PATENT SPECIFICATION

NO DRAWINGS

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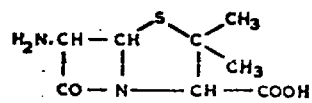
COMPLETE SPECIFICATION

Improvements in or relating to Penicillin Derivatives

We, BEECHAM RESEARCH LABORATORIES LIMITED, a British Company, formerly of Brockham Park, Betchworth, Surrey, now of Great West Road, Brentford, Middlesex, 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 penicillin derivatives and is particularly concerned with derivatives of 6-aminopenicillanic acid.

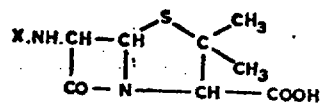
6-Aminopenicillanic acid has the structural formula:—



(I)

It has now been found according to the present invention that new penicillin derivatives having valuable antibiotic activity can be obtained by introducing aminoacyl substituent groups into the amino group of 6-aminopenicillanic acid.

Accordingly, the present invention provides new penicillin derivatives of the general formula:



(II)

where X is an amino-substituted acyl group containing up to 20 carbon atoms and having the formula

$$R(CH_2)_nCH(NH_2).CO-$$

where R is hydrogen or an amino, carboxyl or substituted or unsubstituted alkyl, aryl, aralkyl or heterocyclic group and n is zero or an integer, and non-toxic salts thereof.

[*Price 3s. 6d.*]

The compounds of the present invention are of value as antibacterial agents, as nutritional supplements in animal feeds, as agents for the treatment of mastisis in cattle and as therapeutic agents in poultry and animals, including man, in the treatment especially of infectious diseases caused by Gram-positive and Gram-negative bacteria.

The salts are non-toxic salts including non-toxic metallic salts such as sodium, potassium, calcium and aluminium, the ammonium salt and substituted ammonium salts, e.g. salts of such non-toxic amines as trialkylamines, including triethylamine, procaine, dibenzylamine, N-benzyl-beta-phenethylamine, 1-ephenamine N,N^1 - dibenzylethylenediamine, dehydroabietylamine, N,N^1 - bis - dehydroabietylethylenediamine, and other amines which have been used to form salts with benzylpenicillin.

The present invention also includes a process for the preparation of penicillin derivatives of the general formula II where X is as hereinbefore defined, and non-toxic salts thereof, which comprises coupling 6-aminopenicillanic acid with an acid of the general formula XOH having its amino group or groups protected, or a salt thereof, and thereafter removing the protecting group or groups under sufficiently mild conditions to avoid destruction of the penicillin nucleus.

Examples of the group X in the general formula II are:

(i) $R(CH_2)_nCH(NH_2).CO-$

where n is zero or an integer from 1 to 6 and R is hydrogen or an alkyl, aryl, aralkyl or heterocyclic group which may also be substituted. This group will include derivatives of the amino acids, alanine, valine, *nor*-valine, leucine, *iso*-leucine, phenylalanine, tyrosine, tryptophane, threonine, histidine, asparagine and glutamine. A preferred class of compound

Price 4s 6d.

falling within this group is when *n* is zero and R is phenyl, chlorophenyl, methoxyphenyl, furyl or cyclohexyl group. This class will include α-aminobenzylpenicillin.

(ii) $HOOC.(CH_2)_n.CH(NH_2).CO-$

This group will include derivatives of aspartic acid, glutamic acid and α-aminoadipic acid.

(iii) $H_2N.(CH_2)_n.CH(NH_2).CO-$

wherein n is an integer from 1 to 4. This group will include derivatives of lysine and ornithine.

The majority of the derivatives mentioned above contain at least one asymmetric carbon atom and will exist in D- and L- forms. It is to be understood that the present invention includes both the D- and L- forms and the DL-mixture. When the derivative contains two asymmetric carbon atoms then there will be four isomers and two DL-mixtures.

The protected aminoacyl derivatives of 6-aminopenicillanic acid are preferably prepared by reacting 6-aminopenicillanic acid with a mixed anhydride prepared by reacting the amino-substituted carboxylic acid or a salt thereof, having its amino group or groups protected, with an ester of chlorocarbonic acid, e.g. ethyl chlorocarbonate. Alternatively, the protected amino-substituted carboxylic acid may be converted to a reactive acid halide.

Other methods used to form the protected aminoacyl derivative of 6-aminopenicillanic acid are standard procedures employed in peptide synthesis and include the use of a reactive acid azide or a carbodi-imide reagent (cf. Sheehan and Hess, *J. Amer. Chem. Soc.*, 1955 *77*, 1067).

The subsequent removal of the protecting group or groups, to form the free amino-substituted penicillin may be effected by catalytic hydrogenation and suitable protecting groups are of the general formula $R^{11}O.CO-$, where R^{11} is an allyl, benzyl, substituted benzyl, phenyl or substituted phenyl group, or the trityl group Ph_3C-. The abbreviation "Ph" as used herein represents the phenyl group.

The removal of the protecting group or groups is effected by allowing the protected aminoacyl derivative to react with hydrogen in the presence of a catalyst. This hydrogenation is normally carried out at room temperature and at atmospheric pressure, the pH of the reaction mixture being from 5 to 9. The solvent for the hydrogenation reaction is normally water, but other non-reducible solvents such as ethyl alcohol or dioxane or mixtures of these with water may be employed.

The preferred hydrogenation catalyst is palladium but other catalysts such as platinum or rhodium may be used. The catalyst is preferably employed on an inert support, e.g. of barium carbonate or carbon.

When a carbon atom next to that carrying the carbobenzyloxyamino group to be reduced forms part of an aromatic ring, as in Group (i) above when R is aromatic and *n* is zero, the hydrogenation step is normally completed in a single treatment with hydrogen and catalyst. However, in all other cases, i.e. when the carbon atom next to that carrying the carbobenzyloxyamino group to be reduced is of an aliphatic nature, in order to effect complete reduction it may be necessary to hydrogenate in the presence of two or more successive portions of catalyst.

When the amino-substituted carboxylic acid contains more than one carboxyl group, e.g. α-aminoadipic acid, in order to prepare an unambiguous acyl derivative it is necessary either to protect one of the carboxyl groups before reaction with 6-aminopenicillanic acid or to prepare an unambiguous reactive derivative of one carboxyl group only.

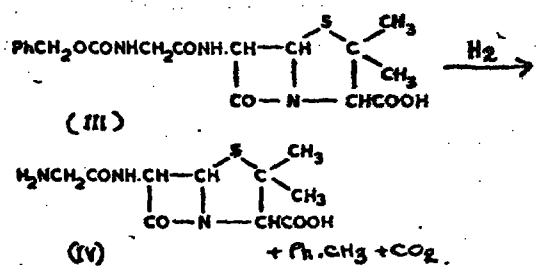
Since some of the antibiotic substances obtained by the process of this invention are relatively unstable compounds which readily undergo chemical changes resulting in the loss of antibiotic activity, it is desirable to choose reaction and isolation conditions which are sufficiently moderate to avoid their decomposition.

The following Example illustrate the invention.

Example 1.

Preparation of aminomethyl penicillin.

A suspension of palladium on barium carbonate (7.3 g., of 30%) in water (200 ml.) was shaken in an atmosphere of hydrogen at room temperature and atmospheric pressure for 2 hours. After this treatment the catalyst was collected and washed well with water, taking care that it never became dry. The sodium salt of carbobenzyloxyaminomethyl penicillin (III) (4.5 g., of a material assaying at a purity of 40%) was added to the aqueous suspension of the pre-treated catalyst and the pH adjusted to 8.0 with sodium hydrogen carbonate solution (3%). The mixture was then shaken under hydrogen at room temperature and atmospheric pressure for 45 minutes. A second quantity of palladium on barium carbonate (7.3 g., of 30%), previously reduced as before, was added and the hydrogenation continued for a further 45 minutes. The catalyst was filtered off, washed with water, and the filtrate, after adjusting to pH 7.0 with N hydrochloric acid, was evaporated to dryness under reduced pressure at a temperature below 20° C. The aminomethyl penicillin (IV) was obtained as a yellow powder (3.0 g.) which assayed for a purity of 33%. Paper chromatography showed that this material contained only one antibiotic which had a considerably different R_F value from the starting material. The product was shown to be stable in acid solution and to inhibit *Staph. aureus* at a concentration of 1.25 mcg./ml.



(III)

(IV) + $Ph.CH_3$ + CO_2

The sodium salt of carbobenzyloxyaminomethyl penicillin was prepared as follows:

Ethyl chlorocarbonate (1.9 ml.) was added dropwise to an ice-cold stirred solution of N-carbobenzyloxyglycine (4.5 g.) and triethylamine (3 ml.) in dry acetone (170 ml.). After 5 minutes the resulting suspension containing the mixed anhydride was treated slowly with an ice-cold solution of 6-aminopenicillanic acid (4.3 g.) in 3% aqueous sodium bicarbonate (170 ml.). Throughout the addition the mixture was maintained at 0° C., but thereafter it was allowed to attain room temperature during a further one hour's stirring. The mixture was washed with ether (3 × 250 ml.) and the washings were discarded. The aqueous phase was treated with hydrochloric acid to pH 2 and the free penicillin acid was extracted into butanol (40 ml., then 2 × 10 ml.). The sodium salt of carbobenzyloxyaminomethyl penicillin was recovered by re-extracting the washed butanol solution with water (3 × 5 ml.), to each portion of which sufficient 3% aqueous sodium bicarbonate had been added to bring the aqueous phase to pH 7. The combined aqueous extracts were washed with ether, then evaporated below 20° C. *in vacuo* to leave the solid sodium salt (4.7 g.).

EXAMPLE 2.

Preparation of α-aminopentylpenicillin.

The sodium salt of α-carbobenzyloxyaminopentylpenicillin (56% pure) was hydrogenated in the presence of three successive portions of pre-treated palladium on barium carbonate catalyst as described in Example 1 to give α-aminopentylpenicillin, which assayed at 34% pure, in 56% yield. Paper chromatography showed only one antibiotic with a considerably different R_F value from the starting material. It was stable in acid solution and was shown to inhibit *Staph. aureus* at a concentration of 0.12 mcg./ml.

The sodium salt of α-carbenzyloxyaminopentyl penicillin used in this Example was prepared in 64% yield from 6-aminopenicillanic acid and a mixed anhydride prepared from N-carbobenzyloxy - DL - *nor*leucine and ethyl chlorocarbonate according to the general procedure of Example 1.

N - Carbobenzyloxy - DL - *nor*leucine, m.p. 106—107° C., was obtained in 70% yield from DL-*nor*leucine and benzyl chlorocarbonate in cold aqueous sodium hydroxide solution.

EXAMPLE 3.

Preparation of α-aminoheptylpenicillin.

The sodium salt of α-carbobenzyloxyaminoheptylpenicillin (68% pure) was hydrogenated in the presence of three successive portions of pre-treated palladium on barium carbonate catalyst as described in Example 1 to give α-aminoheptylpenicillin, which assayed at 47% pure, in 44% yield. Paper chromatography showed only one antibiotic with a considerably different R_F value from the starting material. It was stable in acid solution and was shown to inhibit *Staph. aureus* at a concentration of 0.012 mcg./ml.

The sodium salt of α-carbobenzyloxyaminoheptypenicillin used in this Example was prepared in 43% yield from 6-aminopenicillanic acid and a mixed anhydride prepared from DL - α - carbobenzyloxyamino - octoic acid and ethyl chlorocarbonate according to the general procedure of Example 1.

The DL - α - carbobenzyloxyamino - octoic acid required for this preparation was obtained by the reaction of benzyl chloro-carbonate with DL-α-amino-octoic acid in dilute sodium hydroxide solution. It was obtained as colourless crystals (63%), m.p. 91—92° C. (Found: C, 65.7; H, 7.9; N, 4.9. $C_{16}H_{23}NO_4$ requires C, 65.5; H, 7.9; N, 4.7%).

EXAMPLE 4.

Preparation of α-aminocyclohexylmethylpenicillin.

The sodium salt of α-carbobenzyloxyaminocyclohexylmethylpenicillin (75% pure) was hydrogenated in the presence of three successive portions of pre-treated palladium on barium carbonate catalyst as described in Example 1 to give α-aminocyclohexylmethylpenicillin, which assayed at 46% pure, in 51% yield. Paper chromatography showed only one antibiotic with a considerably different R_F value from the starting material. It was stable in acid solution and was shown to inhibit *Staph. aureus* at a concentration of 0.12 mcg./ml.

The sodium salt of α-carbobenzyloxyaminocyclohexylmethylpenicillin used in this example was prepared in 51% yield from 6-aminopenicillanic acid and a mixed anhydride prepared from DL - α - carbobenzyloxyaminocyclohexylacetic acid and ethyl chlorocarbonate according to the general procedure of Example 1.

The DL - α - carbobenzyloxyaminocyclo-

hexylacetic acid, m.p. 136—137° C. was prepared in 80% yield from DL - α - aminocyclohexylacetic acid and benzyl chlorocarbonate in sodium hydroxide solution. (Found: C, 66.2; H, 7.5; N, 4.6. $C_{16}H_{21}NO_4$ requires C, 65.9; H,7.3; N, 4.8%).

EXAMPLE 5.

Preparation of α - amino - β - phenylethylpenicillin.

The sodium salt of α - carbobenzyloxyamino-β - phenylethylpenicillin (53% pure) was hydrogenated in the presence of three successive portions of pre-treated palladium on barium carbonate catalyst as described in Example 1 to give α-amino-β-phenylethyl penicillin, which assayed at 30% pure, in 49% yield. Paper chromatography showed that this material contained only one antibiotic which had a considerably different R_F value from that of the starting material. The product was stable in acid solution and was shown to inhibit *Staph. aureus* at a concentration of 0.6 mcg./ml.

The sodium salt of α-carbobenzyloxyamino-β-phenylethyl penicillin was prepared by reacting N - carbobenzyloxy - β - phenyl - DL-alanine with ethyl chlorocarbonate to give the reactive mixed anhydride. This was then allowed to react with 6-amino-penicillanic acid under the conditions described in Example 1.

EXAMPLE 6.

Preparation of α - amino - β - 3 - indolylethylpenicillin.

This was obtained by hydrogenating the sodium salt of α - carbobenzyloxyamino - β-3 - indolylethylpenicillin (77% pure) as in Example 2 in 47% yield and assayed at 44%. Paper chromatography showed only one antibiotic with a considerably different R_F value from the starting material. It was stable in acid solution and was shown to inhibit *Staph. aureus* at a concentration of 0.5 mcg./ml.

The sodium salt of α-carbobenzyloxyamino-β - 3 - indolylethylpenicillin used in this Example was prepared in 83% yield from 6-aminopenicillanic acid and a mixed anhydride derived from N-carbobenzyloxy - DL - tryptophane and ethyl chlorocarbonate according to the general procedure of Example 1. N-Carbobenzyloxy - DL - tryptophane had m.p. 167° C. (Found: C, 67.6; H, 5.5; N, 8.2. $C_{19}H_{18}O_4N_2$ requires C, 67.5; H, 5.4; N, 8.3%).

EXAMPLE 7.

Preperation of α - amino - β - hydroxyethylpenicillin.

This was obtained by hydrogenating the sodium salt of α - carbobenzyloxyamino - β-hydroxyethylpenicillin (70% pure) as in Example 2 in 29% yield and assayed at 21% pure. Paper chromatography showed only one antibiotic with a considerably different R_F value from the starting material. It was stable in acid solution and was shown to inhibit *Staph. aureus* at a concentration of 5.0 mcg./ml.

The sodium salt of α-carbobenzyloxyamino-β - hydroxyethylpenicillin used in this Example was prepared in 67% yield from 6-aminopenicillanic acid and a mixed anhydride derived from N-carbobenzyloxy-DL-serine and ethyl chlorocarbonate, according to the general procedure of Example 1.

EXAMPLE 8.

Preparation of α - amino - β - hydroxy - β-phenylethylpenicillin.

This was prepared by hydrogenating the sodium salt of α - carbobenzyloxyamino - β-hydroxy-β-phenylethylpenicillin (60% pure) as in Example 2 in 53% yield and assayed at 39% pure. Paper chromatography showed only one antibiotic with a considerably different R_F value from the starting material. It was stable in acid solution and was shown to inhibit *Staph. aureus*.

The sodium salt of α-carbobenzyloxyamino-β - hydroxy - β - phenylethylpenicillin used in this Example was prepared in 46% yield from 6-aminopenicillanic acid and a mixed anhydride prepared from N - carbobenzyloxy-DL - *threo* - β - phenylserine and ethyl chlorocarbonate according to the general procedure of Example 1.

N - Carbobenzyloxy - DL - *threo* - β-phenylserine, m.p. 63—64° C., was prepared in 30% yield from DL - *threo* - β - phenylserine and benzyl chlorocarbonate in aqueous sodium hydroxide solution.

EXAMPLE 9.

Preparation of α-aminobenzylpenicillin.

A suspension of palladium on barium carbonate (3.7 g. of 30%) in water (20 ml.) was shaken in an atmosphere of hydrogen at room temperature and atmospheric pressure for 1½ hours. The catalyst was then filtered and washed ~~well with~~ water, making sure that it did not become dry. A solution of the sodium salt of α-carbobenzyloxyaminobenzylpenicillin (4 g.) in water (20 ml.) was added to the pretreated catalyst and the suspension shaken in an atmosphere of hydrogen at room temperature and pressure for one hour. The catalyst was then filtered off, washed well with water, and the combined filtrate and washings adjusted to pH 7.0 with N hydrochloric acid. The resulting solution was then evaporated *in vacuo* at a temperature below 20° C. to give α-aminobenzylpenicillin (internal salt) (2.4 g., 74%) which assayed as 48% pure. Paper chromatography showed that this material contained only one antibiotic which had a considerably different R_F value from that of the starting material. It was stable in acid solution and was shown to inhibit *Staph. aureus* at a concentration of 0.12 mcg./ml.

The sodium salt of α-carbobenzyloxyaminobenzylpenicillin required for the above preparation was obtained by reacting DL-α-carbobenzyloxyaminophenylacetic acid (1 equiv.) with ethyl chlorocarbonate (1 equiv.) to give

the reactive mixed anhydride. This was then allowed to react with 6-aminopenicillanic acid under the conditions described in Example 1. It was obtained as a yellow solid (65%) which assayed as 56% pure.

EXAMPLE 10.

Preparation of α - amino - *p* - methoxybenzylpenicillin.

This was obtained according to the procedure described in Example 9 by the hydrogenation of the sodium salt of α-carbobenzyloxyamino - *p* - methoxybenzyl penicillin (59% pure) in 58% yield and assayed as 41% pure. Paper chromatography showed only one antibiotic with a considerably different R_F value from the starting material. It was stable in acid solution and was shown to inhibit *Staph. aureus* at a concentration of 0.025 mcg./ml. The sodium salt of DL - α - Carbobenzyloxyamino - *p* - methoxyphenylacetic acid was obtained from the corresponding α-amino acid in 60% yield in accordance with the method of Example 1, m.p. 105° C. (Found: C, 65.0; H, 5.5; N, 4.1. $C_{17}H_{17}NO_5$ requires C, 64.8; H, 5.4; N, 4.4%).

EXAMPLE 11.

Preparation of α - amino - *p* - chlorobenzylpenicillin.

This was obtained according to the procedure described in Example 9 by the hydrogenation of the sodium salt of α-carbobenzyloxyamino - *p* - chlorobenzylpenicillin (42% pure) in 88% yield and assayed as 45% pure. It was stable in acid solution and was shown to inhibit *Staph. aureus* at a concentration of 0.06 mcg./ml. The sodium salt of DL-α-carbobenzyloxyamino - *p* - chlorophenylacetic acid was obtained from the corresponding α-amino acid in 85% yield in accordance with the method of Example 1, m.p. 132—133° C. (Found: C, 60.5; H, 4.8; N, 4.7. $C_{16}H_{14}ClNO_4$ requires C, 60.0; H, 4.4; N, 4.4%).

EXAMPLE 12.

Preparation of α - amino - 1 - naphthylmethylpenicillin.

This was obtained according to the procedure described in Example 9 by the hydrogenation of the sodium salt of α-carbobenzyloxyamino - 1 - naphthylmethylpenicillin (64% pure) in 61% yield and assayed as 40% pure. It was stable in acid solution and was shown to inhibit *Staph. oxford* at a concentration of 0.025 mcg./ml.

α - Amino - 1 - naphthylacetic acid, m.p. 219—220° C. was prepared by the Bergs-Bucherer synthesis from α-naphthaldehyde (cf. Harvill and Herbst, *J. Org. Chem.*, 1944, *9*, 21). With benzyl chlorocarbonate in aqueous sodium hydroxide solution it gave α-carbobenzyloxyamino - 1 - naphthylacetic acid, m.p. 144—145° C. in 40% yield. The mixed anhydride prepared from this acid and ethyl chlorocarbonate was coupled with 6-aminopenicillanic acid according to the general procedure of Example 1 to give the sodium salt of α - carbobenzyloxyamino - 1 - naphthylmethylpenicillin used in this Example.

EXAMPLE 13.

Preparation of α - amino - 2 - furylmethylpenicillin.

This was obtained according to the procedure described in Example 9 by the hydrogenation of the sodium salt of α-carbobenzyloxyamino - 2 - furylmethyl penicillin (40% pure) in 74% yield and assayed as 32% pure. Paper chromatography showed only one antibiotic with a considerably different R_F value from the starting material. It was stable in acid solution and was shown to inhibit *Staph. aureus* at a concentration of 0.5 mcg./ml.

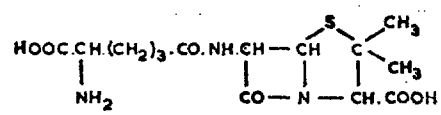
The sodium salt of α-carbobenzyloxyamino-2 - furylmethyl penicillin required for the above preparation was obtained by treating DL - α - carbobenzyloxyamino - 2 - furylacetic acid (1 equiv.) with ethyl chlorocarbonate (1 equiv.) to give the reactive mixed anhydride. This was then allowed to react with 6-aminopenicillanic acid under the conditions described in Example 1. There was obtained a 32% yield of a solid which was 40% pure.

DL - α - Carbobenzyloxyamino - 2 - furylacetic acid, m.p. 121—122° C. was obtained in 40% yield by treating α - amino - 2 - furylacetic acid with benzyl chlorocarbonate in dilute sodium hydroxide solution. (Found: C, 61.3; H, 4.7; N, 4.9. $C_{14}H_{13}O_5N$ requires C, 61.1; H, 4.8; N, 5.1%).

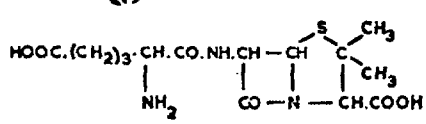
EXAMPLE 14.

Preparation of a mixture of the sodium salts of α- and δ-amino-δ-carboxybutyl penicillins.

A mixture of the sodium salts of α- and δ-carbobenzyloxyamino - δ - carboxybutyl penicillins (9.0 g. of 55% purity) was reduced with three successive 10 g. quantities of pretreated palladium on barium carbonate catalyst in water (3 × 150 ml.) at pH 8.0 as described in Example 1. The product (7.0 g., 82%) assayed for a purity of 42% and was shown by paper chromatography to contain two zones of anti-bacterial activity, one of which corresponded closely with that of the natural δ-amino - δ - carboxybutyl penicillin, i.e. Cephalosporin N (V). The other slightly faster running zone was acid stable and is probably the other isomer, α - amino - δ - carboxybutyl penicillin (VI).



(V)



(VI)

The starting material for this Example, i.e. the mixed sodium salts of α- and δ-carbobenzyl-

oxyamino - δ - carboxybutyl penicillins, was prepared by reacting N - carbobenzyloxy-DL - α - aminoadipic acid (1 equiv.) with ethyl chlorocarbonate (2 equivs.) to give the reactive mixed anhydride and then allowing this to react with 6-aminopenicillanic acid under the conditions described in Example 1. The product was shown by paper chromatography to contain two main constituents with slightly different R_F values, and to inhibit *Staph. aureus* at a concentration of 5.0 mcg./ml.

EXAMPLE 15.

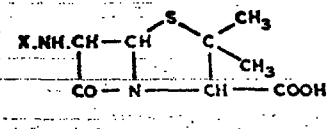
Preparation of α,ε-diaminopentyl penicillin.—The sodium salt of α,ε-dicarbobenzyloxyaminopentyl penicillin (2.0 g., of 68% purity) was reduced with two quantities (2 × 4.4 g.) of pre-treated palladium on barium carbonate in water (2 × 50 ml.) at pH 8.0 as in Example 1. The total time taken for the hydrogenation was 2 hours, the second quantity of catalyst being added after one hour. Isolation as in Example 1 gave α-ε-diaminopentylpenicillin as a hygroscopic solid (0.65 g.) which assayed for a purity of 22%. Paper chromatography showed that this material had a considerably different R_F value from that of the starting material. The product was shown to inhibit *Staph. aureus* at a concentration of 1.25 mcg./ml.

The α,ε - dicarbobenzyloxyaminopentyl penicillin was prepared by reacting N,N^1 - dicarbobenzyloxy - DL - lysine (1 equiv.) with ethyl chlorocarbonate (1 equiv.) to give the reactive mixed anhydride. This was then allowed to react with 6-aminopenicillanic acid under the conditions described in Example 1.

Following the above procedure, alanine, valine, *nor*-valine, leucine, *iso*-leucine, threonine, tyrosine, histidine, aspartic acid, glutamic acid, α-aminopimelic acid, α:β-diaminopropionic acid, ornithine and α:ε-diaminocaproic acid respectively, can be employed to produce α-aminoethyl penicillin, α-amino*iso*butyl penicillin, α-amino-*n*-butyl penicillin, α-amino-γ-methylbutyl penicillin, α-amino - β - methylbutyl penicillin, α - amino-β - hydroxypropyl penicillin, α - amino - β-(*p* - hydroxyphenyl)ethyl penicillin, α - amino-β - (4 - iminazolyl) ethyl penicillin, α - amino-β - carboxyethyl penicillin α - amino - γ - carboxypropyl penicillin, α - amino - ε - carboxypentyl penicillin, α:β - diaminoethyl penicillin, α:δ - diaminobutyl penicillin and α:ε-diaminopentyl penicillin respectively.

WHAT WE CLAIM IS:

1. Penicillin derivatives of the general formula:



(II)

where X is an amino-substituted acyl group containing up to 20 carbon atoms and having the formula

$$R(CH_2)_n.CH(NH_2).CO-$$

where R is hydrogen or an amino, carboxyl or substituted or unsubstituted alkyl, aryl, aralkyl or heterocyclic group and *n* is zero or an integer, and non-toxic salts thereof.

2. Penicillin derivatives as claimed in claim 1 wherein X in the general formula (II) is

$$R.(CH_2)_n.CH(NH_2).CO-$$

where *n* is zero or an integer from 1 to 6 and R is hydrogen or an alkyl, aryl, aralkyl or heterocyclic group which may also be substituted.

3. Penicillin derivatives as claimed in claim 2 wherein *n* is zero and R is a phenyl, chlorophenyl, methoxyphenyl, furyl or cyclohexyl group.

4. Penicillin derivatives as claimed in claim 1 wherein X in the general formula (II) is

$$H_2N.(CH_2)_n.CH(NH_2).CO-$$

where *n* is an integer from 1 to 4.

5. α-Aminobenzylpenicillin.

6. α-Amino-*p*-methoxybenzyl penicillin.

7. α-Amino-2-furylmethyl penicillin.

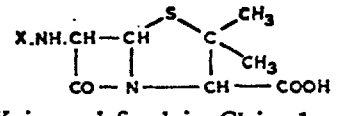
8. α-Aminoheptyl penicillin.

9. α-Aminopentyl penicillin.

10. α-Aminocyclohexylmethyl penicillin.

11. α-Amino-*p*-chlorobenzyl penicillin.

12. The process for the preparation of penicillin derivatives of the general formula:



where X is as defined in Claim 1, and non-toxic salts thereof, which comprises coupling 6-amino-penicillanic acid with an acid of the general formula XOH having its amino group or groups protected, or a salt thereof, and thereafter removing the protecting group or groups under sufficiently mild conditions to avoid destruction of the penicillin nucleus.

13. A process as claimed in claim 12 wherein the 6-aminopenicillanic acid is reacted with a mixed anhydride prepared by reacting the amino-substituted carboxylic acid, or a salt thereof, having its amino group or groups protected, with an ester of chlorocarbonic acid.

14. A process as claimed in claims 12 or 13 wherein the group or groups used to protect the amino-substituted carboxylic acid, or a salt thereof, is a trityl group or a group of the general formula $R^{11}O.CO-$ where R^{11} is an alkyl, benzyl, substituted benzyl, phenyl or substituted phenyl group.

15. A process as claimed in any one of the preceding claims 12 to 14 wherein the removal of the protecting group or groups is effected by catalytic hydrogenation.

oxyamino - δ - carboxybutyl penicillins, was prepared by reacting N - carbobenzyloxy-DL - α - aminoadipic acid (1 equiv.) with ethyl chlorocarbonate (2 equivs.) to give the reactive mixed anhydride and then allowing this to react with 6-aminopenicillanic acid under the conditions described in Example 1. The product was shown by paper chromatography to contain two main constituents with slightly different R_F values, and to inhibit *Staph. aureus* at a concentration of 5.0 mcg./ml.

EXAMPLE 15.

Preparation of α,ε-diaminopentyl penicillin.

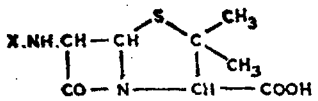
The sodium salt of α,ε-dicarbobenzyloxy-aminopentyl penicillin (2.0 g., of 68% purity) was reduced with two quantities (2 × 4.4 g.) of pre-treated palladium on barium carbonate in water (2 × 50 ml.) at pH 8.0 as in Example 1. The total time taken for the hydrogenation was 2 hours, the second quantity of catalyst being added after one hour. Isolation as in Example 1 gave α-ε-diaminopentylpenicillin as a hygroscopic solid (0.65 g.) which assayed for a purity of 22%. Paper chromatography showed that this material had a considerably different R_F value from that of the starting material. The product was shown to inhibit *Staph. aureus* at a concentration of 1.25 mcg./ml.

The α,ε - dicarbobenzyloxyaminopentyl penicillin was prepared by reacting N,N¹ - dicarbobenzyloxy - DL - lysine (1 equiv.) with ethyl chlorocarbonate (1 equiv.) to give the reactive mixed anhydride. This was then allowed to react with 6-aminopenicillanic acid under the conditions described in Example 1.

Following the above procedure, alanine, valine, *nor*-valine, leucine, *iso*-leucine, threonine, tyrosine, histidine, aspartic acid, glutamic acid, α-aminopimelic acid, α:β-diaminopropionic acid, ornithine and α:ε-diaminocaproic acid respectively, can be employed to produce α-aminoethyl penicillin, α-amino*iso*butyl penicillin, α-amino-*n*-butyl penicillin, α-amino-γ-methylbutyl penicillin, α-amino - β - methylbutyl penicillin, α - amino-β - hydroxypropyl penicillin, α - amino - β-(*p* - hydroxyphenyl)ethyl penicillin, α - amino-β - (4 - iminazolyl) ethyl penicillin, α - amino-β - carboxyethyl penicillin α - amino - γ - carboxypropyl penicillin, α - amino - ε - carboxypentyl penicillin, α:β - diaminoethyl penicillin, α:δ - diaminobutyl penicillin and α:ε-diaminopentyl penicillin respectively.

WHAT WE CLAIM IS:

1. Penicillin derivatives of the general formula:



(II)

where X is an amino-substituted acyl group containing up to 20 carbon atoms and having the formula

$$\underset{\displaystyle NH_2}{R(CH_2)_nCH.CO—}$$

where R is hydrogen or an amino, carboxyl or substituted or unsubstituted alkyl, aryl, aralkyl or heterocyclic group and *n* is zero or an integer, and non-toxic salts thereof.

2. Penicillin derivatives as claimed in claim 1 wherein X in the general formula (II) is

$$\underset{\displaystyle NH_2}{R.(CH_2)_n.CH.CO—}$$

where *n* is zero or an integer from 1 to 6 and R is hydrogen or an alkyl, aryl, aralkyl or heterocyclic group which may also be substituted.

3. Penicillin derivatives as claimed in claim 2 wherein *n* is zero and R is a phenyl, chlorophenyl, methoxyphenyl, furyl or cyclohexyl group.

4. Penicillin derivatives as claimed in claim 1 wherein X in the general formula (II) is

$$\underset{\displaystyle NH_2}{H_2N.(CH_2)_n.CH.CO—}$$

where *n* is an integer from 1 to 4.

5. α-Aminobenzylpenicillin.

6. α-Amino-*p*-methoxybenzyl penicillin.

7. α-Amino-2-furylmethyl penicillin.

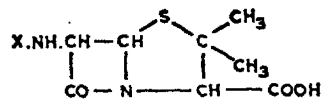
8. α-Aminoheptyl penicillin.

9. α-Aminopentyl penicillin.

10. α-Aminocyclohexylmethyl penicillin.

11. α-Amino-*p*-chlorobenzyl penicillin.

12. The process for the preparation of penicillin derivatives of the general formula:



where X is as defined in Claim 1, and non-toxic salts thereof, which comprises coupling 6-amino-penicillanic acid with an acid of the general formula XOH having its amino group or groups protected, or a salt thereof, and thereafter removing the protecting group or groups under sufficiently mild conditions to avoid destruction of the penicillin nucleus.

13. A process as claimed in claim 12 wherein the 6-aminopenicillanic acid is reacted with a mixed anhydride prepared by reacting the amino-substituted carboxylic acid, or a salt thereof, having its amino group or groups protected, with an ester of chlorocarbonic acid.

14. A process as claimed in claims 12 or 13 wherein the group or groups used to protect the amino-substituted carboxylic acid, or a salt thereof, is a trityl group or a group of the general formula $R^{11}O.CO—$ where R^{11} is an alkyl, benzyl, substituted benzyl, phenyl or substituted phenyl group.

15. A process as claimed in any one of the preceding claims 12 to 14 wherein the removal of the protecting group or groups is effected by catalytic hydrogenation.

16. A process as claimed in claim 15 wherein the catalytic hydrogenation is carried out employing a catalyst comprising palladium on a barium carbonate support.

17. A process for the preparation of penicillin derivatives as claimed in claim 1 substantially as described in any one of Examples 1, 5, 14 and 15.

18. A process for the preparation of penicillin derivatives as claimed in claim 1 substantially as described in any of Examples 2 to 4, 9 to 11 and 13.

19. A process for the preparation of penicillin derivatives as claimed in claim 1 substantially as described in any one of Examples 6 to 8 and 12.

20. Penicillin derivatives when prepared by the process claimed in any one of claims 12 to 19.

RONALD SMITHER,
Agent for the Applicants,
Chartered Patent Agent.

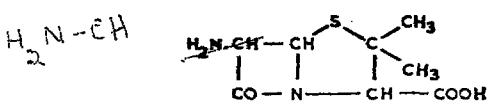
PROVISIONAL SPECIFICATION
No. 31847 A.D. 1958

Improvements in or relating to Penicillin Derivatives

We, BEECHAM RESEARCH LABORATORIES LIMITED, a British Company, of Brockham Park, Betchworth, Surrey, do hereby declare this invention to be described in the following statement:—

This invention relates to penicillin derivatives and is particularly concerned with derivatives of 6-aminopenicillanic acid.

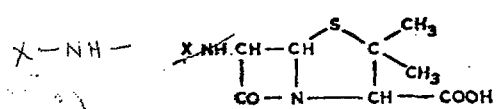
In our co-pending Application No. 26957/60 (Serial No. 870,396) we have described and claimed 6-aminopenicillanic acid having the structural formula:



(I)

It has now been found according to the present invention that new penicillin derivatives having valuable antibiotic activity can be obtained by introducing aminoacyl substituent groups into the amino group of 6-aminopenicillanic acid.

Accordingly, the present invention provides new penicillin derivatives of the general formula:



(II)

where X is an amino-substituted acyl group containing up to 20 carbon atoms, the carbon chain of which may be substituted by further amino groups and part may be present as an aromatic or heterocyclic ring system, and salts thereof. When the carbon chain is substituted by two amino groups or when the amino group is in the α position it may also contain a carboxyl group.

The present invention also includes a process for the preparation of penicillin derivatives of the general formula II where X is an amino-substituted acyl group containing up to 20 carbon atoms, the carbon chain of which may be substituted by a carboxyl group and/or further amino groups and part may be present as an aromatic or heterocyclic ring system, which process comprises reacting a protected amino-substituted carboxylic acid or a salt thereof with 6-aminopenicillanic acid and thereafter removing the protecting group or groups under sufficiently mild conditions to avoid destruction of the penicillin nucleus.

Examples of the group X in the general formula II are:

(i) $NH_2(CH_2)_nCO—$,

where n is an integer from 1 to 20.

(ii) $R(CH_2)_n.CH(NH_2).CO—$,

where n is zero or an integer from 1 to 6 and R is hydrogen or an alkyl, aryl, aralkyl or heterocyclic group which may also be substituted.

This group will include derivatives of the amino acids, alanine, valine, leucine, phenylalanine, tyrosine and tryptophane.

(iii) $HOOC.CH(NH_2)(CH_2)_nCO—$

and

$HOOC.(CH_2)_n.CH(NH_2).CO—$

This group will include derivatives of aspartic acid, glutamic acid and α-aminoadipic acid.

(iv) $H_2N(CH_2)_n.CH(NH_2).(CH_2)_mCO—$

This group will include derivatives of lysine.

(v) $HOOC.CH(NH_2).(CH_2)_n.CH(NH_2).CO—$

This group will include derivatives of α:δ-diamino adipic acid.

The majority of the derivatives mentioned above contain at least one asymmetric carbon atom and will exist in D- and L- forms. It is to be understood that the present invention includes both the D- and L- forms and the DL-mixture. When the derivative contains two asymmetric carbon atoms then there will be four isomers and two DL-mixtures.

The protected aminoacyl derivatives are preferably prepared in the manner described in

our co-pending Application No. 24607/57 (Serial No. 870,395). Thus the 6-aminopenicillanic acid may be reacted with a mixed anhydride prepared by reacting the amino-substituted carboxylic acid, or a salt thereof, having its amino group or groups protected, with ethyl cholorcarbonate. Alternatively, the protected amino-substituted carboxylic acid may be converted to a reactive acid halide.

Other methods used to form the protected aminoacyl derivative are standard procedures employed in peptide synthesis and include the use of a reactive acid azide $R^1.CO.N_3$ (where R^1 is a hydrocarbon group) or a carbodi-imide reagent (cf. Sheehan and Hess, *J. Amer. Chem. Soc.*, 1955, *77*, 1067).

The subsequent removal of the protecting group or groups to form the free amino-substituted penicillin is effected by catalytic hydrogenation and suitable protecting groups are of the general formula $R^{11}O.CO$—, where R^{11} is an allyl, benzyl, substituted benzyl, phenyl or substituted phenyl group, or the trityl group Ph$_3$C—. The abbreviation "Ph" as used herein represents the phenyl group.

The removal of the protecting group or groups is effected by allowing the protected aminoacyl derivative to react with hydrogen in the presence of a catalyst. This hydrogenation is normally carried out at room temperature and at atmospheric pressure, the pH of the reaction mixture being from 5 to 9. The solvent for the hydrogenation reaction is normally water, but other non-reducible solvents such as ethyl alcohol or dioxane or mixtures of these with water may be employed.

The preferred hydrogenation catalyst is palladium but other catalysts such as platinum or rhodium may be used. The catalyst is preferably employed on an inert support, e.g. of barium carbonate or carbon.

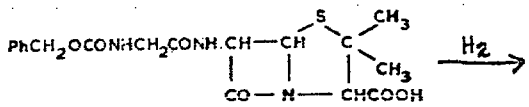
When the amino-substituted carboxylic acid contains more than one carboxyl group, e.g. α-aminoadipic acid, in order to prepare an unambiguous acyl derivative it is necessary either to protect one of the carboxyl groups before reaction with 6-aminopenicillanic acid or to prepare an unambiguous reactive derivative of one carboxyl group only.

The following Examples illustrate the invention:

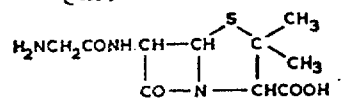
EXAMPLE 1.

Preparation of aminomethyl penicillin.

A suspension of palladium on barium carbonate (7.3 g., of 30%) in water (200 ml.) was shaken in an atmosphere of hydrogen at room temperature and atmospheric pressure for 2 hours. The sodium salt of carbobenzyloxyaminomethyl penicillin (III) (4.5 g., of a material assaying at a purity of 40%) was added and the pH adjusted to 8.0 with sodium hydrogen carbonate solution (3%). The mixture was then shaken under hydrogen at room temperature and atmospheric pressure for 45 mins. A second quantity of palladium on barium carbonate (7.3 g., of 30%), previously reduced as before, was added and the hydrogenation continued for a further 45 mins. The catalyst was filtered off, washed with water, and the filtrate, after adjusting to pH 7.0 with N hydrochloric acid, was evaporated to dryness under reduced pressure at a temperature below 20° C. The aminomethylpenicillin (IV) was obtained as a yellow powder (3.0 g.) which assayed for a purity of 33%. Paper chromatography showed that this material contained only one antibiotic which had a considerably different R_F value from the starting material. It was stable in acid solution.



(III)



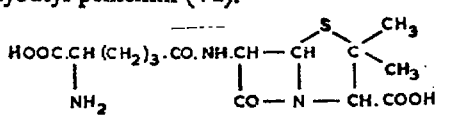
(IV) + Ph.CH$_3$ + CO$_2$

The preparation of the sodium salt of carbobenzyloxyaminomethyl penicillin is described in Example 11 of our co-pending Application No. 24607/57 (Serial No. 870,395).

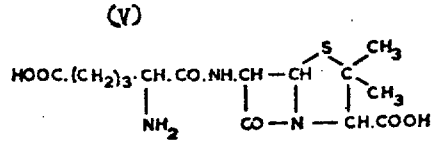
EXAMPLE 2.

Preparation of a mixture of the sodium salt of α- and δ-amino-δ-carboxybutyl penicillin.

A mixture of the sodium salts of α- and δ - carbobenzyloxyamino - δ - carboxybutyl penicillins (9.0 g. of 55% purity) was reduced with three quantities (3 × 10 g.) of pre-treated palladium on barium carbonate catalyst in water (3 × 150 ml.) at pH 8.0 as described in Example 1. The total time taken for the hydrogenation in this instance was 4 hours, the second quantity of catalyst being added at the end of 2 hours and the third quantity at the end of 3 hours. Isolation as in Example 1 gave the product (7.0 g., 82%) which assayed for a purity of 42%. This material was shown by paper chromatography to contain two zones of antibacterial activity, one of which corresponded closely with that of the natural δ-amino - δ - carboxybutyl penicillin, i.e. Cephalosporin N (V). The other slightly faster running zone was acid stable and is probably the other isomer, α - amino - δ - carboxybutyl penicillin (VI).



(V)



(VI)

The starting material for this Example, i.e.

the mixed sodium salts of α- and δ-carbobenzyloxyamino - δ - carboxybutyl penicillins, was prepared by reacting N-carbobenzyloxy-DL - α - aminoadipic acid (1 equiv.) with ethyl chlorocarbonate (2 equivs.) to give the reactive mixed anhydride and then allowing this to react with 6-aminopenicillanic acid under the conditions described in our co-pending Application No. 24607/57 (Serial No. 870,395) (cf. Example 11). The product was shown by paper chromatography to contain two main constituents with slightly different R_F values.

EXAMPLE 3.

Preparation of α,ε-diaminopentyl penicillin.

The sodium salt of α,ε - dicarbobenzyloxyaminophenyl penicillin (2.0 g., of 68% purity) was reduced with two quantities (2 × 4.4 g.) of pre-treated palladium on barium carbonate in water (2 × 50 ml.) at pH 8.0 as in Example 1. The total time taken for the hydrogenation was 2 hours, the second quantity of catalyst being added after 1 hour. Isolation as in Example 1 gave α-ε-diaminopentyl penicillin as a hygroscopic solid (0.65 g.) which assayed for a purity of 22%. Paper chromatography showed that this material had a considerably different R_F value from that of the starting material.

The α,ε - dicarbobenzyloxyaminopentyl penicillin was prepared by reacting N,N¹-dicarbobenzyloxy - DL-lysine (1 equiv.) with ethyl chlorocarbonate (1 equiv.) to give the reactive mixed anhydride. This was then allowed to react with 6-aminopenicillanic acid under the conditions described in our co-pending Application No. 24607/57 (Serial No. 870,395) (cf. Example 11.).

EXAMPLE 4.

Preparation of α - amino - β - phenylethyl penicillin.

The sodium salt of α-carbobenzyloxyamino-β - phenylethyl penicillin (7.6 g. of 53% purity) was reduced with three quantities (3 × 7.0 g.) of pre-treated palladium on barium carbonate in water (3 × 50 ml.) at pH 8.0, as in Example 1. The total time taken for the hydrogenation was 3 hours, the second quantity of catalyst and water being added after 1 hour and the third quantity of catalyst and water being added after 2 hours. Isolation as in Example 1 gave α - amino - β - phenylethyl penicillin as a hyrgoscopic solid (4.6 g.) which assayed for a purity of 30%. Paper chromatography showed that this material contained only one antibiotic which had a considerably different R_F value from that of the starting material.

The α - carbobenzyloxyamino - β - phenylethyl penicillin was prepared by reacting N-carbobenzyloxy - β - phenyl - DL - alanine (1 equiv.) with ethyl chlorocarbonate (1 equiv.) to give the reactive mixed anhydride. This was then allowed to react with 6-aminopenicillanic acid under the conditions described in our co-pending Application No. 24607/57 (Serial No. 870,395) (cf. Example 11).

RONALD SMITHER,
Agent for the Applicants,
Chartered Patent Agent.

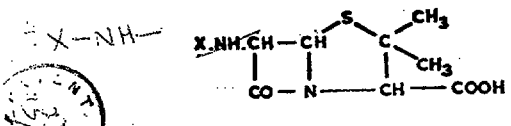
PROVISIONAL SPECIFICATION
No. 16303 A.D. 1959

Improvements in or relating to Penicillin Derivatives

We, BEECHAM RESEARCH LABORATORIES LIMITED, a British Company, of Brockham Park, Betchworth, Surrey, do hereby declare this invention to be described in the following statement: —

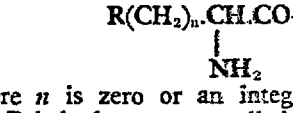
This invention relates to penicillin derivatives and is particularly concerned with derivatives of 6-aminopenicillanic acid.

In our co-pending Application No. 31847/58 we have described new penicillin derivatives of the general formula:



(I)

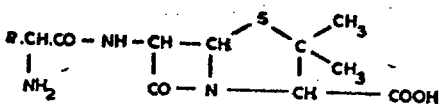
where X is an amino-substituted acyl group containing up to 20 carbon atoms, the carbon chain of which may be substituted by a carboxylic group and/or by further amino groups and part may be present as an aromatic or heterocyclic ring system, and salts thereof. An example of the group X in the general formula (I) is



where n is zero or an integer from 1 to 6 and R is hydrogen or an alkyl, aryl, aralkyl, or heterocyclic group which may also be substituted.

We have now found that certain compounds falling within the general formula (I) possess particularly desirable properties.

Accordingly, the present invention provides new penicillin derivatives of the general formula:



(II)

where R is a butyl, phenyl, chlorophenyl, methoxyphenyl, furyl, hexyl or cyclohexyl group.

The penicillin derivatives of the present invention may be prepared in the manner

31647/58 873,049

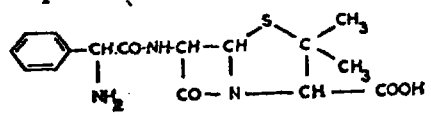
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described in Application No. 31847/58.

The following Examples illustrate the invention:

EXAMPLE 1.

Preparation of α-aminobenzylpenicillin.

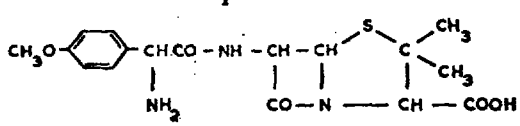


A suspension of palladium on barium carbonate (3.7 g. of 30%) in water (20 ml.) was shaken in an atmosphere of hydrogen at room temperature and atmospheric pressure for 1½ hrs. The catalyst was then filtered off, washed well with water, making sure that the catalyst did not become dry. A solution of the sodium salt of α-carbobenzyloxyaminobenzylpenicillin (4 g.) in water (20 ml.) was added to the pre-treated catalyst and the suspension shaken in an atmosphere of hydrogen at room temperature and pressure for 1 hr. The catalyst was then filtered off, washed well with water, and the combined filtrate and washings adjusted to pH 7.0 with N. hydrochloric acid. The resulting solution was then evaporated *in vacuo* at a temperature below 20° C. to give α-aminobenzylpenicillin (internal salt) (2.4 g. 74%) which assayed as 48% pure. Paper chromatography showed that this material contained only one antibiotic which had a considerably different R_f value from that of the starting material. It was stable in acid solution.

The sodium salt of α-carbobenzyloxyaminobenzylpenicillin required for the above preparation was obtained by reacting DL-α-carbobenzyloxyaminophenylacetic acid (1 equiv.) with ethyl chlorocarbonate (1 equiv.) to give the reactive mixed anhydride. This was then allowed to react with 6-aminopenicillanic acid under the conditions described in Example 11 of our co-pending Application No. 24607/57 (Serial No. 870,395). It was obtained as a yellow solid (65%) which assayed as 56% pure.

EXAMPLE 2.

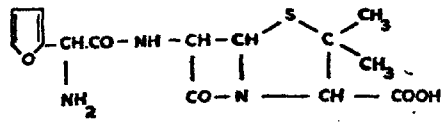
Preparation of α-amino-*p*-methoxybenzyl penicillin.



This was obtained according to the procedure described in Example 1 by the hydrogenation of the sodium salt of α-carbobenzyloxyamino - *p* - methoxybenzyl penicillin (59% pure) in 58% yield and assayed as 41% pure. Paper chromatography showed only one antibiotic with a considerably different R_f value from the starting material. It was stable in acid solution. DL-α-Carbobenzyloxyamino-*p*-methoxyphenylacetic acid was obtained in 60% yield, m.p. 105° C. (Found: C, 65.0; H, 5.5; N, 4.1. $C_{17}H_{17}NO_5$ requires C, 64.8; H, 5.4; N, 4.4%).

EXAMPLE 3.

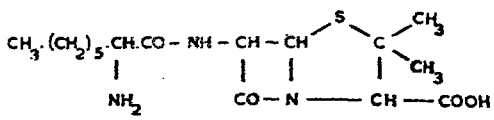
Preparation of α-amino-2-furylmethyl penicillin.



This was obtained according to the procedure described in Example 1 by the hydrogenation of the sodium salt of α-carbobenzyloxyamino - 2 - furylmethyl penicillin (40% pure) in 74% yield and assayed as 32% pure. Paper chromatography showed only one antibiotic with a considerably different R_f value from the starting material. It was stable in acid solution.

EXAMPLE 4.

Preparation of α-aminoheptyl penicillin.

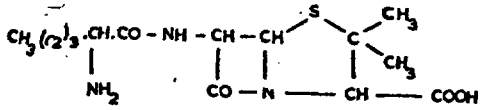


The sodium salt of α-carbobenzyloxyaminoheptyl penicillin (5.1 g., 68% pure) was added to a suspension of palladium on barium carbonate (4.7 g., 30%), which had been pre-treated as in Example 1, in water (150 ml.). The suspension was shaken in an atmosphere of hydrogen at room temperature and pressure for 1 hr. A second quantity (4.7 g., 30%) of pre-treated catalyst was added and the hydrogenation continued for a further hour. A third quantity (4.7 g., 30%) of pretreated catalyst was then added and again the hydrogenation continued for 1 hr. The catalyst was filtered off and the solution treated as in Example 1 to give α-aminoheptyl penicillin (internal salt) as a colourless solid (2.3 g., 44%) which assayed at 47% pure. Paper chromatography showed that this material contained only one antibiotic which had a considerably different R_f value from that of the starting material. It was stable in acid solution. The sodium salt of α-carbobenzyloxyaminoheptyl penicillin required for the above example was obtained by the reaction of DL-α-carbobenzyloxyaminooctanoic acid (1 equiv.) with ethyl chlorocarbonate (1 equiv.) to give the reactive mixed anhydride. This was then allowed to react with 6-aminopenicillanic acid under the conditions described in Example 11 of our co-pending Application No. 24607/57 (Serial No. 870,395). It was obtained as a yellow solid (43% yield) which assayed as 68%.

The DL-α-carbobenzyloxyamino-octanoic acid required for this preparation was obtained by the reaction of benzylchlorocarbonate with DL - α - amino - octanoic acid in dilute sodium hydroxide solution. It was obtained as colourless crystals (63%), m.p. 91—92° C. (Found: C, 65.7; H, 7.9; N, 4.9. $C_{16}H_{23}NO_4$ requires C, 65.5; H, 7.9; N, 4.7%).

EXAMPLE 5.

Preparation of α-aminopentyl penicillin.

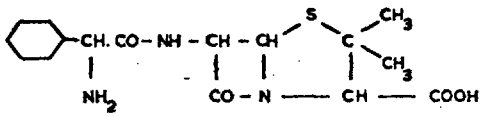


This was obtained according to the procedure described in example 4 by the hydrogenation of the sodium salt of α-carbobenzyloxyaminopentyl penicillin (56% pure) in 56% yield (assaying 34% pure). Paper chromatography showed only one antibiotic with a considerably different R_f value from the starting material. It was stable in acid solution.

DL - N - Carbobenzyloxy nor-leucine was obtained in 70% yield, m.p. 106—107° C.

EXAMPLE 6.

Preparation of α-aminocyclohexylmethyl penicillin.

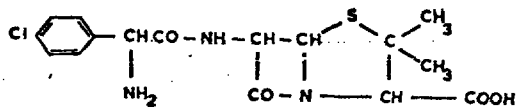


This was obtained according to the procedure described in Example 4 by the hydrogenation of the sodium salt of α-carbobenzyloxyaminocyclohexylmethyl penicillin (75%) in 51% yield assaying as 46% pure. Paper chromatography showed only one antibiotic with a considerably different R_f value from the starting material. It was stable in acid solution.

DL - α - Carbobenzyloxyaminocyclohexylacetic acid was obtained in 80% yield, m.p. 136—137° C. (Found: C, 66.2; H, 7.5; N, 4.6. $C_{16}H_{21}NO_4$ requires: C, 65.9; H, 7.3; N, 4.8%).

EXAMPLE 7.

Preparation of α-amino-*p*-chlorobenzyl penicillin.



This was obtained according to the procedure described in Example 1 by the hydrogenation of the sodium salt of α-carbobenzyloxyamino - *p* - chlorobenzyl penicillin (42% pure) in 88% yield and assayed as 45% pure.

It was stable in acid solution. DL-α-Carbobenzyloxyamino - *p* - chlorophenylacetic acid was obtained in 85% yield, m.p. 132—133° C. (Found: C, 60.5; H, 4.8; N, 4.7 $C_{16}H_{11}ClNO_4$ requires: C, 60.0; H, 4.4; N, 4.4%).

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