicillins, this penicillin is not destroyed by the acid contents of the stomach, but can pass through the stomach without substantial loss of potency into the intestine where it can be absorbed. Since, moreover, the free acid form of the said penicillin is only sparingly soluble in water, it can readily be prepared in solid crystalline form, for instance by merely adding a mineral acid to a solution of an alkali metal salt of the said penicillin.

Although the penicillin having the formula stated above in which R is phenyl, has occasionally been termed phenoxy penicillin in literature, which term is in accordance with the formula given above, it is preferred hereinafter to refer to it as "phenoxymethyl penicillin" and to represent it by the formula:

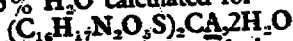


where R' is a phenoxymethyl radical: $C_6H_5-O-CH_2$. This manner of designating

the penicillin in question is analogous to the commonly applied designation of benzylpenicillin for the penicillin which has the formula $C_{16}H_{17}CH_2C_6H_7N_2O_4S$.

- 5 There has now been discovered a hitherto unknown calcium salt of phenoxymethyl penicillin which is characterised in that it has the form of crystals having a solubility in water at 20° C. of about 1.1%. It has no well defined melting point since, unlike the solid crystalline phenoxymethyl penicillin acid which is stated to melt at about 120—128° C., it will be destroyed by heating. Thus, it begins to acquire a yellow coloration at about 175° C. and becomes brownish black at about 200—210° C., a slight development of gas being observable.

On determining the water content of the solid crystalline calcium salt of phenoxymethyl penicillin by the Karl Fischer method, values between 4 and 5% are found; the values observed appear to centre around the value 4.65% H_2O calculated for



- 25 and it is believed that the salt is represented by this formula.

- By producing unsaturated aqueous solutions of the calcium salt of phenoxymethyl penicillin, and subjecting such solutions to freeze-drying in vacuum, solid dry products will be obtained which are not crystalline. By dissolution in water these products will form clear solutions from which, after a short time, the new crystalline calcium salt of phenoxymethyl penicillin will crystallise. The freeze-dried product cannot generally be obtained in an anhydrous state, but a substantial content of water, say 6% as determined by the Karl Fischer titration, will be found therein. As mentioned above, however, the product is not crystalline and moreover it differs from the salt according to the invention by its capability of dissolving in water to form solution from which the crystalline salt will precipitate.

- 45 By drying the freeze-dried calcium salt over phosphorus pentoxide it is possible to reduce its water content to below 0.5% determined as hereinbefore referred to, but still no crystalline product will be obtained.

- 50 The crystalline calcium salt of phenoxymethyl penicillin hereinbefore described can be produced by adding a calcium salt or calcium hydroxide to an aqueous solution of a substance dissociating into phenoxymethyl penicillin ions, allowing the resultant calcium salt to precipitate and removing the precipitate by any suitable known method. Thereby, the concentration of the solution should exceed the solubility of the desired salt in the solvent used. The reaction may be performed in water or an aqueous medium or in an organic solvent in which the free phenoxymethyl penicillin acid or the salt thereof is soluble, provided that the amount of water necessary for the crystallisation is present in the solu-

tion.

When using the free phenoxymethyl penicillin acid in the form of a solution in butyl or amyl acetate, such as is obtained during the commonly used method for concentrating and purifying the crude phenoxymethyl penicillin formed in the culture medium, the said solution may be treated with an aqueous solution or suspension of calcium hydroxide, in the latter case preferably by adding the suspension to the solution of phenoxymethyl penicillin while stirring the mixture vigorously.

When the penicillin acid, for instance in the form of a solution of the said kind, is reacted with a calcium salt, said salt should be a salt of an acid having a lower dissociation constant than that of phenoxymethyl penicillin acid.

When a water-soluble salt of phenoxymethyl penicillin is available, such as the sodium, potassium, or ammonium salt, or an amine salt of the penicillin, such as the cyclohexylamine-salt, it is preferable to produce the calcium salt by mixing an aqueous solution of the phenoxymethyl penicillin salt with an aqueous solution of a water-soluble calcium salt, for example calcium chloride. All the reactions described must be carried out at a sufficiently low temperature to avoid loss of penicillin activity, viz., at a temperature preferably below 40° C.

The invention will be illustrated by the following Examples:—

EXAMPLE 1.

58.5 g of potassium phenoxymethyl penicillinate were dissolved at 20° C in 365 ccs of water to which were previously added 10 ccs of a 2-normal aqueous solution of sodium acetate in order to maintain the pH of the solution between 7 and 8 during the subsequent addition of calcium chloride, which may contain some HCl. Thereafter, 60 ccs of a half-saturated aqueous solution of calcium chloride at 20° C were slowly added whilst stirring. The crystals which precipitated out, were washed with water, and then with acetone and dried in vacuo over phosphorus pentoxide. Yield: 55.0 g. The product assayed 1554 international penicillin units per mg, when determined iodometrically, and it contained 4.60% of water as determined by the Karl Fischer titration. The solubility in water at 20° C was 1.10% and at 0° C 1.09%, the difference being, however, within the reliability of the determination.

EXAMPLE 2.

To a solution of 0.37 g (corresponding to 0.001 mole) sodium phenoxymethyl penicillinate in 6 ccs 80% aqueous ethanol was added a solution of 0.17 g calcium chloride in 2 ccs 80% ethanol. The calcium phenoxymethyl penicillinate was filtered off, washed with water and acetone and dried. Yield: 0.27 g corresponding to 69% of the theoretical.



EXAMPLE 3.

To a solution of 0.37 g (corresponding to 0.001 mole) ammonium phenoxymethyl penicillinate in 4 ccs water was added a suspension of 0.04 g calcium hydroxide in water. The mixture was shaken vigorously for 15 minutes and the calcium phenoxymethyl penicillinate was filtered off, washed with water and dried. Yield: 0.20 g.

EXAMPLE 4.

To a solution of 0.35 g (corresponding to 0.001 mole) phenoxymethyl penicillin in a mixture of 8 ccs amyl acetate and 2 ccs ethanol was added a suspension of 0.04 g calcium hydroxide in 4 ccs water. The mixture was shaken for 15 minutes and the crystals were collected. Yield: 0.25 g corresponding to 64% of the theoretical.

EXAMPLE 5.

A solution of 0.42 g (corresponding to 0.001 mole) diisopropylammonium phenoxymethyl penicillinate in a mixture of 3 ccs water and 2 ccs ethanol was mixed with 0.4 ccs of a saturated aqueous solution of calcium chloride. The calcium phenoxymethyl penicillinate was collected by filtration, washed with water and dried. Yield: 0.30 g corresponding to 77.5% of the theoretical.

EXAMPLE 6.

To a solution of 0.42 g (corresponding to 0.001 mole) tert. butylammonium phenoxymethyl penicillinate in 3 ccs water was added 0.4 ccs of a saturated, aqueous solution of calcium chloride. The yield of calcium phenoxymethyl penicillinate was 0.37 g corresponding to 95.5% of the theoretical.

EXAMPLE 7.

To a solution of 0.41 g (corresponding to 0.001 mole) isopropylammonium phenoxymethyl penicillinate in a mixture of 3 ccs water and 1 cc ethanol was added 0.4 ccs of a saturated, aqueous solution of calcium chloride. The calcium phenoxymethyl penicillinate was collected by filtration, washed with water and dried. Yield: 0.37 g.

EXAMPLE 8.

To a solution of 0.44 g (corresponding to 0.001 mole) N.N-dimethyl- β -hydroxyethylammonium phenoxymethyl penicillinate in 3 ccs water was added 0.4 ccs of a saturated, aqueous solution of calcium chloride. The yield of calcium phenoxymethyl penicillinate was 0.37 g.

EXAMPLE 9.

To a solution of 0.44 g (corresponding to 0.001 mole) 3-methoxypropylammonium phenoxymethyl penicillinate in 3 ccs water was added a saturated aqueous solution of calcium chloride. The yield of calcium phenoxymethyl penicillinate was 0.37 g.

EXAMPLE 10.

To a suspension of 0.45 g cyclohexylammonium phenoxymethyl penicillinate in 5 ccs 96% aqueous ethanol was added a solution of 0.1 g calcium acetate in 1 cc water. A clear

solution resulted and the calcium phenoxymethyl penicillinate began to crystallise. Yield: 0.20 g corresponding to 51.5% of the theoretical.

EXAMPLE 11.

To a solution of 0.35 g phenoxymethyl penicillin in 5 ccs 96% aqueous ethanol was added a solution of 0.1 g calcium acetate in 1 cc water. The calcium phenoxymethyl penicillinate was filtered off, washed with water, acetone and dried. Yield: 0.30 g corresponding to 77.5% of the theoretical.

EXAMPLE 12.

To a suspension of 0.35 g phenoxymethyl penicillin in 2 ccs water was added a solution of 0.3 g calcium acetate in 3 ccs water. The phenoxymethyl penicillin dissolved and calcium phenoxymethyl penicillinate began to crystallise. Yield: 0.36 g corresponding to 93% of the theoretical.

EXAMPLE 13.

To a suspension of 0.35 g phenoxymethyl penicillin in 5 ccs water was added 0.05 g precipitated calcium carbonate. The mixture was shaken vigorously for 30 minutes, whereafter the calcium phenoxymethyl penicillinate was collected by filtration. Yield: 0.30 g corresponding to 77.5% of the theoretical.

EXAMPLE 14.

To a solution of 0.35 g phenoxymethyl penicillin in a mixture of 5 ccs amyl acetate and 1.5 ccs ethanol was added a suspension of 0.05 g precipitated calcium carbonate in 5 ccs water. The mixture was shaken vigorously for one hour and the calcium phenoxymethyl penicillinate was filtered off, washed with water and acetone and dried. Yield: 0.25 g.

EXAMPLE 15.

To a solution of 11.5 g sodium phenoxymethyl penicillinate in 72 ccs water was added dropwise while stirring, a solution of 3.66 g calcium chloride in 12 ccs water. The calcium phenoxymethyl penicillinate was filtered off, washed with water and acetone and dried at 80° C. Yield: 11.1 g corresponding to 95.7% of the theoretical.

Owing to the low solubility of the described calcium salt of phenoxymethyl penicillin a protracted penicillin effect can be obtained by subcutaneous or intramuscular injection of aqueous suspensions of this salt mixed with a suspending and/or dispersing agent. Alternatively, the penicillin salt mixed with the suspending and/or dispersing agent may be put up as such and diluted with sterile water when required for use. In order to increase the chemical stability of the phenoxymethyl penicillin, a content of Ca-ions exceeding that derived from the calcium salt of the phenoxymethyl penicillin may be incorporated with the suspension medium, for instance by adding to the suspension a soluble salt of calcium other than the phenoxymethyl penicillin salt for instance calcium chloride. This reduces the solubility of the calcium salt of phenoxymethyl

- penicillin and in consequence thereof augments its chemical stability. In order to prevent precipitation of the suspended crystals in the form of a layer or cake which cannot readily be re-suspended in the suspension medium, agents capable of increasing the viscosity of the suspension medium may be added. Such agents are well known, examples of them being carboxymethyl cellulose, polyvinyl pyrrolidone and gelatin. Furthermore, to facilitate re-suspension a surface active substance may be added, for instance lecithin, surface active amine salts, such as cetyl pyridinium chloride, or the substance known under the Registered Trade Mark "Tweens."

Examples of suspensions are:

EXAMPLE 16.

- An aqueous solution of polyvinyl pyrrolidone containing 20 g per 100 ccs is prepared and heated to 120° C. for 20 minutes in an autoclave. To the solution thus sterilised is added a growth-inhibiting agent, for instance phenyl mercuric acetate in such an amount that the concentration will be 1:20000.

- Sterile calcium salt of phenoxymethyl penicillin containing particles of sizes between the limits 20 and 60 μ is passed under aseptic conditions through a sieve the mesh width of which is 0.13 mm. 30 g of the substance that have passed the sieve are stirred into 50 ccs of the polyvinyl pyrrolidone solution until uniform suspension has been attained, after which more polyvinyl pyrrolidone solution is added until the volume is 100 ccs and the suspension is homogenised in a homogenisation apparatus.

EXAMPLE 17.

- 1.2 g lecithin are dissolved in 60 ccs ether and the solution is sterilised through a glass filter G 5. 60 g sterile calcium phenoxymethyl penicillinate having a particle size of 20—60 μ are stirred into the solution until all the particles are moistened therewith and subsequently the ether is removed by evaporation in vacuo or at slight heat. The dried substance is passed aseptically through a sieve the mesh width of which is 0.13 mm.

- 2.7 g carboxymethyl cellulose are dissolved in water with vigorous agitation in a Waring-blender and the solution obtained is heated to 120° C. for 20 minutes after which phenyl mercuric acetate is added to make a concentration of this substance of 1:20000 in the solution. The viscosity measured by a rotation viscosimeter should be 35—40 cP at 20° C.

- 30.0 g of the sterile calcium salt of phenoxymethylpenicillin treated with lecithin as described above is stirred into 50 ccs of the sterile solution of carboxymethyl cellulose to form a uniform suspension, after which more of the solution is added to form 100 ccs of the solution which is then homogenised in a homogenisation apparatus. The resulting suspension contains 300 mg of the calcium salt per cc.

- By using such suspensions for intramuscular

injections in normal persons the following penicillin concentrations in the serum are found after the intervals stated, each injection containing 200 mg of the calcium salt of phenoxymethyl penicillin, the concentrations of penicillin in the serum measured after injection of 300 mg procaine benzyl penicillin being given for comparison.

TABLE I.

phenoxymethyl penicillin, given as calcium salt (10⁻⁶ g per cc serum)

Sub-jects	1 h	2 h	4 h	8 h	12 h	24 h
A	1.9	1.1	0.2	0.06	0.06	0.06
B	0.7	0.6	0.3	0.25	0.15	0.06
C	1.7	1.1	0.5	0.08	0.06	0.06
D	1.8	0.8	0.1	0.07	0.06	0.06
E	0.3	0.2	0.2	0.28	0.17	0.06
Average	1.3	0.76	0.26	0.15	0.10	0.06
300 mg procaine penicillin	0.59	0.42	0.40	0.45	0.38	0.04

Table I shows that during the first two hours the calcium salt produces higher penicillin concentrations in the serum than does the procaine salt of benzyl penicillin and that the calcium salt has a protracted effect a little less pronounced than that of procaine benzyl penicillinate.

Preparations for oral use can be produced in the well known way with pharmaceutical carriers or diluents and in well known forms such as pills, tablets, or capsules. An example showing a manner of producing a tablet suitable for oral use is as follows:

EXAMPLE 18.

200 g of the new calcium salt of phenoxymethyl penicillin are thoroughly mixed with 60 g potato starch and 20 g kaolin and the mixture is granulated with an aqueous solution of 4% gelatin after which the granulate is completely dried. It is then mixed with 35 g potato starch and 35 g talcum after which the mixture is analysed and compressed to tablets of such a weight, that each tablet will contain 200 mg of phenoxymethyl penicillin.

The serum concentrations in normal persons after oral administration of one such tablet are compared in the following Table II with the serum concentration obtained by administering tablets of the potassium salt of phenoxymethyl penicillin in the amount of one tablet containing 200 mg of phenoxymethyl penicillin.

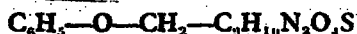
TABLE II.

Phenoxymethyl penicillin, given as calcium salt
(10⁻⁴ g per cc serum).

Sub- jects	½ h	1 h	2 h	4 h	6 h	7 h
A	0.11	1.3	1.4	0.20	—	—
B	0.24	1.2	0.9	0.15	—	—
C	0.22	1.8	2.4	0.28	0.04	0.03
D	0.08	1.3	0.61	0.12	—	—
E	0.35	1.8	0.52	0.10	—	—
Average	0.20	1.5	1.2	0.17	—	—

What I claim is:—

1. A crystalline calcium salt of phenoxy-methyl penicillin corresponding to the formula Pen_2Ca , where Pen represents the phenoxy-methyl penicillin ion:



- said crystals containing 4 to 5% water of crystallisation.

2. A method for producing the calcium salt claimed in Claim 1, which comprises adding a calcium salt or calcium hydroxide to a

solution of a substance dissociating into phenoxymethyl penicillin ions in the presence of water, allowing the resultant calcium salt to precipitate and removing the precipitate by any suitable known method.

3. Method of producing the crystalline salt of phenoxymethyl penicillin claimed in Claim 1, substantially as described with reference to the foregoing Examples.

4. A penicillin preparation comprising the compound claimed in Claim 1, together with a pharmaceutical carrier or diluent suitable for oral administration.

5. A penicillin preparation in tablet form comprising a tableted mixture of the compound claimed in Claim 1.

6. An injectable penicillin preparation comprising an aqueous suspension of the compound claimed in Claim 1.

7. A penicillin preparation suitable for injection after dilution with sterile water, comprising a compound as claimed in Claim 1, and a suspending and/or dispersing agent.

8. A penicillin preparation as claimed in Claim 6, in which the aqueous medium contains an amount of additional calcium ions exceeding that derived from the compound claimed in Claim 1.

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Agent for the Applicant.

PROVISIONAL SPECIFICATION

No. 29909 A.D. 1955

A new Salt of Phenoxymethyl Penicillin and methods for its Production

- I, KNUD ABILDGAARD-ELLING, a Danish Subject, trading as LØVENS KEMISKE FABRIK VED A. KONGSTED, of 19, Brønshøjvej, Copenhagen, Denmark, do hereby declare this invention to be described in the following statement:—

- This invention relates to a new salt of penicillin and to methods of producing the same.

- Specification No. 643,514 describes a method of producing novel penicillins which comprises growing a penicillin-producing mould in a culture medium containing an effective amount of less than about one per cent, of a precursor compound represented by the formula: $\text{R}(\text{CH}_2)_n-\text{R}'$, 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-hydroxy-phenyl radical, and R' represents a carboxylic acid group of functional derivative thereof, such as, a carboxy, carboxy-ester, carboxy-salt or carboxy-amide group; a carbinol; a carbinylamine; acetal; or aldehyde group, R and R' 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 $-(\text{CH}_2)_n\text{R}'$ portion of the precursor compound by oxygen.

According to Example 18 of the said Specification the sodium salt of phenoxymethylpenicillin (which in the said Example is denominated "phenoxypenicillin") is prepared by growing a penicillin mould of the notatum-chrysogenum group in the presence of N-(2-hydroxyethyl)-phenoxylacetamide, and subsequently isolating the compound in the form of its sodium salt.

It has now been found that, in contradistinction to the alkali metal and the alkaline earth metal salts of phenoxymethylpenicillin, and also to the salts of the said bases with other penicillins, such as benzylpenicillin, the calcium salt of phenoxymethylpenicillin is only very sparingly soluble in water. It was found that the solubility of the calcium salt of phenoxymethylpenicillin in water at pH = about 7 is about 11 grams per litre at 20° C. Moreover, it was found that the solubility in anhydrous ethanol is 2.8 grams per litre at 20° C., and that the salt after drying in vacuo over phosphorus pentoxide contained 4.21% water.

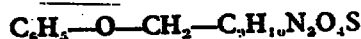
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C	0.22	1.8	2.4	0.28	0.04	0.03
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solution of a substance dissociating into phenoxymethyl penicillin ions in the presence of water, allowing the resultant calcium salt to precipitate and removing the precipitate by any suitable known method.

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It has now been found that, in contradistinction to the alkali metal and the alkaline earth metal salts of phenoxymethylpenicillin, and also to the salts of the said bases with other penicillins, such as benzylpenicillin, the calcium salt of phenoxymethylpenicillin is only very sparingly soluble in water. It was found that the solubility of the calcium salt of phenoxymethylpenicillin in water at pH = about 7 is about 11 grams per litre at 20° C. Moreover, it was found that the solubility in anhydrous ethanol is 2.8 grams per litre at 20° C., and that the salt after drying in vacuo over phosphorus pentoxide contained 4.21% water.



Thus, the salt appears to contain about 2 molecules of water of crystallisation. The calcium salt of phenoxymethylpenicillin has not hitherto been described in the literature.

5 Owing to the low solubility of the calcium salt of phenoxymethylpenicillin, protracted penicillin effects can be obtained by intramuscular injections of suspensions of the said salt. Furthermore, the use of the said calcium
10 salt offers the advantage that the protracted effect can be obtained without employing salts of penicillin with organic bases, for example procaine, which bases in some cases may cause serious, or even deadly, cases of illness due to
15 hypersensitivity towards the organic base.

The calcium salt of phenoxymethylpenicillin can be produced by reacting a solution containing a substance capable of furnishing phenoxymethylpenicillin ions with a calcium
20 salt or with calcium hydroxide. The reaction may be performed in an aqueous medium or in an organic solvent in which the free phenoxymethyl penicillin acid, or the salt thereof is, at least to some extent, soluble, and which may
25 contain certain amounts of water. When the free phenoxymethylpenicillin is reacted with a calcium salt, that salt should be a salt of an acid having a lower dissociation constant than that of penicillin acid. When using the free penicillin acid in the form of a solution of the
30 same in butyl or amyl acetate, such as obtained during the commonly used method for concentrating and purifying the crude penicillin formed in the culture medium, the said solution may be treated with an aqueous solution
35 or suspension of calcium hydroxide, in the last case preferably by adding the suspension to the solution of phenoxymethylpenicillin while vigorously stirring the mixture.

When a water-soluble salt of phenoxymethylpenicillin is available, such as the sodium, potassium or ammonium salt, or an amine salt of the same, such as the salt of cyclohexylamine, it is preferable to produce
40 the calcium salt by mixing an aqueous solution of the penicillin salt with an aqueous solution of a water-soluble calcium salt, for example calcium chloride. It is also possible to produce
45 the calcium salt of phenoxymethylpenicillin by treating an aqueous solution of the ammonium salt of phenoxymethylpenicillin with an aqueous suspension of calcium hydroxide, preferably by slowly adding the suspension to the solution. This method offers the advantage that
50 the ammonia liberated during the reaction can be removed from the reaction mixture by evaporation.

The invention will be illustrated by the following Example:—

EXAMPLE.

58.5 g potassium phenoxymethylpenicillinate were dissolved in 365 ml water to which had previously been added 10 ml of a 2-normal aqueous solution of sodium acetate in order
60 to keep the pH of the solution between 7—8, as the calcium chloride may contain some HCl. Thereafter, 60 ml of a half saturated aqueous solution of calcium chloride was added slowly while stirring. The crystals precipitated out
65 were washed with water, subsequently with acetone and dried in vacuo over phosphorus pentoxide. Yield: 55.0 g. The product contained 1554 international penicillin units per mg, when determined iodometrically.

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This invention, which is complementary with that described in the Provisional Specification of our co-pending Application No. 29909/
85 1955, relates to novel penicillin compounds which are sparingly soluble in water and, on account thereof, can be used for the production of pharmaceutical preparations of penicillin which form by parenteral injection, for instance subcutaneously or intramuscularly, a
90 depot from which penicillin is slowly resorbed.

The novel salts of penicillin have other valuable properties which make them suitable for other purposes.

Sparingly soluble penicillin salts are widely used for producing pharmaceutical preparations having a retarded penicillin effect. The salt of penicillin most commonly employed for this purpose is procaine penicillinate which, however, suffers from certain drawbacks. Thus the basic component thereof is in itself an anaesthetic widely used in medical practice, and in some patients allergic effects are noted. In such cases fatal results may be caused by administering the penicillin salt thereof. Other examples of well-known penicillin salts sparingly soluble in water are the penicillin salts of substituted alkylenediamines, such as N,N'-dibenzylethylenediamine. However, among other disadvantages this salt also presents the disadvantage of containing an amine component, which may have undesirable physiological effects.

In the case of the abovenamed salts it has generally been claimed that they can be produced from various kinds of penicillin, although the products in actual use have generally been the salts of benzyl penicillin.

- 5 It has, however, been known for some time that a great variety of different penicillins may be produced by growing a penicillin-producing mould in association with a culture medium
10 containing a nutrient material and a selected precursor compound, said penicillins being represented by the formula:



- 15 in which R is a member of the class consisting of aliphatic radicals containing from 2 to 6 carbon atoms, phenyl and monosubstituted phenyl.

- 20 The free acid of the penicillin of the latter kind in which R is phenyl has recently acquired considerable interest, because it has been found that this free penicillin acid displays a remarkable stability against acids. Consequently this compound is of special utility for oral administration since, unlike the ordinary penicillins,
25 this penicillin is not destroyed by the acid contents of the stomach, but can pass through the stomach without considerable losses of potency into the intestine where it can be absorbed. Since, moreover, the free acid form of the said penicillin is only slightly soluble in
30 water, it can readily be prepared in solid crystalline form, for instance merely by adding a mineral acid to a solution of an alkali metal salt of the said penicillin.

- 35 Although the penicillin having the formula stated above in which R is phenyl has occasionally been termed phenoxy penicillin in literature which term is in accordance with the formula given above, it is here preferred to call it
40 phoxymethyl penicillin and to represent it by the formula:



- 45 in which R¹ is a phoxymethyl radical: $C_6H_5-O-CH_2$. This manner of designating the penicillin in question is analogous to the commonly applied designation of benzyl-penicillin for the penicillin which has the formula $C_6H_5-CH_2-C_{10}H_{11}N_2O_4S$.

- 50 I have now discovered a hitherto unknown calcium salt of phoxymethyl penicillin which is characterised in that it has the form of crystals having a solubility in water at 20° C. of about 1.1%. It has no well defined melting point since, unlike the solid crystalline
55 phoxymethyl penicillin acid which is stated to melt at about 120—128° C., it will be destroyed by heating. Thus, it begins to take a yellow colour at about 175° C. and becomes brownish black at about 200—210° C., a slight development of gas being observable.

60 On determining the water content of the

solid crystalline calcium salt of phoxymethyl penicillin by the Karl Fischer method, values between 4 and 5% are found; the values observed appear to centre around the value of 4.65% H₂O calculated for $(C_{16}H_{17}N_2O_5)_2Ca \cdot 2H_2O$ and it is believed that the salt is represented by this formula. 65

The present invention accordingly provides a solid crystalline sparingly water-soluble calcium salt of phoxymethyl penicillin. 70

The invention also provides a crystalline calcium salt of phoxymethyl penicillin corresponding to the formula Pen_2Ca , where Pen represents the phoxymethyl penicillin ion: $C_6H_5-O-CH_2-C_{10}H_{11}N_2O_4S$, said crystals containing 4 to 5% water of crystallisation. 75

The crystalline calcium salt of phoxymethyl penicillin can be produced by reacting a solution containing a substance dissociating phoxymethyl penicillin ions with a calcium salt or with calcium hydroxide, the concentration exceeding the solubility of the crystalline calcium salt of phoxymethyl penicillin in the solvent used. The reaction may be performed in water or an aqueous medium or in an organic solvent in which the free phoxymethyl penicillin acid or the salt thereof is soluble, provided that the amount of water necessary for the crystallisation is present in the solution. 80

It has been found that the crystalline calcium salt precipitated from the aforesaid aqueous solutions and having a content of water in the neighbourhood of 4.65% in accordance with the invention does not lose significant amounts of water when dried over phosphorus pentoxide in an exsiccator in vacuo thus additionally indicating that the product is a dihydrate. 85 90 100

When using the free phoxymethyl penicillin acid in the form of a solution of the same in butyl or amyl acetate, such as obtained during the commonly used method for concentrating and purifying the crude phoxymethyl penicillin formed in the culture medium, the said solution may be treated with an aqueous solution or suspension of calcium hydroxide, in the latter case preferably by adding the suspension to the solution of phoxymethyl penicillin while stirring the mixture vigorously. 105 110

When the penicillin acid, for instance in form of a solution of the said kind, is reacted with a calcium salt, this salt should be a salt of an acid having a lower dissociation constant than that of phoxymethyl penicillin acid. 115

When a water-soluble salt of phoxymethyl penicillin is available, such as the sodium, potassium, or ammonium salt, or an amine salt of the penicillin, such as the cyclohexylamine salt, it is preferable to produce the calcium salt by mixing an aqueous solution of the phoxymethyl penicillin salt with an aqueous solution of a water-soluble calcium salt, for example calcium chloride. All the reactions described must be carried out at a sufficiently 120 125

low temperature to avoid loss of penicillin activity, preferably below 40° C.

EXAMPLE.

- 5 58.5 g of potassium phenoxymethyl penicillinate were dissolved at 20° C. in 365 ccs of water to which were previously added 10 ccs of a 2-normal aqueous solution of sodium acetate in order to keep the pH of the solution between 7 and 8 during the subsequent addition of calcium chloride, which may contain some HCl. Thereafter, 60 ccs of a half-saturated aqueous solution of calcium chloride at 20° C. were slowly added under stirring. The crystals, which precipitated out, were washed with water, and then with acetone and dried in vacuum over phosphorus pentoxide. Yield: 55.0 g. The product assayed 1554 international penicillin units pro mg, when determined iodometrically, and it contained 4.60% of water as determined by the Karl Fischer titration. The solubility in water at 20° C. was 1.10% and at 0° C. 1.09%, the difference being, however, within the reliability of the determination.
- 15 The new product of the present invention can be used for oral administration in the form of tablets.
- 20 Such tablets may comprise for example the crystalline calcium phenoxymethyl penicillin

30 dihydrate in admixture with suitable quantities of potato starch, light kaolin, gelatine and talcum.

Alternatively the product of the present invention can be used for subcutaneous or intramuscular injection. In such case the surface of the crystals may for example be covered with a layer of lecithin and the thus covered crystals suspended in a viscous aqueous solution of carboxy methyl cellulose. Micro-organic growth-inhibiting substances such as phenyl mercuric nitrate may be incorporated with the suspension.

Owing to the low solubility of the described crystalline calcium salt of phenoxymethyl penicillin a protracted penicillin effect can be obtained by subcutaneous or intramuscular injection of suspensions of this salt. When using the salt for oral administration free phenoxymethyl penicillin acid will be formed in the stomach in a finer state of division, and thus more readily resorbable from the intestinal tract, than if the free acid had been administered orally as a preparation consisting of previously prepared crystals of the free acid.

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PROVISIONAL SPECIFICATION

No. 37443 A.D. 1955

A new Salt of Phenoxymethyl Penicillin and methods for its Production

- 55 I, KNUD ABILDGAARD-ELLING, a Danish Subject, trading as LØVENS KEMISKE FABRIK VED A. KONGSTED, of 19, Brønshøjvej, Copenhagen, Denmark, do hereby declare this invention to be described in the following statement:—

60 This invention relates to novel penicillin compounds which are sparingly soluble in water and, on account thereof, can be used for the production of pharmaceutical preparations of penicillin which form by parenteral injection, for instance subcutaneously or intramuscularly, a depot from which penicillin is slowly resorbed.

- 65 This invention is also complementary to those described in the Provisional Specifications of our co-pending Applications Nos. 29909/1955 and 34334/1955 and is intended to provide Examples supplementing those disclosed in the Provisional Specifications of said Applications.

EXAMPLE 1.

- 70 To a solution of sodium phenoxymethyl penicillinate (0.37 g corresponding to 0.001 mole) in 80% ethanol (6 ccs) is added a solution of calcium chloride (0.17 g) in 80% ethanol (2 ccs). The calcium phenoxymethyl penicillinate is filtered off, washed with water and acetone and dried. Yield 0.27 g corresponding to 69% of the theoretical.

EXAMPLE 2.

85 To a solution of ammonium phenoxymethyl penicillinate (0.37 g corresponding to 0.001 mole) in water (4 ccs) is added a suspension of calcium hydroxide (0.04 g) in water. The mixture is shaken vigorously for 15 minutes and the calcium phenoxymethyl penicillinate is filtered off, washed with water and dried. Yield 0.20 g.

EXAMPLE 3.

90 To a solution of phenoxymethyl penicillin (0.35 g corresponding to 0.001 mole) in a mixture of amyl acetate (8 ccs) and ethanol (2 ccs) is added a suspension of calcium hydroxide (0.04 g) in water (4 ccs). The mixture is shaken for 15 minutes and the crystals are collected. Yield 0.25 g corresponding to 64% of the theoretical.

EXAMPLE 4.

95 A solution of diisopropylammonium phenoxymethyl penicillinate (0.42 g corresponding to 0.001 mole) in a mixture of water (3 ccs) and ethanol (2 ccs) is mixed with a saturated, aqueous solution of calcium chloride (0.4 ccs). The calcium phenoxymethyl penicillinate is collected by filtration, washed with water and dried. Yield 0.30 g corresponding to 77.5% of the theoretical.

EXAMPLE 5.

100 To a solution of tert. butylammonium

- phenoxymethyl penicillinate (0.42 g corresponding to 0.001 mole) in water (3 ccs) is added a saturated, aqueous solution of calcium chloride (0.4 ccs). The yield of calcium phenoxymethyl penicillinate is 0.37 g corresponding to 95.5% of the theoretical.

EXAMPLE 6.

- To a solution of isopropylammonium phenoxymethyl penicillinate (0.41 g corresponding to 0.001 mole) in a mixture of water (3 ccs) and ethanol (1 cc) is added a saturated, aqueous solution of calcium chloride (0.4 ccs). The calcium phenoxymethyl penicillinate is collected by filtration, washed with water and dried. Yield 0.37 g.

EXAMPLE 7.

- To a solution of N,N-dimethyl- β -hydroxyethylammonium phenoxymethyl penicillinate (0.44 g corresponding to 0.001 mole) in water (3 ccs) is added a saturated, aqueous solution of calcium chloride (0.4 ccs). The yield of calcium phenoxymethyl penicillinate is 0.37 g.

EXAMPLE 8.

- To a solution of 3-methoxypropylammonium phenoxymethyl penicillinate (0.44 g corresponding to 0.001 mole) in water (3 ccs) is added a saturated aqueous solution of calcium chloride. The yield of calcium phenoxymethyl penicillinate is 0.37 g.

EXAMPLE 9.

- To a suspension of cyclohexylammonium phenoxymethyl penicillinate (0.45 g) in 96% ethanol (5 ccs) is added a solution of calcium acetate (0.1 g) in water (1 cc). A clear solution results and the calcium phenoxymethyl penicillinate begins to crystallise. Yield 0.20 g corresponding to 51.5% of the theoretical.

EXAMPLE 10.

- To a solution of phenoxymethyl penicillin (0.35 g) in 96% ethanol (5 ccs) is added a solution of calcium acetate (0.1 g) in water (1 cc). The calcium phenoxymethyl penicillinate is filtered off, washed with water, acetone and dried. Yield 0.30 g corresponding to 77.5% of the theoretical.

EXAMPLE 11.

- To a suspension of phenoxymethyl penicillin (0.35 g) in water (2 ccs) is added a solution of calcium acetate (0.3 g) in water (3 ccs). The phenoxymethyl penicillin dissolves and calcium phenoxymethyl penicillinate begins to crystallise. Yield 0.36 g corresponding to 93% of the theoretical.

EXAMPLE 12.

- To a suspension of phenoxymethyl penicillin (0.35 g) in water (5 ccs) is added precipitated calcium carbonate (0.05 g). The mixture is shaken vigorously for 30 minutes, whereafter the calcium phenoxymethyl penicillinate is collected by filtration. Yield 0.30 g corresponding to 77.5% of the theoretical.

EXAMPLE 13.

- To a solution of phenoxymethyl penicillin (0.35 g) in a mixture of amyl acetate (5 ccs) and ethanol (1.5 ccs) is added a suspension

of precipitated calcium carbonate (0.05 g) in water (5 ccs). The mixture is shaken vigorously for one hour and the calcium phenoxymethyl penicillinate is filtered off, washed with water and acetone and dried. Yield 0.25 g.

EXAMPLE 14.

To a solution of sodium phenoxymethyl penicillinate (11.5 g) in water (72 ccs) is added dropwise with stirring a solution of calcium chloride (3.66 g) in water (12 ccs). The calcium phenoxymethyl penicillinate is filtered off, washed with water and acetone and dried at 80° C. Yield 11.1 g corresponding to 95.7% of the theoretical.

Owing to the low solubility of the described calcium salt of phenoxymethyl penicillin a protracted penicillin effect can be obtained by subcutaneous or intramuscular injection of suspensions of this salt. To increase the chemical stability of the phenoxymethyl penicillin a content of Ca-ions exceeding that of the calcium salt of the phenoxymethyl penicillin may be incorporated with the suspension medium, for instance by adding to the suspension a soluble salt of calcium other than the phenoxymethyl penicillin salt, for instance calcium chloride. This reduces the solubility of the calcium salt of phenoxymethyl penicillin and in consequence thereof augments its chemical stability. In order to prevent precipitation of the suspended crystals in the form of a layer or cake which cannot readily be re-suspended in the suspension medium, agents capable of increasing the viscosity of the suspension medium may be added. Such agents are well known, examples of them being carboxymethyl cellulose, polyvinyl pyrrolidone and gelatin. Furthermore, to facilitate resuspension a surface active substance may be added, for instance lecithin, surface active amine salts, such as cetyl pyridinium chloride, or tweens.

Examples of suspensions are:

EXAMPLE 15.

An aqueous solution of polyvinyl pyrrolidone containing 20 g per 100 ccs is prepared and heated to 120° C. for 20 minutes in an autoclave. To the solution thus sterilised a growth-inhibiting agent is added, for instance phenyl mercuric acetate in such an amount that the concentration will be 1:20000.

Sterile calcium salt of phenoxymethyl penicillin containing particles of sizes between the limits 20 and 60 μ is strained under aseptic conditions through a sieve the mesh width of which is 0.13 mm. 30 g of the substance that have passed the sieve are stirred into 50 ccs of the polyvinyl pyrrolidone solution until uniform suspension has been obtained, after which more polyvinyl pyrrolidone solution is added until the volume is 100 ccs and the suspension is homogenised in a homogenisation apparatus.

EXAMPLE 16.

1.2 g of lecithin are dissolved in 60 ccs ether and the solution is sterilised through a glass

filter G 5. 60 g sterile calcium phenoxy-methyl penicillinate having a particle size of 20-60 μ are stirred into the solution until all the particles are moistened therewith and subsequently the ether is removed by evaporation in vacuo or at slight heat. The dried substance is strained aseptically through a sieve the mesh width of which is 0.13 mm.

2.7 g carboxymethyl cellulose are dissolved in water with vigorous agitation in a Waring-blender and the solution obtained is heated to 120° C. for 20 minutes after which phenyl mercuric acetate is added to make a concentration of this substance of 1:20000 in the solution. The viscosity measured by a rotation viscosimeter should be 35-40 cP at 20° C.

30.0 g of the sterile calcium salt of phenoxy-methyl penicillin treated with lecithin as described above is stirred into 50 ccs of the sterile solution of carboxymethyl cellulose to form a uniform suspension, after which more of the solution is added to form 100 ccs of the solution which is then homogenised in a homogenisation apparatus. The resulting suspension contains 300 mg of the calcium salt per ml.

By using such a suspension for intramuscular injections in normal persons the following penicillin concentrations in the serum were found after the intervals stated, each injection containing 200 mg of the calcium salt of phoxymethylpenicillin, the concentrations of benzylpenicillin in the serum measured after injection of 300 mg of the procain salt of benzylpenicillin being given for comparison:

TABLE I

Phoxymethylpenicillin (given as calcium salt)
(μ g per cc serum).

Sub- jects	1 h	2 h	4 h	8 h	12 h	24 h
A	1.9	1.1	0.2	0.06	0.06	0.06
B	0.7	0.6	0.3	0.25	0.15	0.06
C	1.7	1.1	0.5	0.08	0.06	0.06
D	1.8	0.8	0.1	0.07	0.06	0.06
E	0.3	0.2	0.2	0.28	0.17	0.06
Average	1.3	0.76	0.26	0.15	0.10	0.06
300 mg procain benzyl- penicillin	0.59	0.42	0.40	0.45	0.38	0.04

Table I shows that the calcium salt of phoxymethylpenicillin during the first two hours produces higher penicillin concentrations

in the serum than those obtained after injections of the procain salt of benzylpenicillin, and that the calcium salt of phoxymethylpenicillin has a protracted effect a little less pronounced than that of the procain salt of benzylpenicillin.

Preparations for oral use can be produced in the form of pills, tablets, or capsules. An example showing a manner of producing a tablet suitable for oral use is as follows:—

EXAMPLE 17.

200 g of the new calcium salt of phenoxy-methylpenicillin are thoroughly mixed with 60 g potato starch and 20 g kaolin and the mixture is granulated with an aqueous solution of 4% gelatin after which the granulate is completely dried. It is then mixed with 35 g potato starch and 35 g talcum after which the mixture is analysed and compressed to tablets of such a weight that each tablet contains 200 mg of phoxymethylpenicillin.

The serum concentrations in normal persons after oral administration of one such tablet are compared in the following Table II with the serum concentrations obtained by administering tablets of the potassium salt of phoxymethylpenicillin in the amount of one tablet containing 200 mg of phoxymethylpenicillin.

TABLE II.

Phoxymethylpenicillin (given as calcium salt)
(μ g per cc serum).

Sub- jects	$\frac{1}{2}$ h	1 h	2 h	4 h	6 h	7 h
A	0.11	1.3	1.4	0.20	—	—
B	0.24	1.2	0.9	0.15	—	—
C	0.22	1.8	2.4	0.28	0.04	0.03
D	0.08	1.3	0.61	0.12	—	—
E	0.35	1.8	0.52	0.10	—	—
Average	0.20	1.5	1.2	0.17	—	—

Phoxymethylpenicillin (given as potassium salt) (μ g per cc serum)

Sub- jects	$\frac{1}{2}$ h	1 h	2 h	4 h	6 h	7 h
A	0.12	0.35	0.76	0.43	—	—
B	0.26	1.1	0.82	0.14	—	—
C	0.07	0.22	1.7	0.50	0.07	0.05
D	0.40	1.1	0.90	0.13	—	—
E	0.10	0.48	0.46	0.04	—	—
Average	0.19	0.65	0.93	0.25	—	—

The Table shows that the calcium salt is equivalent to the potassium salt. The calcium salt is, however, free of foreign penicillins produced by the fungi, for instance penicillin K.

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