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PATENT SPECIFICATION

DRAWINGS ATTACHED

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COMPLETE SPECIFICATION

Improvements in and relating to α-Aminobenzylpenicillin

We, AMERICAN HOME PRODUCTS CORPORATION, a Corporation organized and existing under the laws of the State of Delaware, United States of America, of 685, Third Avenue, New York 17, New York, United States of America, (Assignee of NORMAN HOWARD GRANT and HARVEY EUGENE ALBURN), 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 generally to the production of derivatives of penicillanic acids and more particularly to a novel form of D-6-(2-amino-2-phenyl-acetamido) penicillanic acid and a method of preparing such form.

One form of D-6-(2-amino-2-phenyl-acetamido) penicillanic acid is already known to the art from British Patent Specification No. 873049, in which a comparatively complex method for the preparation thereof is disclosed.

The previously known hydrated forms of D-6-(2-amino-2-phenyl-acetamido) penicillanic acid, hereinatfer referred to as "ampicillin A", is now of proven value in its broad spectrum antibacterial activity and is useful as a therapeutical agent in poultry and in mammals, particularly in man, in the treatment of infectious diseases caused by gram-positive and gram-negative bacteria, upon parenteral or oral administration. It also has use as a nutritional supplement in animal feed. The similarly known form, L-6-(2-amino-2-phenyl-acetamido) penicillanic acid, has been found to be of appreciably less value for the aforesaid purposes because of its inherently lesser antibiotic activity.

As disclosed in said British Patent 873049, ampicillin A may be prepared by a method generally comprising the reaction of 6-amino penicillanic acid with the α-aminobenzyl acid chloride or anhydride in which the amino group has previously been provided with a protecting acyl group, such as PhCH2OCO-, or some other functionally equivalent protecting group. To recover the desired ampicillin A, it is then necessary to remove the protecting group by catalytic hydrogenation under sufficiently mild conditions to avoid destruction of the penicillin nucleus. In accordance with the teachings in said patent, the less active L-6-(2-amino-2-phenyl acetamido) penicillanic acid is prepared in similar manner.

With respect to the mild conditions stated to be necessary for the method, the patent warns that since some of the antibiotic substances obtained by the process disclosed therein are relatively unstable compounds which readily undergo chemical changes resulting in the loss of antibiotic activity, it is desirable to choose the reaction conditions which are sufficiently moderate to avoid their decomposition. In this connection, it is further stated in the patent that the temperature chosen for the process of preparation of the derivatives of penicillanic acid should not exceed 30° C., and that in many cases a suitable temperature is ambient temperature. Further in this connection, each of the Examples disclosed in the patent states the conditions under which recovery of the desired product is obtained is by evaporation in vacuum at a temperature below 20° C. with the exception that D-6-(2-amino-2-phenyl-acetamido) penicillanic acid monohydrate, ampicillin A, may be obtained by evaporation in vacuum at a temperature of 32° C.

Ampicillin A prepared by the foregoing procedures and all other procedures as far as known contain from 2.5% to 10% water as determined by the Karl Fischer method. This further indicates that ampicillin A as prepared heretofore has been in the form of the hemihydrate or the monohydrate as referred to in British Patent 873049, and possibly also as dihydrate, and certainly also as trihydrate as we have found.

We have now made the surprising discovery that ampicillin, i.e., D-6-(2-amino-2-phenyl-

[*Price* 4*s.* 6*d.*]

acetamido) penicillanic acid, may be prepared in a previously unknown anhydrous form designated hereinafter as "ampicillin B". This new compound is characterized by the fact that it has very little, if any, water, up to no more than 2.5%. It is much less soluble in either water or dimethylsulfoxide than is ampicillin A. The differing crystal structure of the ampicillin B form is demonstrated by its spectograph, an example of which is shown in Fig. 1 of the drawings, when compared with the spectograph of ampicillin A, an example of which is shown in Fig. 2. Moreover, X-ray diffraction analyses of single crystals of ampicillin B have indicated a molecular weight of 346±3 (the theoretical molecular weight of the monomeric anhydrous compound being 349), as compared with the molecular weight of samples of one form of ampicillin A of 367 (precisely the theoretical weight of the monohydrate) determined by osmometrical analyses.

The ampicillin B compound is further distinguished in that, due to its lesser solubility in water, it is of greater stability on storage than is ampicillin A. Because of this stability, coupled with its denseness, the efficiency of production of the compound in capsule dosage form is increased. As a further advantage that is linked to its lesser solubility in water, ampicillin B exhibits slower absorption in the gut and hence provides prolonged blood levels and more effective action against intestinal pathogens. The foregoing differences and advantages of ampicillin B over ampicillin A are of enhanced significance, since to utilize them, therapeutic effectiveness is not at all sacrificed. On the contrary, effectiveness of ampicillin B is substantially equal to that of ampicillin A on a weight-for-weight basis. For example, when mice were challenged intraperitoneally with a penicillin sensitive strain of *S. aureus* and a virulent strain of *S. typhosa* and both types of ampicillin were administered by the oral route to the separately infected mice, equal protection was afforded by each drug.

Ampicillin B has been demonstrated experimentally to have very little affinity for water. For example, one gram suspended in 10 ml. of water at 0° C. for 3 days dissolved only to about 20%, and the remaining 80% was easily dried to 0.67% H_2O and retained the ampicillin B infra red spectrum. This appears to suggest a reason why ampicillin B is so much more stable on storage than is ampicillin A, since water seems to be an important factor in reducing the stability of ampicillin.

After actual hydration, ampicillin B appears to change back to ampicillin A as has been indicated by the infra red spectrum, paper and thin layer chromatography, and paper electrophoresis. It has not yet been possible to convert the solid form of ampicillin A to ampicillin B using a variety of drying techniques.

To demonstrate the stability in the solid state of ampicillin B as compared with ampicillin A, a series of samples was subjected to varying conditions as given below in Table A. The assays were for β-lactam based on the hydroxamate assay to indicate per cent loss tabulated below:

TABLE A

Conditions	Time	Per cent loss A	Per cent loss B
50°C.	10 days	19	0
70°C.	65 hrs.	39	0
70°C.	160 hrs.	61	0
107°C.	18 hrs.	66	2
107°C.	90 hrs.	79	1
107°C.	166 hrs.	94	3
70°C., 100% humidity	18 hrs.	69	2
120°C., 100% humidity (Autoclaved)	15 mins.	40	6

Generally, the method for preparing the novel compound ampicillin B comprises heating ampicillin A in the presence of free water at a temperature of from 40° to about 100° C., until ampicillin B is formed. The heating may best be carried out with the charge of ampicillin A plus water at a pH of from about 3.0 to 7.0. Preferably the free water is present in

amount that is at least 50% by weight of the charge, and the heating is applied to the charge under vacuum until the dry ampicillin B product is obtained. In an alternative procedure, the required heating conditions may be applied to an aqueous reaction mixture comprising the reactants which normally result in formation of ampicillin A. In another procedure, the required heat and water may be supplied by directly steaming crystals of ampicillin A. It has been found most advantageous from the standpoint of feasibility and of economic processing to carry on the heating operation at a pH of from 5.0 to 5.5 and at a temperature within the range of 50—55° C.

The aqueous environment necessary to the method of obtaining anhydrous ampicillin B may advantageously comprise an organic solvent, which is preferably entirely or partially miscible with water. For example, acetone, ethanol, isopropanol, n-propanol, n-butanol, ethylene glycol, ethylene glycolmonomethyl ether, and dioxane have been found eminently suitable as said organic solvent and, in certain instances, even when included in proportions up to 95% of the aqueous environment.

The presence of free water in the charge of ampicillin A to be transformed into ampicillin B, and the minimum heating temperature of 40° C. to effect the transformation, have both been demonstrated to be essential to the method of the invention. In this connection, it has been proven that drying alone will not cause the desired transformation. Several lines of evidence were followed in such proof. Thus, it was found exceedingly difficult to lower the water content of previously dried ampicillin A below 2% by a variety of procedures, including Abderhalden drying at 55° C. with P_2O_5, azeotropic distillation with benzene, reaction with 2,2-dimethoxypropane and acid, the vacuum oven over P_2O_5 at 55° C., and treatment of dimethylformamide solution with Linde "molecular sieve" followed by dry ether crystallization. In all cases, the end products were still ampicillin A. On the other hand, water or dilute acid extraction of ampicillin B gave an insoluble component (78% of the total at pH 4 and 20% at pH 2 in 10% suspensions) which remained ampicillin B even when mildly dried *in vacuo* at room temperature over P_2O_5. The foregoing is a further indication that one difference in kind between ampicillin A and ampicillin B is due to the process of crystal formation.

As already referred to, ampicillin B is less soluble in water and in dimethylsulfoxide. For example, the ratio of solubility in water is about 4 : 7. However, once ampicillin B is dissolved into water, it is then apparently identical to ampicillin A and can be made to crystallize as ampicillin A by the lower temperature, lesser water content crystallization procedures known heretofore. No evidence has been found that ampicillin B changes to ampicillin A in the solid state. Further, ampicillin B is readily converted to form ampicillin A by dissolving in water at any pH and crystallizing below 40° C. or freeze-drying. On the other hand, of course, conversion of ampicillin A to ampicillin B requires heat and presence of water. The reaction is influenced by the period of heating, water content and pH as will appear in greater detail hereinafter.

The following examples are illustrative of the invention, but are not to be considered necessarily limitative thereof:

Example I

Mix 120 grams of 6-aminopenicillanic acid with 15 liters of cold water, 27.6 ml. of 8.5 N KOH, and 49.5 grams of D-phenylglycine-N-carboxyanhydride. Stir for 1 hour at 2° C. Filter, and concentrate the filtrate (pH 5.2) to 2.9 liters. Remove the precipitate, and pass the filtrate through a 3.2 x 55 cm. Dowex 1-x 10 column (acetate form, 50—100 mesh). Concentrate the effluent (3.14 liters) to 275 ml. at 50—55° C. and dry the insoluble product *in vacuo* to obtain a product weighing 41 grams and containing 1% water. Subject the product to infrared analysis to show the spectrograph of ampicillin B.

Example II

Dissolve a sample of substantially pure commercially obtainable ampicillin A in water at pH 2.0 to form a 10% solution. Adjust the solution to pH 3.0 and then heat to 65° C. At this temperature, adjust the pH with dilute sodium hydroxide solution to approximately 4.8 to cause rapid formation of granular crystals. Filter off the crystals and then dry. Take separate infrared spectra of the dried product and of the starting material to result in respective spectrographs for each as shown in Fig. 1 and Fig. 2 respectively.

In connection with the considerably different infrared spectrum found for ampicillin B, the importance of such difference as an indication of the differences in spectrum between ampicillin B and ampicillin A appears in the *Chemistry of Penicillin*, H. W. Thompson, et al, Princeton University Press, Princeton, New Jersey, 1949, wherein it is stated at page 383 as follows:

> "Absorption bands in the 2-25μ region arise from the excitation of molecular vibrations, the fundamentals, overtones, or combinations being taken up. A molecule as complex as penicillin has many possible vibrational modes and the spectrum is accordingly highly complex. In principle, however, this spectrum remains a unique property of the molecule, and for this reason, the identity of the infrared spectra of natural and synthetic samples may, if studied under high resolution, be the best proof of structure available."

EXAMPLE III

Warm each sample of a series of 10% aqueous suspensions or solutions of commercially obtainable ampicillin A at pH 2.0, 3.0, 4.8 and 7.0 for 10 minutes at the temperatures set forth in Table B below. Adjust each to pH 4.8 and warm each at the same given temperature for 5 minutes, filter rapidly, dry at room temperature and subject the insoluble fraction to infrared analysis of crystal form to obtain the results set forth in said Table B below:

TABLE B

pH	25°	30°	35°	40°	45°	50°	55°	70°	81°
2.0					No ppt	No ppt	B	No ppt	
3.0	A	A	A	B	B	B	B	B - A	A decomp.
4.8		A		A	A - B	B	B	B	B
7.0		A	A	B	B	B	B	B	A decomp.

EXAMPLE IV

When 10% aqueous suspensions were warmed at 55°, pH 4.8 (iso-electric region) for various periods, the changed rate of solubilization of ampicillin B, as well as the time dependence of the conversion, became apparent, as shown in the following tabulation:

TABLE C

Warming period minutes	Insoluble Fraction %	Crystal type
2	14	A - B
4	31	B
6	36	B
8	37	B
15	44	B
30	50	B
60	44	B
120	44	B
180	42	B

EXAMPLE V

Prepare separate 10% and 20% aqueous suspensions of ampicillin A and warm both at 50° C., then, without filtering, pour into Petri dishes and dry overnight at 52° C. and 100° C., respectively, to obtain ampicillin B in each dish in evidence that ampicillin A may be converted quantitatively to ampicillin B.

EXAMPLE VI

Introduce one ml. of a 50% aqueous suspension of ampicillin A crystals into a series of five beakers each having an 8 cm.^{2} floor. Dry each suspension through a heating range starting at 40° C. and increasing the temperature in 3° C. increments after respective periods of 2 1/4, 1 2/3, 1, 1, and 1 hours while testing the product in one beaker after each interval to achieve a maximum temperature of 55° C. in the last remaining beaker. The product in each beaker is ampicillin A.

In an identical container, dry 2 ml. of a 25% suspension of ampicillin A (75% water) for 2 hours at 55° C. to form ampicillin B.

The foregoining demonstrates that both sufficient water and time of heating are necessary to formation of ampicillin B.

EXAMPLE VII

A group of healthy dogs were given orally

equivalent doses by weight of ampicillin A and ampicillin B and serum concentration determines with respect to each dog were made over a period of six hours. The data with respect to these experiments is given below in Table D:

TABLE D

50 mg./kg. dose for each

	Ampicillin A			Ampicillin B		
Dog Identification	W	X		Y	Z	
Dog Wt. (kg.)	12.6	13.0	Av.	12.4	11.1	Av.
Hours						
0	0	0		0	0	
1	2.15	1.70	1.92	1.58	7.65	4.62
2	5.40	6.80	6.10	3.88	8.92	6.40
3	3.40	3.50	3.45	5.62	5.38	5.00
4	1.85	1.50	1.68	2.41	2.92	2.67
6	0.632	0.30	0.47	0.53	0.675	0.60

The foregoing demonstrated the sustained high blood level concentrations obtainable with the use of ampicillin B when compared to equivalent use of ampicillin A.

EXAMPLE VIII

Prepare unit dosage forms containing ampicillin B with the use of known compounding procedures for use of such forms in human therapy by oral ingestion from the following formulations in Tables E and F:

TABLE E

Tablet Formula	
Crystalline ampicillin B	250.0 mg.
Calcium Carbonate, Heavy	150.0 mg.
Cab-O-Sil M-5 (a type of colloidal silica)	5.0 mg.
Sodium Citrate, Anhydrous	20.0 mg.
Magnesium Stearate	7.5 mg.
Amberlite XE-88 ("Amberlite" is a Registered Trade Mark)	5.0 mg.
Microcel—C	90.0 mg.
Total Wt.	527.5 mg.

TABLE F

Capsule Formulation	
Crystalline ampicillin B	250.0 mg.
Cab-O-Sil M-5 (a type of colloidal silica)	15.0 mg.
Magnesium Stearate U.S.P.	20.0 mg.
Lactose U.S.P.	250.0 mg.
Total Wt.	535.0 mg.

Alternatively, prepare capsules from the formulation given in Table F above.

Use the unit dosage forms for treating gram-negative or gram-positive infections in humans. Administer the forms orally and in a daily regimen of from 500 mg. to 10 gm. depending upon therapeutic requirements. For example, the 10 gm. dosage may be administered to a known typhoid carrier. Preferably, to treat gram-negative infections, administer from 4-6 unit dosage forms daily, and to treat gram-positive infections, administer from 2-4 unit dosage forms daily for sustained high blood level concentrations of the antibiotic.

EXAMPLE IX

Suspend separate batches of ampicillin A in various mixtures of water and organic solvent, as set forth in Table G below, and under atmospheric conditions, heat each mixture at the temperature also designated in said Table, to obtain ampicillin B in each case.

TABLE G

Organic Solvent	Concentration of Solvent	Temperature
Acetone	75%	85°
Acetone	50%	85°
Acetone	50%	50°
Acetone	75%	85°
Ethanol	50%	50°
Dioxane	75%	85°
Dioxane	60%	69°
Ethanol	60%	71°
Ethanol	75%	60°
Ethylene glycol	75%	71°
Ethylene glycol	75%	85°
Ethylene glycol monomethyl ether	75%	85°
Isopropanol	75%	70°
Isopropanol	75%	85°
Isopropanol	90%	80°
Isopropanol	95%	85°
n-propanol	60%	80°
n-propanol	75%	71°
n-propanol	75%	85°
n-butanol	85%	85°

EXAMPLE X

Pump 85 liters of 99% isopropanol into a 50 gallon stainless steel kettle. Add 15 liters of tap water. Start the stirer of the kettle, open the manhole, and warm the contents of the kettle to 70°. At 70°, with the stirer in operation, add 14.58 kg. of ampicillin A at a rate of about 1.5 kg. per minute. Close the manhole and raise the temperature until refluxing is observed (at a temperature reading of about 74°). Reflux the system for 20 minutes at which time stop the heating, continue the

stirring and open the bottom valve of the kettle to release the contents to a 20 inch stainless steel table top Buchner with a canvas pad. Filter the suspension.

Wash the first 40% of filter cake with 6 liters of 85% isopropanol (at room temperature). Suck the filter cake dry and transfer to drying trays. The subsequent portion (60%), which receives longer exposure to hot solvent is washed with 9 liters of 85% isopropanol, and this is followed by an extra wash with 5 liters of 85% isopropanol.

Both cakes are dried at 48° in a Stokes vacuum oven for 2 days and are then milled. Upon assays showing the two portions to have practically the same potency, they are pooled by thorough mixing to form the combined product weighing 10.70 kg. and having the following characteristics:

Hydroxymate Assay (%)	93.2
Moisture (K-F, %)	1.5
Optical Rotation $(\alpha)_D^{25}$	+269°
Form indicated by IR Spectrum	anhydrate
Yield of Activity (%)	87
Appearance	fine powder
Color	off-white
Odor	none

WHAT WE CLAIM IS:—

1. Ampicillin B, a crystalline D-6(2-amino-2-phenyl-acetamido) penicillanic acid having a bound water content of not more than 2.5%, a molecular weight of about 349, and an infrared spectrograph as given in Figure 1 of the accompanying drawings.

2. A process for the production of ampicillin B which comprises heating ampicillin A as hereinbefore defined in the presence of free water at a temperature of at least 40° until ampicillin B is formed.

3. A process for the production of ampicillin B which comprises heating the reaction product of 6-amino penicillanic acid and α-aminobenzyl acid chloride or anyhydride in the presence of free water at a temperature of at least 40° until ampicillin B is formed.

4. A process as claimed in claim 2 or 3, in which the temperature is from 50 to 55°C.

5. A process as claimed in any of claims 2 to 4 in which the heating is carried out at a pH of from about 3.0 to 7.0.

6. A process as claimed in claim 5, in which the heating is carried out at a pH of from 5.0 to 5.5.

7. A process as claimed in any of claims 2 to 6 in which the free water is present in an amount at least 50% by weight of the charge.

8. A process as claimed in any of claims 2 to 7, in which the heating is applied under vacuum until dry ampicillin B is obtained.

9. A process as claimed in any of claims 2 to 8 in which there is present an organic solvent in the reaction mixture.

10. A process as claimed in claim 9, in which the organic solvent is entirely or partially miscible with water.

11. A process as claimed in claim 9 or 10 in which the amount of organic solvent is up to 95% of the aqueous environments.

12. A process as claimed in any of claims 2 to 11, in which the process takes place under a pressure from *vacuo* to atmospheric.

13. A process for the production of ampicillin B substantially as hereinbefore described with particular reference to any of Examples I to VI.

14. A process for the production of ampicillin B substantially as hereinbefore described with particular reference to Example IX and X.

15. Ampicillin B whenever produced by a process as claimed in any of claims 2 to 8 and 13.

16. Ampicillin B whenever produced by a process as claimed in any of claims 9 to 12 and 14.

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stirring and open the bottom valve of the kettle to release the contents to a 20 inch stainless steel table top Buchner with a canvas pad. Filter the suspension.

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6. A process as claimed in claim 5, in which the heating is carried out at a pH of from 5.0 to 5.5.

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8. A process as claimed in any of claims 2 to 7, in which the heating is applied under vacuum until dry ampicillin B is obtained.

9. A process as claimed in any of claims 2 to 8 in which there is present an organic solvent in the reaction mixture.

10. A process as claimed in claim 9, in which the organic solvent is entirely or partially miscible with water.

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12. A process as claimed in any of claims 2 to 11, in which the process takes place under a pressure from *vacuo* to atmospheric.

13. A process for the production of ampicillin B substantially as hereinbefore described with particular reference to any of Examples I to VI.

14. A process for the production of ampicillin B substantially as hereinbefore described with particular reference to Example IX and X.

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16. Ampicillin B whenever produced by a process as claimed in any of claims 9 to 12 and 14.

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