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PATENT SPECIFICATION

NO DRAWINGS

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International Classification:—C07d.

COMPLETE SPECIFICATION

Improvements in or relating to Penicillins

We, BEECHAM RESEARCH LABORATORIES LIMITED of Great West Road, Brentford, Middlesex, a British Company, 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:—

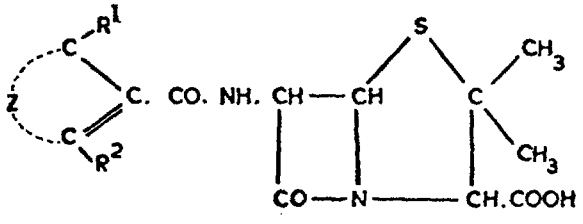
This invention relates to new penicillins, and is concerned with an improvement in or modification of the invention of our Specification No. 24607/57 (Serial No. 870,395).

The said Specification describes and claims a process for the preparation of 6-acylamino derivatives of penicillanic acid in which 6-aminopenicillanic acid or a salt thereof is reacted with a carboxylic acid chloride or bromide, a sulphonic acid chloride, an ester of chlorocarbonic acid, an acid anhydride of a carboxylic acid or a mixed anhydride derived from a carboxylic acid.

The present invention is particularly concerned with a new class of penicillins which are derivatives of 6-aminopenicillanic acid and which are of value as antibacterial agents, as nutritional supplements in animal food, as agents for the treatment of mastitis in cattle and as therapeutic agents in poultry and animals, including man, in the treatment especially of infectious diseases caused by Gram-positive bacteria.

Antibacterial agents such as benzylpenicillin have proved highly effective in the past in the therapy of infections due to Gram-positive bacteria but such agents suffer from serious drawbacks including that of being unstable in aqueous acid, e.g. upon oral administration, and of being ineffective against numerous so-called resistant strains of bacteria, e.g. penicillin-resistant strains of *Staphylococcus aureus*, which produce penicillinase. Many of the new penicillins of the present invention, in addition to their potent anti-bacterial activity, exhibit resistance to destruction by penicillinase and are thereby effective against resistant strains of bacteria. Additionally, some of the new penicillins are stable to acids.

According to the present invention there are provided new penicillins of the general formula:



(I)

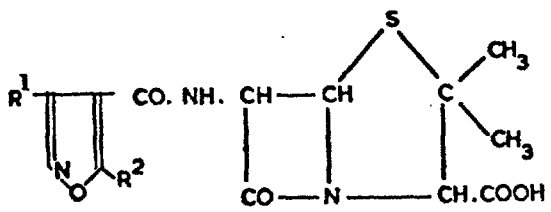
and non-toxic salts thereof, where Z is the residue of a substituted or unsubstituted heterocyclic ring system and R^1 and R^2 are the same or different and are each an alkyl, aryl, acyl, aralkyl, cycloalkyl, heterocyclic, alkoxy, aryloxy, aralkoxy, alkylthio, arylthio or aralkylthio group which may be substituted or a halogen atom, or R^1 or R^2 may form part of a further substituted or unsubstituted cyclic system fused to the ring containing Z.

The salts are non-toxic salts including non-toxic metallic salts such as sodium potassium, calcium and aluminium, ammonium and substituted ammonium salts, e.g. salts of such non-toxic amines as trialkylamines, including triethylamine, procaine, dibenzylamine, N-

[*Price 4s. 6d.*]

benzyl-beta-phenethylamine, 1-ephenamine, N,N¹-dibenzylethylenediamine, dehydroabietylamine, N,N¹ - bis - dehydroabietylethylenediamine, and other amines which have been used to form salts with benzylpenicillin.

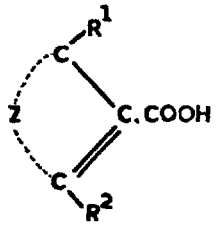
A preferred class of compounds has the general formula:



(II)

where R^1 and R^2 are the same or different and are each an alkyl, aryl, acyl, aralkyl, cycloalkyl, heterocyclic, alkoxy, aryloxy, aralkoxy, alkylthio, arylthio or aralkylthio group which may be substituted or a halogen atom. Of this class the compounds in which one of the groups R^1 and R^2 is a substituted or unsubstituted phenyl group and the other a lower alkyl group are of particular interest.

The present invention also provides a process for the preparation of the new penicillins of the general formula (I) and non-toxic salts thereof, wherein 6-aminopenicillanic acid or a liquor containing 6-aminopenicillanic acid, is reacted with an acid chloride, bromide, anhydride or mixed anhydride derived from a carboxylic acid of the general formula:



(III)

where Z, R^1 and R^2 are as defined above.

One method of preparing compounds of the present invention by way of a mixed anhydride of an alkyl chlorocarbonate comprises mixing an acid of the general formula (III) with the alkyl chlorocarbonate and a tertiary hydrocarbonyl or aliphatic amine such as triethylamine in an anhydrous, inert and preferably water-miscible solvent such as dioxane and if desired a small amount of pure, dry acetone. To this solution of the mixed anhydride there in then added a chilled solution of 6-aminopenicillanic acid and tertiary hydrocarbonyl amine, ~~i.e.~~ e.g. triethylamine, in, for example, a solvent such as water to form the substituted ammonium salt of the desired product. The mixture may then, if desired, be extracted at alkaline pH with a water-immiscible solvent such as ether to remove unreacted starting materials. The product in the aqueous phase is then converted to the free acid, preferably in the cold under a layer of ether by the addition of dilute mineral acid. The free acid is then extracted into a water-immiscible, neutral organic solvent such as ether and the extract is washed with water and then dried. The product in the ethereal extract in its free acid form is then converted to any desired metal or amine salt by treatment with the appropriate base, e.g. a free amine such as procaine base or a solution of potassium 2-ethylhexanoate in dry *n*-butanol. These salts are usually insoluble in solvents such as ether and can be recovered in pure form by simple filtration.

Another method of preparing an ethereal solution of the acid form of a compound of the present invention comprises preparing an aqueous solution of 6-aminopenicillanic acid and sodium bicarbonate and then adding the acid chloride. The mixture is then extracted with ether to remove unreacted or hydrolysed starting materials. The solution is then acidified and the free acid form of the product is extracted into ether. This etheral extract is dried, e.g. with anhydrous sodium sulphate, and the drying agent is removed to leave a dry ethereal solution from which the product is easily isolated, preferably in the form of an ether-insoluble salt such as the potassium salt. This procedure is used when the acid chloride reacts with a primary amine more rapidly than it does with water, as determined by simple test. In this procedure the acid chloride may be replaced by equimolecular amount of the corresponding acid bromide or acid anhydride.

In the case in which the acid chloride reacts more rapidly with water than with the 6-aminopenicillanic acid, it is necessary to use anhydrous conditions. Thus the 6-aminopenicillanic acid and triethylamine are mixed with an anhydrous solvent, e.g. acetone, chloroform or methylene dichloride and the acid chloride in the same solvent is added thereto. The mixture is then acidified and the aqueous layer removed. The solvent layer is then treated with sodium or potassium bicarbonate solution and the aqueous bicarbonate layer separated and concentrated to isolate the sodium or potassium salt of the penicillin.

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 conditions which are sufficiently moderate to avoid their decomposition. The reaction conditions chosen will, of course,

depend largely upon the reactivity of the chemical reagent being used. In most instances, a compromise has to be made between the use of very mild conditions for a lengthy period and the use of more vigorous conditions for a shorter time with the possibility of decomposing some of the antibiotic substances.

The temperature chosen for the process of the present invention should in general not exceed 30° C. and in many cases a suitable temperature is ambient temperature. Since the use of strongly acid or alkaline aqueous conditions in the process of this invention should be avoided, it has been found preferable to perform the process at a pH of from 6 to 9, and this can conveniently be achieved by using a buffer, for example a solution of sodium bicarbonate, or a sodium phosphate buffer. In addition to the use of aqueous media for the reaction, including filtered fermentation broths or aqueous solutions of crude 6-aminopenicillanic acid, use can be made of organic solvents, e.g. dimethylformamide, dimethylacetamide, chloroform, acetone, methylene dichloride, methyl isobutyl ketone and dioxane. Frequently it is highly satisfactory to add an aqueous solution of a salt of 6-aminopenicillanic acid to a solution of the acylating agent in an inert solvent which is miscible with water, such as acetone or dimethyl formamide. Vigorous stirring is, of course, advisable when more than one phase is present, e.g. solid and liquid or two liquid phases.

At the conclusion of the reaction, the products are isolated if desired by the techniques used with benzylpenicillin and phenoxymethylpenicillin. Thus, the product can be extracted into diethyl ether or *n*-butanol at acid pH and then recovered by lyophilisation or by conversion to a solvent-insoluble salt, as by neutralisation with an *n*-butanol solution of sodium or potassium 2-ethylhexanoate, or the product can be precipitated from aqueous solution as a water insoluble salt of an amine or recovered directly by lyophilisation, preferably in the form of a sodium or potassium salt. When formed as the triethylamine salt, the product is converted to the free acid form and thence to other salts in the manner used with benzylpenicillin and other penicillins. Thus, careful treatment of such a triethylamine compound in water with sodium hydroxide converts it to the sodium salt and the triethylamine may be removed by extraction, as with toluene. Treatment of the sodium salt with strong aqueous acid converts the compound to the acid form, which can be converted to other amine salts, e.g. procaine, by reaction with the amine base. Salts so formed are isolated by lyophilisation or, if the product is insoluble, by filtration. One method of isolating the product as a crystalline potassium salt comprises extracting the product from an acidic, aqueous solution (e.g. pH 2) into diethyl ether, drying the ether and adding at least one equivalent of a concentrated solution of potassium 2-ethylhexanoate in dry *n*-butanol. The potassium salt forms, precipitates, usually in crystalline form, and is collected by filtration or decantation.

The following Examples illustrate the invention: In these Examples essentially three different acylation procedures have been employed, designated Methods A, B and C.

Example I

Method A

4:6-Dimethyl-α-pyron-5-yl penicillin (sodium salt)

A solution of isodehydroacetyl chloride (Salemink, Rec. Trav. chim., 1959, *78*, 364) (7.5 g.) in dry chloroform (60 ml.) was slowly added to a stirred ice-cold mixture of 6-aminopenicillanic acid (8.64 g.), triethylamine (11 ml.) and dry chloroform (60 ml.), then the mixture was stirred at room temperature for 3 hours. After concentration *in vacuo* at room temperature, the mixture was shaken with water (120 ml.) and the layers were separated. The aqueous phase was brought to pH 7 by the addition of sodium bicarbonate and washed with ether (2 × 100 ml.) followed by butanol (2 × 30 ml.). It was then covered with butanol (30 ml.) and acidified to pH 3 with N hydrochloric acid. The layers were separated and the aqueous phase was extracted with two further 20 ml. portions of butanol. The combined butanol extracts (which at this stage contained the free penicillin acid) were washed with water (25 ml.) and then neutralised by adding 8% sodium bicarbonate solution (20 ml.) with vigorous shaking. The layers were separated and the aqueous phase was evaporated at low temperature and pressure to leave the impure sodium salt of 4:6-dimethyl-α-pyron-5-yl-penicillin as a hygroscopic brown solid which was finally dried in a vacuum desiccator. Yield, 3.55 g.

The product inhibited *Staph. Oxford* at a concentration of 5 mcg./ml.

Example II

Method B

2:4:5-Triphenyl-3-furylpenicillin (sodium salt)

A solution of 2:4:5-triphenyl-3-furoyl chloride (5.77 g.) in dry acetone (95 ml.) was added during 15 minutes to a stirred solution of 6-aminopenicillanic acid (3.46 g.) in 3% aqueous sodium bicarbonate (135 ml.) and acetone (40 ml.). The mixture was stirred for 4 hours and then filtered. The filtrate was extracted with ether (2 × 150 ml). The aqueous phase was then covered with ether (75 ml.) and brought to pH 2 by adding dilute hydrochloric acid. The layers were

separated and the aqueous phase was extracted with two further 50 ml. portions of ether. The combined ether extracts (which at this stage contained the free penicillin acid) were washed with water (3 × 25 ml.) and then shaken with sufficient 8% sodium bicarbonate solution to give a neutral aqueous phase (pH 7). The neutral aqueous layer was separated, washed with ether, and evaporated at low temperature and pressure. The residue was dried *in vacuo* over phosphorus pentoxide to leave the crude sodium salt of 2:4:5-triphenyl-3-furylpenicillin as a white powder (7.42 g.). Colorimetric assay with hydroxylamine against a benzylpenicillin standard indicated a purity of 36%.

The product inhibited *Staph. Oxford* at 5 mcg./ml., *Staph. 1* at 25 mcg./ml., and *Staph. 2* at 25 mcg./ml.

The 2:4:5-triphenyl-3-furoyl chloride used in this Example was prepared by warming the corresponding acid with thionyl chloride and a trace of pyridine, removing excess reagent *in vacuo* and crystallising the residue from light petroleum to give colourless needles, m.p. 97—98°C.

Example III

Method C

Preparation of 2,4-dimethoxy-3-quinolylpenicillin

Triethylamine (5.8 ml.) was added to a stirred suspension of 2,4-dimethoxyquinoline-3-carboxylic acid (4.66 g.) in dry chloroform (32 ml.) to give a clear yellow solution. The latter was cooled to −20° C. and treated during 5 minutes with a solution of thionyl chloride (1.64 ml.) in dry chloroform (6.5 ml.). The mixture was stirred for 30 minutes at −20°.

The resulting yellow solution, containing 2,4-dimethoxyquinoline-3-carbonyl chloride, was added during 10 minutes to a stirred suspension of 6-aminopenicillanic acid (4.33 g.) in dry chloroform (100 ml.) containing triethylamine (2.9 ml). The mixture was stirred for 3 hours, then filtered. The solution was evaporated at low temperature and pressure to give a brown gummy residue which was shaken with water (40 ml.) and ether (150 ml.).

The ether extract was evaporated at low temperature and pressure to leave a pale yellow solid residue of the crude triethylamine salt of 2,4-dimethyl-3-quinolylpenicillin (3.02 g.).

Colorimetric assay with hydroxylamine against a benzylpenicillin standard indicated it to be about 39% pure.

It inhibited *Staph. Oxford* at a concentration of 12.5 mg./ml., the benzylpenicillin-resistant *Staph. 1* at 25 mcg./ml., and the benzylpenicillin-resistant *Staph. 2* at 50 mcg./ml.

The 2,4-dimethoxyquinoline-3-carboxylic acid, m.p. 151—153°, was prepared by the action of methanolic sodium methoxide on ethyl 2,4-dichloroquinoline-3-carboxylate, followed by saponification.

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Example IV

5-Methyl-3-phenyl-4-isoxazolylpenicillin

A solution of 5-methyl-3-phenylisoxazole-4-carbonyl chloride (4.43 g.) in acetone (120 ml.) was added gradually to a stirred solution of 6-aminopenicillanic acid (4.32 g.) in 3% aqueous sodium bicarbonate (168 ml.) and acetone (50 ml.). When addition was complete the mixture was stirred at room temperature for 4 hours and then extracted with ether (2 × 200 ml.), only the aqueous phase being retained. This aqueous solution was covered with ether (50 ml.) and adjusted to pH 2 by the addition of N. hydrochloric acid. After separating the layers, the aqueous phase was extracted with two further 50 ml. portions of ether. The combined ether solutions (which at this stage contained the free penicillin acid) were washed with water and then neutralised by shaking with N. sodium bicarbonate solution (20 ml.). The aqueous phase was separated, washed with ether, and evaporated at low temperature and pressure to leave the crude sodium salt of 5-methyl-3-phenyl-4-isoxazolylpenicillin as a white solid, which was finally dried *in vacuo* over phosphorous pentoxide. The product (7.34 g.) was estimated by colourimetric assay with hydroxylamine against a benzylpenicillin standard to be about 66% pure.

It inhibited *Staph. Oxford* at a concentration of 0.25 mcg./ml, the benzylpenicillin-resistant *Staph. 1* at 1.25 mcg./ml. and the benzylpenicillin-resistant *Staph. 2* at 1.25 mcg./ml.

Example V

3:5-Diphenyl-4-isoxazolylpenicillin

A solution of 3:5-diphenylisoxazole-4-carbonyl chloride (750 mg) in acetone (16 ml.) was added dropwise to a stirred solution of 6-aminopenicillanic acid (575 mg.) in 3% aqueous sodium bicarbonate solution (23 ml.) and acetone (7 ml.). The mixture was stirred for 3 hours at room temperature and then worked up as described in Example IV, to give the sodium salt of 3:5-diphenyl-4-isoxazolylpenicillin as a cream-coloured solid (890 mg.). Colourimetric assay with hydroxylamine against a benzylpenicillin standard indicated a purity of 51%.

Example VI

Preparation of 3-methyl-5-phenyl-4-isoxazolylpenicillin

A solution of 3-methyl-5-phenylisoxazole-4-carbonyl chloride (9.95 g.) in dry acetone (140 ml.) was added during 15 minutes to

a stirred solution of 6-aminopenicillanic acid (9.75 g.) in 6% aqueous sodium bicarbonate (189 ml.) and acetone (50 ml.). The mixture was stirred for 4 hours and then extracted with ether (3 × 150 ml.), only the aqueous phase being retained. The latter was covered with ether (100 ml.) and adjusted to pH 2 by the addition of N. hydrochloric acid. After separating the layers, the aqueous phase was extracted with two further 75 ml. portions of ether. The combined ether solutions, containing the free penicillin acid, were washed with water (2 × 75 ml.) and then shaken with sufficient N. aqueous sodium bicarbonate solution to give a neutral aqueous phase (pH 7.). The aqueous phase was separated, washed with ether, and evaporated at low temperature and pressure to leave a solid residue of the crude sodium salt of 3 - methyl - 5 - phenyl - 4 - isoxazolylpenicillin (18.4 g.).

Colorimetric assay with hydroxylamine against a benzylpenicillin standard indicated it to be about 65% pure.

It inhibited *Staph. Oxford* at a concentration of 0.1 mcg./ml., the benzylpenicillin resistant *Staph. 1* at 1.25 mcg./ml., and the benzylpenicillin-resistant *Staph. 2* at 1.25 mcg./ml.

Example VII

3,5-Dimethyl-4-isoxazolylpenicillin

A solution of 3,5-dimethylisoxazole-4-carbonyl chloride (3.71 g.) in acetone (136 ml.) was added during 10 minutes to a stirred solution of 6-aminopenicillanic acid (4.98 g.) in 3% aqueous sodium bicarbonate (193 ml.) and acetone (57 ml.). The mixture was stirred for 4 hours and then worked up as described in Example VI to give the sodium salt of 3,5 - dimethyl - 4 - isoxazolylpenicillin (6.23 g.).

Colorimetric assay with hydroxylamine against a benzylpenicillin standard indicated a purity of 68%.

It inhibited *Staph. Oxford* at 0.25 mcg./ml.

Example VIII

5-Benzyl-3-methyl-4-isoxazolylpenicillin

The reaction between 6-aminopenicillanic acid (4.33 g.) and 5-benzyl-3-methylisoxazole-4-carbonyl chloride (4.75 g.) under the conditions described in Example VI, gave the sodium salt of 5-benzyl-3-methyl-4-isoxazolylpenicillin (7.85 g.).

Colorimetric assay with hydroxylamine against a benzylpenicillin standard indicated a purity of 68%.

It inhibited *Staph. Oxford* at 0.5 mcg./ml.

5 - Benzyl - 3 - methylisoxazole - 4 - carboxylic acid, m.p. 139—140° C., was obtained from the reaction between ethyl α-(phenylacetyl)acetoacetate and hydroxylamine hydrochloride in aqueous ethanol followed by hydrolysis of the ester product. Reaction of the acid with thionyl chloride gave 5-benzyl - 3 - methylisoxazole - 4 - carbonyl chloride, b.p. 144—146° C at 2 mm.

Example IX

3-Methyl-5-styryl-4-isoxazolylpenicillin

The reaction between 6-aminopenicillanic acid (7.15 g.) and 3 - methyl - 5 - styrylisoxazole-4-carbonyl chloride (8.35 g. crude product, m.p. 108—109° C. from treatment of the acid with thionyl chloride) under the conditions described in Example VI gave the sodium salt of 3 - methyl - 5 - styryl - 4-isoxazolylpenicillin (13.16 g.).

Colorimetric assay with hydroxylamine against a benzylpenicillin standard indicated a purity of 73%.

it inhibited *Staph. Oxford* at 0.1 mcg./ml., *Staph. 1* at 2.5 mcg./ml., and *Staph. 2* at 1.25 mcg./ml.

Example X

5-tert-Butyl-3-phenyl-4-isoxazolyl penicillin

The reaction between 6-aminepenicillanic acid (3.90 g.) and 5 - tert - butyl - 3 - phenylisoxazole-4-carbonyl chloride (4.95 g., b.p. 140—141°/1.75 mm., from treatment of the acid with thionyl chloride) under the conditions described in Example VI gave the sodium salt of 5 - tert - butyl - 3 - phenyl-4-isoxazolylpenicillin (7.45 g.).

Colorimetric assay with hydroxylamine against a benzylpenicillin standard indicated a purity of 44%.

It inhibited *Staph Oxford* at 5 mcg./ml., *Staph. 1* at 12.5 mcg./ml., and *Staph. 2* at 12.5 mcg./ml.

Example XI

5-(2-Furyl)-3-methyl-4-isoxazolylpenicillin

The reaction between 6-aminopenicillanic acid (10.3 g.) and 5 - (2 - furyl) - 3 - methylisoxazole-4-carbonyl chloride (9.6 g; crude product from treatment of the acid with thionyl chloride), under the conditions described in Example VI gave the sodium salt of 5 - (2 - furyl) - 3 - methyl - 4-isoxazolylpenicillin (17.06 g.).

Colorimetric assay with hydroxylamine against benzylpenicillin standard indicated a purity of 69%.

It inhibited *Staph Oxford* at 0.1 mcg./ml., *Staph. 1* at 2.5 mcg./ml. and *Staph. 2* at 2.5 mcg./ml.

The 5 - (2 - furyl) - 3 - methylisoxazole-4-carboxylic acid, m.p. 222—224° C. from which the acid chloride was prepared, resulted from the reaction between ethyl α-(2-furoyl)-acetoacetate and hydroxylamine hydrochloride in aqueous ethanol, followed by hydrolysis of the ester product.

Example XII

3-Methyl-5-(3¹,5¹-dimethyl-4¹-isoxazolyl)-4-isoxazolyl penicillin.

The reaction between 6-aminopenicillanic

acid and (9.35 g.) and 3 - methyl - 5 - (3¹,5¹-dimethyl - 4¹ - isoxazolyl) - isoxazole - 4-carbonyl chloride (10.42 g., b.p. 122—124° at 0.9 mm, from treatment of the acid with thionyl chloride) under the conditions described in Example VI gave the sodium salt of 3 - methyl - 5 - (3¹,5¹ - dimethyl - 4¹-isoxazolyl) - 4 - isoxazolylpenicillin (18.5 g.) as a pale yellow solid.

Colorimetric assay with hydroxylamine against a benzylpenicillin standard indicated a purity of 60%.

It inhibited *Staph. Oxford* at 1.25 mg./ml., *Staph. 1* at 2.5 mcg./ml and *Staph. 2* at 5 mcg/ml.

The 3 - methyl - 5 - (3¹,5¹ - dimethyl - 4¹-isoxazolyl) - isoxazole - 4 - carboxylic acid used to prepare the acid chloride was obtained as white needles, m.p. 187—188° C. from the reaction between ethyl α-(3,5-dimethyl-isoxazole - 4 - carbonyl) - acetoacetate and hydroxylamine hydrochloride in aqueous ethanol and hydrolysis of the ester product.

EXAMPLE XIII

3-Methyl-5-(2-thienyl)-4-isoxazolyl-penicillin.

The reaction between 6-aminopenicillanic acid (6.48 g.) and 3 - methyl - 5 - (2-thienyl)isoxazole - 4 - carbonyl chloride (7.02 g., m.p. 109—110° C, from treatment of the acid with thionyl chloride) under the conditions described in Example VI gave the sodium salt of 3 - methyl - 5 - (2 - thienyl)-4 - isoxazolylpenicillin (11.97 g.) as a pale yellow solid.

Colorimetric assay with hydroxylamine against a benzylpenicillin standard indicated a purity of 70%.

It inhibited *Staph. Oxford* at 0.25 mcg./ml., *Staph. 1* at 1.25 mcg./ml., and *Staph. 2* at 1.25 mcg./ml.

The 3 - methyl - 5 - (2 - thienyl)-isoxazole - 4 - carboxylic acid, m.p. 200—201° C. from which the acid chloride was obtained, was prepared by hydrolysis of the ester product of the reaction between ethyl α-(2-thenoyl)-acetoacetate (from ethyl acetoacetate, sodium, and 2-thenoyl chloride in benzene) and hydroxylamine hydrochloride in aqueous ethanol.

EXAMPLE XIV

3-*p*-Chlorophenyl-5-methyl-4-isoxazolyl-penicillin.

The reaction between 6-aminopenicillanic acid (6.5 g.) and 3 - *p* - chlorophenyl - 5-methylisoxazole-4-carbonyl chloride (7.66 g., m.p. 70—71°C., from treatment of the acid with thionyl chloride) under the conditions described in Example VI gave the sodium salt of 3 - *p* - chlorophenyl - 5 - methyl - 4-isoxazolylpenicillin (9.98 g.) as a pale yellow solid.

Colorimetric assay with hydroxylamine against a benzylpenicillin standard indicated a purity of 68%.

It inhibited *Staph. Oxford* at 0.02 mcg./ml., *Staph. 1* at 0.5 mcg./ml., and *Staph. 2* at 0.25 mcg./ml.

The 3 - *p* - chlorophenyl - 5 - methyl-isoxazole - 4 - carboxylic acid, m.p. 212—213°C., from which the acid chloride was prepared, was obtained by hydrolysis of the ester product of the reaction between p-chlorobenzohydroxamic chloride and ethyl acetoacetate in methanolic sodium methoxide.

EXAMPLE XV

3-Methyl-5-methylmercapto-4-isoxazolyl-penicillin.

A solution of 3 - methyl - 5 - methylmercaptoisoxazole - 4 - carbonyl chloride (5.75 g., crude product from treatment of the acid with thionyl chloride) in dry chloroform was added during 10 minutes to a stirred suspension of 6-aminopenicillanic acid (6.5 g.) in dry chloroform (150 ml.) containing triethylamine (12.6 ml.). The mixture was stirred for 2 hours, then it was filtered. The chloroform solution was shaken with N. hydrochloric acid to give an aqueous phase of pH 2, only the chloroform phase being retained. The latter was washed with water (2 × 100 ml.), treated with charcoal, and filtered.

The chloroform solution was shaken with sufficient N. aqueous sodium bicarbonate solution to give a neutral aqueous phase (pH 7.). The layers were separated, and the chloroform solution was extracted with water. The combined aqueous extracts were evaporated at low temperature and pressure to leave a brown solid residue of the crude sodium salt of 3 - methyl - 5 - methylmercapto - 4 - isoxazolylpenicillin (3.32 g.).

Colorimetric assay with hydroxylamine against a benzylpenicillin standard indicated a purity of 31%.

It inhibited *Staph. Oxford* at 0.2 mcg./ml.

EXAMPLE XVI

5-*p*-Chlorophenyl-3-methyl-4-isoxazolyl-penicillin.

A solution of 5 - *p* - chlorophenyl - 3-methylisoxazole - 4 - carbonyl chloride (13.5 g.) in dry acetone (320 ml.) was added during 10 minutes to a stirred solution of 6-aminopenicillanic acid (11.5 g.) in 3% aqueous sodium bicarbonate (440 ml.) and acetone (130 ml.). The mixture was stirred for 2 hrs. and then extracted with ether (2 × 200 ml.), only the aqueous phase being retained. The latter was covered with ether (100 ml.) and adjusted to pH 2 by the addition of N. hydrochloric acid. After separating the layers, the aqueous phase was extracted with two further 100 ml. portions of ether. The combined ether solutions, containing the free penicillin acid, were washed with water (2 × 50 ml.) and then shaken with sufficient N. aqueous sodium bicarbonate solution to give a neutral aqueous phase (pH 7). The aqueous phase was separated, washed with ether and

evaporated at low temperature and pressure to leave a solid residue of the crude sodium salt of 5 - *p* - chlorophenyl - 3 - methyl - 4-isoxazolylpenicillin (21.4 g.).

Colorimetric assay with hydroxylamine against a benzylpenicillin standard indicated it to be about 69% pure.

The product inhibited *Staph. Oxford* at a concentration of 0.02 mcg./ml., *Staph. 1* at 0.5 mcg./ml., and *Staph. 2* at 0.25 mcg./ml.

EXAMPLE XVII

3-Methyl-5-*o*-nitrophenyl-4-isoxazolylpenicillin.

A solution of 3 - methyl - 5 - *o* - nitrophenyl - isoxazole - 4 - carbonyl chloride (13.6 g.) in dry acetone (300 ml.) was added during 10 mins. to a stirred solution of 6-aminopenicillanic acid (10.8 g.) in 3% aqueous sodium bicarbonate (420 ml.) and acetone (125 ml.). The mixture was stirred for 3 hrs. and then extracted with ether (3 × 200 ml.), only the aqueous phase being retained. The latter was covered with ether (100 ml.) and adjusted to pH 2 by the addition of N. hydrochloric acid. After separating the layers, the aqueous phase was extracted with two further 75 ml. portions of ether. The combined ether solutions were washed with water (2 × 50 ml.) and then shaken with sufficient N. aqueous sodium bicarbonate solution to give a neutral aqueous phase (pH 7). The layers were separated, and the ether solution was extracted with water (25 ml.). The combined aqueous extracts were washed with ether and evaporated at low temperature and pressure to leave a yellow solid residue of the crude sodium salt of 3 - methyl - 5 - *o* - nitrophenyl - 4 - isoxazolylpenicillin (23 g.).

Colorimetric assay with hydroxylamine against a benzylpenicillin standard indicated it to be about 67% pure.

The product inhibited *Staph. Oxford* at a concentration of 0.1 mcg./ml., *Staph. 1* at 1.25 mcg./ml., and *Staph. 2* at 0.5 mcg./ml.

EXAMPLE XVIII

The following penicillins were prepared by reaction of the named acids with 6-aminopenicillanic acid, by way of the acid chloride or other suitable intermediate in accordance with the procedure of Method A, B or C:—

Acid	*Penicillin*
3 - *o* - Chlorophenyl - 5 - methylisoxazole - 4 - carboxylic acid.	3 - *o* - Chlorophenyl - 5 - methyl - 4-isoxazolylpenicillin.
3 - *m* - Chlorophenyl - 5 - methylisoxazole - 4 - carboxylic acid.	3 - *m* - Chlorophenyl - 5 - methyl - 4-isoxazolylpenicillin.
3 - (2,4 - Dichlorophenyl) - 5 - methylisoxazole - 4 - carboxylic acid.	3 - (2,4 - Dichlorophenyl) - 5 - methyl-4 - isoxazolylpenicillin.
3 - (3,4 - Dichlorophenyl) - 5 - methylisoxazole - 4 - carboxylic acid.	3 - (3,4 - Dichlorophenyl) - 5 - methyl-4 - isoxazolylpenicillin.
3 - *p* - Tolyl - 5 - methylisoxazole - 4-carboxylic acid.	3 - *p* - Tolyl - 5 - methyl - 4 - isoxazolylpenicillin.
3 - *o* - Nitrophenyl - 5 - methylisoxazole-4 - carboxylic acid.	3 - *o* - Nitrophenyl - 5 - methyl - 4-isoxazolylpenicillin.
3 - *m* - Nitrophenyl - 5 - methylisoxazole - 4 - carboxylic acid.	3 - *m* - Nitrophenyl - 5 - methyl - 4-isoxazolylpenicillin.
3 - *p* - Nitrophenyl - 5 - methylisoxazole-4 - carboxylic acid.	3 - *p* - Nitrophenyl - 5 - methyl - 4-isoxazolylpenicillin.
3 - *p* - Bromophenyl - 5 - methylisoxazole - 4 - carboxylic acid.	3 - *p* - Bromophenyl - 5 - methyl - 4-isoxazolylpenicillin.
3 - *p* - Fluorophenyl - 5 - methylisoxazole - 4 - carboxylic acid.	3 - *p* - Fluorophenyl - 5 - methyl - 4-isoxazolylpenicillin.
3 - *p* - Methylsulphonylphenyl - 5-methylisoxazole - 4 - carboxylic acid.	3 - *p* - Methylsulphonylphenyl - 5-methyl - 4 - isoxazolylpenicillin.
3 - *p* - Methoxyphenyl - 5 - methylisoxazole - 4 - carboxylic acid.	3 - *p* - Methoxyphenyl - 5 - methyl - 4-isoxazolylpenicillin.

Acid	*Penicillin*
3 - *o* - Methoxyphenyl - 5 - methyl-isoxazole - 4 - carboxylic acid.	3 - *o* - Methoxyphenyl - 5 - methyl - 4-isoxazolylpenicillin.
3 - *p* - Ethoxyphenyl - 5 - methyl-isoxazole - 4 - carboxylic acid.	3 - *p* - Ethoxyphenyl - 5 - methyl - 4-isoxazolylpenicillin.
3 - (3,4 - Dimethoxyphenyl) - 5 - methyl-isoxazole - 4 - carboxylic acid.	3 - (3,4 - Dimethoxyphenyl) - 5 - methyl-4 - isoxazolylpenicillin.
3 - *p* - Dimethylaminophenyl - 5-methylisoxazole - 4 - carboxylic acid.	3 - *p* - Dimethylaminophenyl - 5-methyl - 4 - isoxazolylpenicillin.
3 - α - Naphthyl - 5 - methylisoxazole-4 - carboxylic acid.	3 - α - Naphthyl - 5 - methyl - 4-isoxazolylpenicillin.
3 - β - Naphthyl - 5 - methylisoxazole-4 - carboxylic acid.	3 - β - Naphthyl - 5 - methyl - 4-isoxazolylpenicillin.
3 - Phenyl - 5 - ethylisoxazole - 4-carboxylic acid.	3 - Phenyl - 5 - ethyl - 4 - isoxazolyl-penicillin.
3 - *p* - Chlorophenyl - 5 - ethylisoxazole-4 - carboxylic acid.	3 - *p* - Chlorphenyl - 5 - ethyl - 4-isoxazolylpenicillin.
3 - Phenyl - 5 - isopropyl - isoxazole - 4-carboxylic acid.	3 - Phenyl - 5 - isopropyl - 4 - isoxazolyl-penicillin.
3 - Phenyl - 5 - methylmercaptoisoxazole-4 - carboxylic acid.	3 - Phenyl - 5 - methylmercapto - 4-isoxazolylpenicillin.
3 - Methyl - 5 - *o* - Chlorophenyl-isoxazole - 4 - carboxylic acid.	3 - Methyl - 5 - *o* - chlorophenyl - 4-isoxazolylpenicillin.
3 - Methyl - 5 - *p* - bromophenyl-isoxazole - 4 - carboxylic acid.	3 - Methyl - 5 - *p* - bromophenyl - 4-isoxazolylpenicillin.
3 - Methyl - 5 - *o* - iodophenylisoxazole-4 - carboxylic acid.	3 - Methyl - 5 - *o* - iodophenyl - 4-isoxazolylpenicillin.
3 - Methyl - 5 - (2,4 - dichlorophenyl)-isoxazole - 4 - carboxylic acid.	3 - Methyl - 5 - *o* - chlorophenyl - 4-isoxazolylpenicillin.
3 - Methyl - 5 - *p* - tolylisoxazole - 4-carboxylic acid.	3 - Methyl - 5 - *p* - tolyl - 4 -isoxazolyl-penicillin.
3 - Methyl - 5 - *m* - nitrophenyl-isoxazole - 4 - carboxylic acid.	3 - Methyl - 5 - *m* - nitrophenyl - 4-isoxazolylpenicillin.
3 - Methyl - 5 - *p* - nitrophenylisoxazole-4 - carboxylic acid.	3 - Methyl - 5 - *p* - nitrophenyl - 4-isoxazolylpenicillin.
3 - Methyl - 5 - *p* - methoxyphenyl-isoxazole - 4 - carboxylic acid.	3 - Methyl - 5 - *p* - methoxyphenyl - 4-isoxazolylpenicillin.
3 - Methyl - 5 - *p* - ethoxyphenyl-isoxazole - 4 - carboxylic acid.	3 - Methyl - 5 - *p* - ethoxyphenyl - 4-isoxazolylpenicillin.
3 - Methyl - 5 - (2,6 - dimethoxyphenyl)-isoxazole - 4 - carboxylic acid.	3 - Methyl - 5 - (2,6 - dimethoxyphenyl)-4 - isoxazolylpenicillin.
3 - Methyl - 5 - *p* - methylsulphonyl-isoxazole - 4 - carboxylic acid.	3 - Methyl - 5 - *p* - methylsulphonyl-phenyl - 4 - isoxazolylpenicillin.

Acid	*Penicillin*
3 - Methyl - 5 - *p* - fluorophenyl-isoxazole - 4 - carboxylic acid.	3 - Methyl - 5 - *p* - fluorophenyl - 4-isoxazolylpenicillin.
3 - Methyl - 5 - *p* - cyanophenylisoxazole-4 - carboxylic acid.	3 Methyl - 5 - *p* - cyanophenyl - 4-isoxazolylpenicillin.
3 - Methyl - 5 - *p* - methylmercapto-phenylisoxazole - 4 - carboxylic acid.	3 - Methyl - 5 - *p* - methylmercapto-phenyl - 4 - isoxazolylpenicillin.
3 - Methyl - 5 - *p* - dimethylaminophenyl-isoxazole - 4 - carboxylic acid.	3 - Methyl - 5 - *p* - dimethylamino-phenyl - 4 - isoxazolylpenicillin.
3 - Methyl - 5 - α - naphthylisoxazole-4 - carboxylic acid.	3 - Methyl - 5 - α - naphthyl - 4-isoxazolylpenicillin.
3 - Methyl - 5 - β - naphthylisoxazole-4 - carboxylic acid.	3 - Methyl - 5 - β - naphthyl - 4-isoxazolylpenicillin.
3 - Ethyl - 5 - phenylisoxazole - 4 - carboxylic acid.	3 - Ethyl - 5 - phenyl - 4 - isoxazolyl-penicillin.
3 - Ethyl - 5 - *p* - chlorophenylisoxazole-4 - carboxylic acid.	3 - Ethyl - 5 - *p* - chlorophenyl - 4-isoxazolylpenicillin.
3 - Isopropyl - 5 - phenylisoxazole - 4-carboxylic acid.	3 - Isopropyl - 5 - phenyl - 4 - isoxazolyl-penicillin.
3 - *tert.* Butyl - 5 - methylisoxazole - 4-carboxylic acid.	3 - *tert.* Butyl - 5 - methyl - 4-isoxazolylpenicillin.
3 - Methyl - 5 - cyclohexylisoxazole - 4-carboxylic acid.	3 - Methyl - 5 - cyclohexyl - 4-isoxazolylpenicillin.
3 - Cyclohexyl - 5 - methylisoxazole-4 - carboxylic acid.	3 - Cyclohexyl - 5 - methyl - 4-isoxazolylpenicillin.
3 - α - Furyl - 5 - methylisoxazole - 4-carboxylic acid.	3 - α - Furyl - 5 - methyl - 4 - isoxazolyl-penicillin.
3 - α - Thienyl - 5 - methylisoxazole-4 - carboxylic acid.	3 - α - Thienyl - 5 - methyl - 4-isoxazolylpenicillin.

FURAN SERIES

EXAMPLE XIX

Preparation of 2,4,5-trimethyl-3-furyl-penicillin.

A solution of 2,4,5-trimethyl-3-furoyl chloride (3.45 g.) in dry acetone (120 ml.) was added during 10 minutes to a stirred solution of 6-aminopenicillanic acid (4.33 g.) in 3% aqueous sodium bicarbonate. (168 ml.) and acetone (50 ml.). The mixture was stirred for 3½ hours. It was extracted with ether (2 × 150 ml.) only the aqueous phase being retained. The latter was covered with ether (50 ml.) and adjusted to pH 2 by adding N. hydrochloric acid. The layers were separated and the ether layer was washed with water (2 × 25 ml.). The ether solution, containing the free penicillin acid, was shaken with sufficient N. aqueous sodium bicarbonate to give a neutral aqueous phase (pH 7). The aqueous layer was separated, washed with ether, and evaporated at low temperature and pressure to leave a straw-coloured residue of the crude sodium salt of 2,4,5 - trimethyl - 3-furylpenicillin (1.33 g.).

Colorimetric assay with hydroxylamine against a benzylpenicillin standard indicated it to be about 42% pure.

It inhibited *Staph. Oxford* at a concentration of 0.6 mcg./ml.

The 2:4:5 - trimethyl - 3 - furoyl chloride, b.p. 39—41°/0.02 mm., was prepared from the corresponding acid and thionyl chloride.

EXAMPLE XX

Preparation of 4,5-dimethyl-2-phenyl-3-furylpenicillin.

A solution of 4,5 - dimethyl - 2 - phenyl-3-furoyl chloride (3.0 g.) in dry chloroform (16 ml.) was added during 10 minutes to a stirred suspension of 6-aminopenicillanic acid

(2.6 g.) in dry chloroform (60 ml.) containing triethylamine (5.1 ml.). The mixture was stirred for 4 hours, then filtered. The chloroform solution was shaken with N. hydrochloric acid to give an aqueous phase of pH 2, only the chloroform phase being retained. The latter was washed with water (2 × 50 ml.).

The chloroform solution was shaken with the theoretical amount of N. aqueous sodium bicarbonate solution (12½ml.) to give a thick yellow emulsion which was evaporated at low temperature and pressure to leave a solid residue of the crude sodium salt of 4,5-dimethyl - 2 - phenyl - 3 - furylpenicillin (4.63 g.).

Colorimetric assay with hydroxylamine assay against a benzylpenicillin standard indicated it to be about 48% pure.

It inhibited *Staph. Oxford* at a concentration of 1.25 mcg./ml., the benzylpenicillin-resistant *Staph. 1* at 6 mcg./ml., and the benzylpenicillin-resistant *Staph. 2* at 5 mcg./ml.

The 4,5 - dimethyl - 2 - phenyl - 3-furoyl chloride, b.p. 98—100°/0.01 mm., was prepared by warming 4,5 - dimethyl - 2-phenyl - 3 - furoic acid with thionyl chloride. The acid, m.p. 145—147°, was itself obtained by condensing acetoin with ethyl benzoylacetate in the presence of anhydrous zinc chloride, followed by saponification of the resulting ester.

THIOPHENE SERIES

EXAMPLE XXI

4-Bromo-2,5-dimethyl-3-thienyl-penicillin.

Triethylamine (3.81 g.) was added to a stirred suspension of 6-aminopenicillanic acid (2.98 g.) in dry alcohol-free chloroform (21 ml.). The mixture was cooled to 0° and a solution of 4 - bromo - 2,5 - dimethyl thiophene-3-carbonyl chloride (3.50 g.) in chloroform (21 ml.) was added dropwise over about 10 minutes. The cooling bath was removed and the mixture was stirred for 2½ hours at room temperature, then filtered. The filtrate was washed with 1N and then with 0.1N hydrochloric acid (13.8 ml. of each), the washings being discarded. The chloroform layer was shaken vigorously with 1N. aqueous sodium bicarbonate (14 ml.) and the layers were separated by centrifuging. The bicarbonate extract was evaporated at low temperature and pressure to give the crude sodium salt of 4 - bromo - 2,5 - dimethyl - 3-thienyl-penicillin as a yellow solid (4.07 g.), estimated by colorimetric assay with hydroxylamine against benzylpenicillin standard to be about 54% pure.

It inhibited *Staph. Oxford* at a concentration of 0.6 mcg./ml., the benzylpenicillin-resistant *Staph. 1* and 62.5 mcg./ml., and the benzylpenicillin-resistant *Staph. 2* at 62.5 mcg./ml.

The 4 - bromo - 2,5 - dimethylthiophene-3-carbonyl chloride, b.p. 75—76°/0.05 mm., was prepared by warming the corresponding carboxylic acid with thionyl chloride.

PYRROLE SERIES

EXAMPLE XXII

Preparation of 2-carbethoxy-3,5-dimethyl-4-pyrrylpenicillin.

A suspension of 2 - carbethoxy - 3,5-dimethylpyrrole-4-carbonyl chloride (5.25 g; m.p. 202—204°C: prepared by heating 2-carbethoxy - 3,5 - dimethylpyrrole - 4 - carboxylic acid under reflux with an excess of acetyl chloride) in dry chloroform (100 ml.) was added during 10 minutes to a stirred suspension of 6-aminopenicillanic acid (4.75 g.) in dry chloroform (150 ml.) containing triethylamine (9.3 ml.). The mixture was stirred for 4 hours, then filtered. The chloroform solution was shaken with N. hydrochloric acid to give an aqueous phase of pH 2, only the chloroform phase being retained. The latter was washed with water (2 × 50 ml.).

The chloroform solution was shaken with N. aqueous sodium bicarbonate solution (19 ml.) to give a neutral aqueous phase (pH 7). An emulsion formed which did not separate readily and it was evaporated at low temperature and pressure to leave a light brown solid residue of the crude sodium salt of 2 - carbethoxy - 3,5 - dimethyl - 4 - pyrryl-penicillin (2.98 g.).

Colorimetric assay with hydroxylamine against a benzylpenicillin standard indicated it to be about 48% pure.

It inhibited *Staph. Oxford* at a concentration of 1.25 mcg./ml.

PYRAZOLE SERIES

EXAMPLE XXIII

Preparation of 1,3,5-triphenyl-4-pyrazolyl-penicillin.

A solution of 1,3,5 - triphenylpyrazole - 4-carbonyl chloride (4.75 g. of the crude product from the corresponding carboxylic acid and thionyl chloride) in dry chloroform (100 ml.) was added during 15 minutes to a stirred suspension of 6-aminopenicillanic acid (2.81 g.) in dry chloroform (65 ml.) containing triethylamine (5.5 ml.). The mixture was stirred for 3½ hours, then filtered. The chloroform solution was shaken with N. hydrochloric acid to give an aqueous phase of pH 2, only the chloroform phase being retained. The later was washed with water (2 × 50 ml.).

The chloroform solution was shaken with the calculated amount of N. aqueous sodium bicarbonate solution to give a thick emulsion which was evaporated at low temperature and pressure to leave a solid residue of the

crude sodium salt of 1,3,5 - triphenyl - 4-pyrazolylpenicillin (7.75 g.).

Colorimetric assay with hydroxylamine against a benzylpenicillin standard indicated it to be about 42% pure.

inhibited *Staph. Oxford* at a concentration of 12.5 mcg./ml., the benzylpenicillin-resistant *Staph. 1* at 50 mcg./ml. and the benzylpenicillin-resistant *Staph. 2* at 62.5 mcg./ml.

EXAMPLE XXIV

A solution of 3,5 - dimethyl - 1 phenylpyrazole-4- carbonyl chloride (4.69 g.) in dry acetone (120 ml.) was added during 10 minutes to a stirred solution of 6-aminopenicillanic acid (4.33 g.) in 3% aqueous sodium bicarbonate (168 ml.) and acetone (50 ml.). Some oil separated but this redissolved. The mixture was stirred for 4 hours. The solution was extracted with ether (2 × 200 ml.), only the aqueous phase being retained. The latter was covered with ether (50 ml.) and adjusted to pH 2 by adding N. hydrochloric acid. The layers were separated, and the aqueous phase was extracted with two further 50 ml. portions of ether.

The combined ether extracts were washed with water and then shaken with sufficient N. aqueous sodium bicarbonate solution to give a neutral aqueous phase (pH 7). The aqueous layer was separated, washed with ether, and evaporated at low temperatue and pressure to leave a residue of the crude sodium salt of 3,5 - dimethyl - 1 - phenyl-4-pyrazolylpenicillin (1.82 g.).

Colorimetric assay with hydroxylamine against a benzylpenicillin standard indicated it to be about 25% pure.

It inhibited *Staph. Oxford* at 0.5 mcg./ml.

EXAMPLE XXV

1,5-Dimethyl-3-phenyl-4-pyrazolylpenicillin (sodium salt)

A solution of 1,5 - dimethyl - 3 - phenylpyrazole-4-carbonyl chloride (4.7 g.) in methylene chloride (30 ml.) was added dropwise to a stirred solution of 6-aminopenicillanic acid (4.3 g.) and triethylamine (8.3 g.) in methylene chloride (100 ml.) at 0°, and was then allowed to stand overnight.

Normal hydrochloric acid was added with shaking until a pH of 2 was attained (35 ml.), and the methylene chloride layer was separated. This was shaken with sufficient 1 N sodium bicarbonate to bring the pH to 7 (15 ml.) and the aqueous extract was separated and evaporated to dryness at room temperature *in vacuo*. The penicillin sodium salt was obtained as a light brown solid (5.8 g., 65%). The purity by hydroxylamine assay was 66%.

The product inhibited *Staph. Oxford* at a concentration of 1.25 mcg./ml., *Staph. 1* at 5 mcg./ml., and *Staph. 2* at 5 mcg./ml.

The 1,5 - dimethyl - 3 - phenylpyrazole-4-carbonyl chloride was prepared from the corresponding acid and thionyl chloride.

PYRIDINE SERIES

EXAMPLE XXVI

2,4-Dichloro-6-methyl-3-pyridyl penicillin (sodium salt)

To a stirred solution of 6-aminopenicillanic acid (3.24 g.) in 3% aqueous sodium bicarbonate (100 ml.) and acetone (50 ml.) was added a solution of 2,4 - dichloro - 6 - methylpyridine-3-carbonyl chloride (3.38 g.) in acetone (100 ml.) over 15 minutes. The mixture was allowed to stand overnight at room temperature and was then washed with ether. The aqueous layer was then shaken with a further 100 ml. of ether and sufficient 1 N. hydrochloric acid to bring the pH to about 2. After separating, the ethereal layer was extracted with sufficient 1 N. sodium bicarbonate to bring the pH to 7. The aqueous layer was then evaporated to dryness at room temperature *in vacuo*. The penicillin sodium salt was obtained as a cream coloured powder (92%). The assay by the hydroxylamine procedure indicated it to be about 61% pure.

The product inhibited *Staph. Oxford* at a concentration of 6 mcg./ml., the benzylpenicillin-resistant *Staph. 1* at 125 mcg./ml., and the benzyl penicillin-resistant *Staph. 2* at 125 mcg./ml.

EXAMPLE XXVII

2,4-Dimethoxy-6-methyl-3-pyridylpenicillin (sodium salt)

To a stirred mixture of 2,4 - dimethoxy-6-methylpyridine-3-carboxylic acid (2.95 g.) triethylamine (4.6 ml.), and chloroform (15 ml.) at −30° was added thionyl chloride (1.15 ml.). The solution was kept at −10° for 1½ hours and was then added to a stirred mixture of 6-aminopenicillanic acid (3.25 g.) and triethylamine (2.15 ml.) in chloroform (75 ml.), which was then allowed to reach room temperature and was left overnight. The reaction mixture was filtered and the filtrate evaporated under reduced pressure. Ether (50 ml.) and water (100 ml.) were then added to dissolve the residual gum and the penicillin was then extracted as described under 2,4 - dichloro - 6 - methyl - 3 - pyridyl penicillin. The desired penicillin was obtained as a cream coloured powder (weight yield 27%). Which was estimated by the hydroxylamine procedure to be about 27% pure. The product inhibited *Staph. Oxford* at 6 mcg./ml, *Staph. 1* at 25 mcg./ml., and *Staph. 2* at 25 mcg./ml.

2,4 - Dimethoxy - 6 - methylpyridine - 3-carboxylic acid (m.p. 182°) was prepared by the reaction of 2,4 - dichloro - 3 - ethoxycarbonyl - 6 - methylpyridine with sodium methoxide followed by hydrolysis.

PYRIMIDINE SERIES.

EXAMPLE XXVIII

2-Diethylamino-4,6-dimethyl-5-pyrimidyl penicillin (sodium salt)

Thionyl chloride (0.90 ml.) was added to a solution of 2 - diethylamino - 4,6 - dimethylpyrimidine-5-carboxylic acid (2.75 g.) in methylene chloride (25 ml.) at 0—5° and was allowed to stand for 40 minutes. The mixture was evaporated to dryness at 0° *in vacuo*, and the residue was redissolved in methylene chloride (20 ml.). This solution was added over 10 minutes to a stirred suspension of 6-aminopenicillanic acid in triethylamine (7.36 ml.) and methylene chloride (80 ml.) at 5—10°. After stirring ½ hour at this temperature the mixture was allowed to reach room temperature, when a clear solution was produced. After 3 hours the solution was shaken with sufficient 1 N. hydrochloric acid to give a pH of 2. The solvent layer was then separated and extracted with 1 equivalent of 1 N. sodium bicarbonate and the aqueous extract was evaporated to dryness *in vacuo* at room temperature. The penicillin sodium salt was obtained as a pale brown solid (43%). The assay by the hydroxylamine method was 45%.

The product inhibited *Staph. Oxford* at a concentration of 5 mcg./ml.

2 - Diethylamine - 4,6 - dimethylpyrimidine-5- carboxylic acid (m.p. 146° decomp.) was obtained by treating 5 - bromo - 4,6-dimethyl - 2 - hydroxypyrimidine, first with phosphorus oxychloride to give 5 - bromo-4,6 - dimethyl - 2 - chloropyrimidine (m.p. 82—83°) then with diethylamine to give 5-bromo - 2 - diethylamino - ~~4,6 - dimethyl-amine~~ to give 5 - bromo ~~- 2 - diethylamino-~~ 4,6-dimethylpyrimidine (b.p. 120—1°C/0.6 mm., n_D^{20}—1.5492). The latter compound was treated with butyl lithium followed by CO_2 to give the desired pyrimidine acid.

EXAMPLE XXIX

4,6-Dimethyl-2-methoxy-5-pyrimidyl penicillin (sodium salt)

This compound was prepared from 4,6-dimethyl - 2 - methoxypyrimidine - 5 - carboxylic acid by the method employed for 2 - diethylamino - 4,6 - dimethyl - 5-pyrimidyl penicillin sodium salt except that the initial temperature during the addition of thionyl chloride was −35° to −40°. The desired penicillin salt was obtained as a pale brown powder (37%). The assay by the hydroxylamine method was 37%.

The product inhibited *Staph. Oxford* at a concentration of 12.5 mcg./ml., the benzylpenicillin resistant *Staph. 1* at 125 mcg./ml., and the benzylpenicillin-resistant *Staph. 2* at 125 mcg./ml.

4,6 - Dimethyl - 2 - methoxypyrimidine-5-carboxylic acid (m.p. 152—153° dec.) was obtained from 5 - bromo - 4,6 - dimethyl-2-methoxypyrimidine by reaction with butyl lithium followed by carbonation with carbon dioxide. The 5 - bromo - 4,6 - dimethyl-2-methoxypyrimidine (m.p. 61—62°) was obtained by the reaction of 5 - bromo - 2-chloro - 4,6 - dimethylpyrimidine with sodium methoxide.

EXAMPLE XXX

4,6-Dimethoxy-5-pyrimidyl penicillin (sodium salt)

4,6 - Dimethoxypyrimidine - 5 - carboxylic acid was converted to its acid chloride by standing for one hour at room temperature with excess of thionyl chloride in the presence of dimethylformamide. Excess of thionyl chloride was removed *in vacuo* and the crude residue was dissolved in methylene chloride and condensed with 6-aminopenicillanic acid as described under 2 - diethylamino - 4,6-dimethyl - 5 - pyrimidyl penicillin sodium salt except that the condensation was carried out at room temperature.

The penicillin was obtained as a pale yellow solid (79%). The assay by hydroxylamine method was 69%.

The product inhibited *Staph. Oxford* at 2.5 mcg./ml., *Staph. 1* at 6.25 mcg./ml. and *Staph. 2* at 6.25 mcg./ml.

4,6 - Dimethoxypyrimidine - 5 - carboxylic acid (m.p. 200—201° decomp.) was obtained from 5 - bromo - 4,6 - dimethoxypyrimidine by reaction with butyl lithium followed by carbonation. 5 - Bromo - 4,6 - dimethoxypyrimidine (m.p. 149—150°) was prepared by the reaction of 5 - bromo - 4,6 - dichloropyrimidine with sodium methoxide.

EXAMPLE XXXI

4-Chloro-6-methoxy-5-pyrimidyl-penicillin.

4 - Chloro - 6 - methoxypyrimidine - 5-carboxylic acid was converted to its acid chloride by standing for one hr. at room temperature with an excess of thionyl chloride in the presence of dimethylformamide. Excess of thionyl chloride was removed *in vacuo* and the crude residue was dissolved in methylene chloride and condensed with 6-aminopenicillanic acid at room temperature as described under 4,6 - dimethoxy - 5 - pyrimidlypenicillin sodium salt.

The penicillin was obtained as a white solid (65%). The assay by the hydroxylamine method was 50%.

The product inhibited *Staph. Oxford* at 0.5 mcg./ml., *Staph. 1* at 25 mcg./ml., and *Staph. 2* at 12.5 mcg./ml.

4 - Chloro - 6 - methoxypyrimidine - 5-carboxylic acid (m.p. 145.5° C. decomp.) was obtained from 5 - bromo - 4 - chloro - 6-methoxypyrimidine by reaction with butyl lithium followed by carbonation.

5 - Bromo - 4 - chloro - 6 - methoxypyrimidine (m.p. 77°) was prepared by the reaction of 5 - bromo - 4,6 - dichloropyrimidine with one mole of sodium methoxide in the cold.

BENZOFURAN SERIES

EXAMPLE XXXII

2-Ethyl-3-benzofurylpenicillin (sodium salt)

A solution of crude 2-ethylbenzofuran-3-carbonylchloride (5 g.) in acetone (57 ml.) was added, with stirring, to a solution of 6-aminopenicillanic acid (5.19 g.) in 1N sodium bicarbonate solution (72 ml.), water (30 ml.) and acetone (143 ml.). After stirring for three hours at room temperature the acetone was distilled off under reduced pressure. The remaining solution was filtered and acidified to pH 2 with 1 N hydrochloric acid (60 ml.) whilst shaking with ether. The ether layer was washed with a little water, then extracted with sufficient 1 N sodium bicarbonate to bring the pH to 8 (20 ml.). The aqueous extract was then evaporated to dryness at room temperature under reduced pressure. The residual penicillin sodium salt was dried *in vacuo* to give a pale yellow solid (4.7 g., 48%). Colorimetric assay with hydroxylamine against a benzylpenicillin standard indicated a purity of about 38%.

The product inhibited *Staph. Oxford* at a concentration of 0.1 mcg./ml.

The 2 - ethylbenzofuran - 3 - carbonyl chloride was obtained by the reaction of oxalyl chloride and 2-ethylbenzofuran in carbon disulphide in the presence of aluminium chloride.

QUINOLINE SERIES

EXAMPLE XXXIII

Preparation of 2,4-dichloro-3-quinolyl penicillin.

A solution of 2,4 - dichlorquinoline - 3-carbonyl chloride (3.93 g.) in dry acetone (100 ml.) was added during 15 minutes to a stirred solution of 6-aminopenicillanic acid (3.26 g.) in 3% aqueous sodium bicarbonate (87 ml.) and acetone (10 ml.). Further 3% aqueous sodium bicarbonate (10 ml.) was added after 45 minutes to maintain a pH$\geqslant$7. The solution was stirred for 5 hours. It was then extracted with ether (2 × 100 ml.), only the aqueous phase being retained.

The aqueous solution, containing the sodium salt of the penicillin, was evaporated at low temperature and pressure to leave residue of the crude sodium salt of 2,4 - dichloro - 3-quinolylpenicillin (7.7 g.).

Colorimetric assay with hydroxylamine against a benzylpenicillin standard indicated it to be about 39% pure.

It inhibited *Staph. Oxford* at 2.5 mcg./ml., *Staph. 1* at 12.5 mcg./ml., and *Staph. 2* at 12.5 mcg./ml.

The 2,4 - dichloroquinoline - 3 - carbonyl chloride, m.p. 83—84° was prepared by refluxing 2,4 - dihydroxyquinoline - 3 - carboxylic acid in phosphorus oxychloride for 2 hours.

EXAMPLE XXXIV

3-Ethoxy-2-methyl-4-quinolyl penicillin.

Thionyl chloride (0.95 ml.) was added slowly at room temperature to a solution of 3 - ethoxy - 2 - methylquinoline - 4 - carboxylic acid (2.1 g.) in triethylamine (2.5 ml.) and methylene chloride (50 ml.). The mixture was stirred for 1 hour, then evaporated to dryness at room temperature under reduced pressure.

The pale coloured residue was redissolved in methylene chloride (50 ml.) and added, with stirring, at room temperature to a solution of 6-aminopenicillanic acid (1.9 g.) in triethylamine (5.0 ml.) and methylene chloride (50 ml.). The mixture was allowed to stand overnight and was then filtered. The filtrate was washed with sufficient 1 N hydrochloric acid to produce a pH of about 2. The methylene chloride layer was then extracted with sufficient 1-N sodium bicarbonate to bring the pH to about 8 (8 ml.) and the aqueous extracts were then evaporated to dryness *in vacuo* at room temperature to give the penicillin as a pale solid, yield 2.2 g. (33%) estimated by the hydroxylamine procedure to be about 56% pure.

The product inhibited *Staph. Oxford* at 1.25 mcg./ml., *Staph. 1* at 5 mcg./ml., and *Staph. 2* at 5 mcg./ml.

EXAMPLE XXXV

2-Ethyl-3-methoxy-4-quinolyl penicillin.

Thionyl chloride (0.72 ml.) was added to a stirred solution of 2 - ethyl - 3 - methoxyquinoline-4-carboxylic acid (2.3 g.) in chloroform (15 ml.) and triethylamine (2.8 ml.) at −20°.

The reaction mixture was stirred at room temperature for 2 hours and was then filtered from some insoluble material. The filtrate was evaporated to dryness *in vacuo* and the residue was redissolved in ether. By treating the ethereal solution with 1.N hydrochloric acid followed by 1 N. sodium bicarbonate solution as described under 3-ethoxy - 2 - methyl - 4 - quinolyl penicillin the desired 2 - ethyl - 3 - methoxy - 4-quinolyl penicillin was obtained as the sodium salt (2.4 g. 53%), estimated by the hydroxylamine procedure to be about 19% pure.

It inhibited *Staph. Oxford* at 2.5 mcg./ml., *Staph. 1* at 25 mcg./ml., and *Staph. 2* at 12.5 mcg./ml.

2 - Ethyl - 3 - methoxyquinoline - 4-carboxylic acid (m.p. 214° decomp.) was obtained from 2 - ethyl - 3 - hydroxyquinoline-4-carboxylic acid by treatment with diazomethane in ether, followed by saponification of the resulting methyl ester with alcoholic potassium hydroxide.

EXAMPLE XXXVI

The following quinolyl penicillins when prepared by reaction of the named acids with 6-aminopenicillanic acid by way of the acid chloride or other suitable intermediate in accordance with the procedure of Method A, B or C:—

Acid	*Penicillin*
3 - benzylquinoline - 4 - carboxylic acid.	3 - benzyl - 4 - quinolylpenicillin.
3 - methoxyquinoline - 4 - carboxylic acid.	3 - methoxy - 4 - quinolylpenicillin.
3 - ethoxylquinoline - 4 - carboxylic acid.	3 - ethoxy - 4 - quinolylpenicillin.
2 - methyl - 3 - methoxyquinoline - 4-carboxylic acid.	2 - methyl - 3 - methoxy - 4 - quinolylpenicillin.
2 - methyl - 3 - *n* - propoxyquinoline-4 - carboxylic acid.	2 - methyl - 3 - *n* - propoxy - 4-quinolylpenicillin.
2 - methyl - 3 - *n* - butoxyquinoline-4 - carboxylic acid.	2 - methyl - 3 - *n* - butoxy - 4 - quinolylpenicillin.
2 - ethyl - 3 - ethoxyquinoline - 4 - carboxylic acid.	2 - ethyl - 3 - ethoxy - 4 - quinolylpenicillin.
2 - ethyl - 3 - *n* - propoxyquinoline-4 - carboxylic acid.	2 - ethyl - 3 - *n* - propoxy - 4 - quinolylpenicillin.
2 - ethyl - 3 - *n* - butoxyquinoline - 4-carboxylic acid.	2 - ethyl - 3 - *n* - butoxy - 4 - quinolylpenicillin.
2 - *n* - propyl - 3 - methoxyquinoline-4 - carboxylic acid.	2 - *n* - propyl - 3 - methoxy - 4-quinolylpenicillin.
2 - *n* - propyl - 3 - ethoxyquinoline - 4-carboxylic acid.	2 - *n* - propyl - 3 - ethoxy - 4 - quinolylpenicillin.
2 - ethyl - 3 - methylquinoline - 4 - carboxylic acid.	2 - ethyl - 3 - methyl - 4 - quinolylpenicillin.
2 - *n* - propyl - 3 - ethylquinoline - 4-carboxylic acid.	2 - *n* - propyl - 3 - ethyl - 4 - quinolylpenicillin.
2 - phenyl - 3 - methylquinoline - 4-carboxylic acid.	2 - phenyl - 3 - methyl - 4 - quinolylpenicillin.
2 - phenyl - 3 - ethylquinoline - 4 - carboxylic acid.	2 - phenyl - 3 - ethyl - 4 - quinolylpenicillin.

COUMARIN SERIES

EXAMPLE XXXVII

3,7-Dimethoxy-4-coumarinylpenicillin.

A mixture of 3,7 - dimethoxycoumarin 4-carboxylic acid (1.15 g.), dry alcohol-free chloroform (10 ml.) and thionyl chloride (0.7 ml.) was refluxed until complete solution was obtained (2½ hours). The solution was evaporated under reduced pressure and to the residue, dry alcohol-free chloroform (10 ml.) was added and distilled off again. The residue was left under high vacuum for a few minutes and then dissolved in dry alcohol-free chloroform (10 ml.). This solution was added during 10 minutes to a stirred mixture of 6-aminopenicillanic acid (1 g.) dry alcohol-free chloroform (15 ml.) and triethylamine (1.3 ml.). After stirring for a further one hour, the solution was washed with water (5 ml.) plus enough 1 N. hydrochloric acid (4.6 ml.) to give an aqueous phase of pH 2. The chloroform phase was then extracted with water (10 ml.) plus enough 3% w/v

aqueous sodium bicarbonate (13 ml.) to give an aqueous phase of pH 7. The bicarbonate phase was separated, washed with ether (1 × 25 ml.) and evaporated at 30°/2 mm. The residue was dried in vacuum over phosphorus pentoxide to give the penicillin as a buff powder (1.1 g.); purity (by hydroxylamine assay)—43%.

The product inhibited *Staph. Oxford* at a concentration of 0.6 mcg./ml., *Staph. 1* at 25 mcg./ml. and *Staph. 2* at 12.5 mcg./ml.

ACRIDEN SERIES

EXAMPLE XXXVIII

9-Acridylpenicillin.

Acridine 9-carboxylic acid (2.2 g.) was mixed with thionyl chloride (10 ml.) and refluxed for 2 hours. The solution was then evaporated at 80° under water vacuum and the residual solid filtered with the aid of anhydrous benzene and dissolved in dry alcohol-free chloroform (20 ml.). This solution was then added dropwise during 15 minutes to a stirred mixture of 6-aminopenicillanic acid (2.16 g.), dry alcohol-free chloroform (30 ml.) and triethylamine (2.8 ml.). After stirring for a further one hour, the solution was washed with water (10 ml.) plus enough 1 N. hydrochloric acid (7.5 ml.) to give an aqueous phase of pH 2. The chloroform layer was then extracted with water (20 ml.) plus enough 3% aqueous sodium bicarbonate (20 ml.) to give an aqueous phase of pH 7. Ether (200 ml.) was used to clear an emulsion which formed at this stage. Evaporation of the bicarbonate phase at 30°/2 mm. left a residue which was dried over phosphorus pentoxide under vacuum to give the crude penicillin as a light green powder (0.8 g.): purity (by hydroxylamine assay)—36%.

The product inhibited *Staph. Oxford* at a concentration of 2.5 mcg./ml., *Staph. 1* at 12.5 mcg./ml., and *Staph. 2* at 12.5 mcg./ml.

INDOLE SERIES

EXAMPLE XXXIX

1:2-Dimethyl-3-indolylpenicillin

A solution of 1:2 - dimethylindole - 3-carboxylic acid (3.78 g.) in dry benzene (50 ml.) was treated with thionyl chloride (3 ml.) and pyridine (3 drops) refluxed for 4 hours and evaporated under reduced pressure. The residual crude 1:2-dimethylindole-3-carbonyl chloride was dissolved in dry chloroform (33 ml.) and added during 10 minutes to a stirred suspension of 6-aminopenicillanic acid (4.33 g.) in dry chloroform (100 ml.) and triethylamine (8.4 ml.). The mixture was stirred at room temperature for 4 hours and then filtered. The orange-brown filtrate was shaken with sufficient N hydrochloric acid to give an aqueous phase of pH 2. The layers were separated and the chloroform solution was washed with water (2 × 50 ml.) and then shaken with sufficient 8% aqueous sodium bicarbonate solution to give a neutral aqueous phase (pH 7). This final aqueous solution was separated and evaporated at low temperature and pressure to leave the crude sodium salt of 1:2-dimethyl-3-indolylpenicillin as a yellow-brown solid, which was finally dried over phosphorus pentoxide *in vacuo*. Yield, 4.47 g.

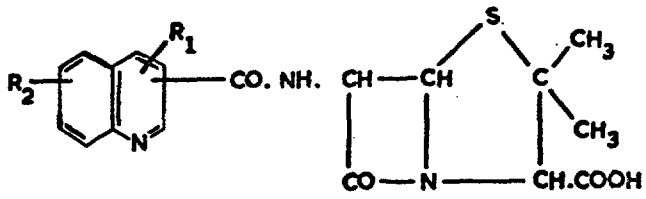
Colorimetric assay with hydroxylamine against a benzylpenicillin standard indicated it to be about 70% pure.

Many of the new penicillins of the present invention possess the useful property of being relatively stable to acids, and thus are suitable for oral administration. As a measure of such stability, the 'half-lives' of several of the new penicillins dissolved in 50% aqueous ethanol at pH 1.3 and 35°C were determined and compared with corresponding figures for Penicillin G. and Penicillin V. The stability to acid of the penicillins tabulated below can readily be seen to compare favourably with that of Penicillin V. and to be vastly greater than that of Penicillin G.

Penicillin	Half-life at pH 1.3 and 35° C.
5-Methyl-3-phenyl-4-isoxazolyl	160 mins.
3-Methyl-5-phenyl-4-isoxazolyl	160 mins.
3-*p*-Chlorophenyl-5-methyl-4-isoxazolyl	150 mins.
2:4-Dichloro-6-methyl-3-pyridyl	>10 hrs.
3-Ethoxy-2-methyl-4-quinolyl	>10 hrs.
3:7-Dimethoxy-4-coumarinyl	>10 hrs.
Penicillin V.	160 mins.
Penicillin G.	3½ mins.

The compounds of the present invention may be employed in admixture with suitable pharmaceutical carriers. The present invention therefore includes a composition comprising a pharmaceutical carrier and a new penicillin of the general formula (I) or a non-toxic salt thereof.

Our Specification No. 876,662 claims acids of the general formula:

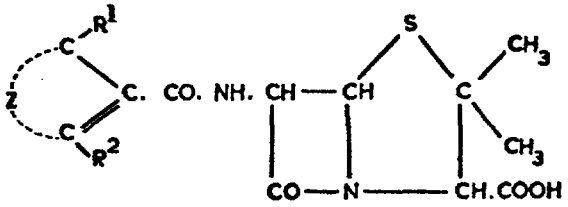


and non-toxic salts thereof wherein R_1 and R_2 are the same or different and each is a hydrogen atom or a lower alkyl, hydroxy, chloro, bromo, phenyl or thienyl group and we make no claim herein to anything claimed in Specification No. 876,662.

Subject to the foregoing disclaimer

WHAT WE CLAIM IS:—

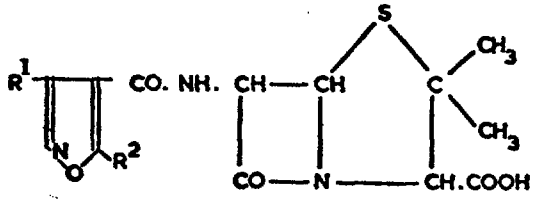
1. New penicillins of the general formula:



(I)

and non-toxic salts thereof, where Z is the residue of a substituted or unsubstituted heterocyclic ring system and R^1 and R^2 are the same or different and are each an alkyl, aryl, acyl, aralkyl, cycloalkyl, heterocyclic, alkoxy, aryloxy, aralkoxy, alkylthio, arylthio or aralkylthio group which may be substituted or a halogen atom, or R^1 or R^2 may form part of a further substituted or unsubstituted cyclic system fused to the ring containing Z.

2. New penicillins and non-toxic salts thereof as claimed in Claim 1 of the general formula:



(II)

where R^1 and R^2 are the same or different and are each an alkyl, aryl, acyl, aralkyl, cycloalkyl, heterocyclic, alkoxy, aryloxy, aralkoxy, alkylthio, arylthio or aralkylthio group which may be substituted or a halogen atom.

3. New penicillins and non-toxic salts thereof as claimed in Claim 2 wherein in the general formula one of the groups R^1 and R^2 is a substituted or unsubstituted phenyl group and the other a lower alkyl group.

4. 5 - Methyl - 3 - phenyl - 4 - isoxazolylpenicillin and non-toxic salts thereof.

5. 3 - Methyl - 5 - phenyl - 4 - isoxazolylpenicillin and non-toxic salts thereof.

6. 5 - (2 - Furyl) - 3 - methyl - 4-isoxazolylpenicillin and non-toxic salts thereof.

7. 3 - *o* - Chlorophenyl - 5 - methyl - 4-isoxazolylpenicillin and non-toxic salts thereof.

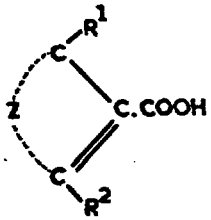
8. 5 - *o* - Chlorophenyl - 3 - methyl - 4-isoxazolylpenicillin and non-toxic salts thereof.

9. 3 - *p* - Chlorophenyl - 5 - methyl - 4-isoxazolylpenicillin and non-toxic salts thereof.

10. 5 - *p* - Chlorophenyl - 3 - methyl - 4-isoxazolylpenicillin and non-toxic salts thereof.

11. 3 - Ethoxy - 2 - methyl - 4 - quinolylpenicillin and non-toxic salts thereof.

12. A process for the preparation of the new penicillins and non-toxic salts thereof claimed in Claim 1, wherein 6-aminopenicillanic acid, or a liquor containing 6-aminopenicillanic acid is reacted with an acid chloride, bromide, anhydride or mixed anhydride derived from a carboxylic acid of the general formula:

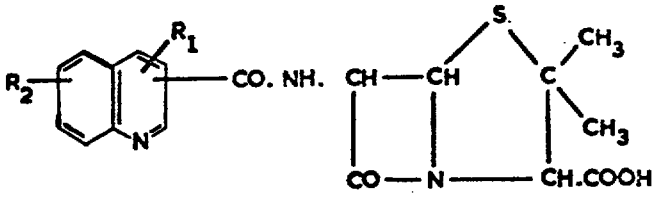


(III)

where Z, R^1 and R^2 are as defined in Claim 1.

The compounds of the present invention may be employed in admixture with suitable pharmaceutical carriers. The present invention therefore includes a composition comprising a pharmaceutical carrier and a new penicillin of the general formula (I) or a non-toxic salt thereof.

Our Specification No. 876,662 claims acids of the general formula:

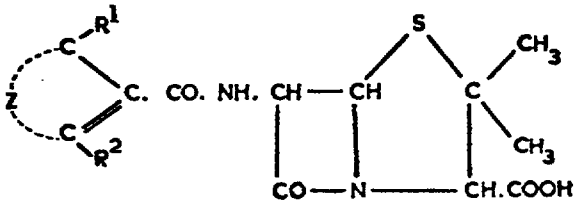


and non-toxic salts thereof wherein R_1 and R_2 are the same or different and each is a hydrogen atom or a lower alkyl, hydroxy, chloro, bromo, phenyl or thienyl group and we make no claim herein to anything claimed in Specification No. 876,662.

Subject to the foregoing disclaimer

WHAT WE CLAIM IS:—

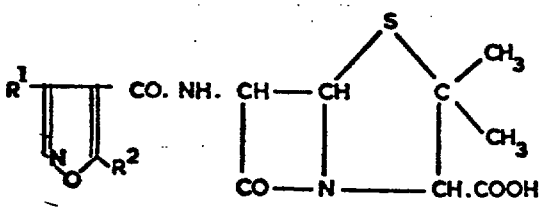
1. New penicillins of the general formula:



(I)

and non-toxic salts thereof, where Z is the residue of a substituted or unsubstituted heterocyclic ring system and R^1 and R^2 are the same or different and are each an alkyl, aryl, acyl, aralkyl, cycloalkyl, heterocyclic, alkoxy, aryloxy, aralkoxy, alkylthio, arylthio or aralkylthio group which may be substituted or a halogen atom, or R^1 or R^2 may form part of a further substituted or unsubstituted cyclic system fused to the ring containing Z.

2. New penicillins and non-toxic salts thereof as claimed in Claim 1 of the general formula:



(II)

where R^1 and R^2 are the same or different and are each an alkyl, aryl, acyl, aralkyl, cycloalkyl, heterocyclic, alkoxy, aryloxy, aralkoxy, alkylthio, arylthio or aralkylthio group which may be substituted or a halogen atom.

3. New penicillins and non-toxic salts thereof as claimed in Claim 2 wherein in the general formula one of the groups R^1 and R^2 is a substituted or unsubstituted phenyl group and the other a lower alkyl group.

4. 5 - Methyl - 3 - phenyl - 4 - isoxazolylpenicillin and non-toxic salts thereof.

5. 3 - Methyl - 5 - phenyl - 4 - isoxazolylpenicillin and non-toxic salts thereof.

6. 5 - (2 - Furyl) - 3 - methyl - 4-isoxazolylpenicillin and non-toxic salts thereof.

7. 3 - *o* - Chlorophenyl - 5 - methyl - 4-isoxazolylpenicillin and non-toxic salts thereof.

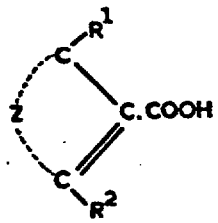
8. 5 - *o* - Chlorophenyl - 3 - methyl - 4-isoxazolylpenicillin and non-toxic salts thereof.

9. 3 - *p* - Chlorophenyl - 5 - methyl - 4-isoxazolylpenicillin and non-toxic salts thereof.

10. 5 - *p* - Chlorophenyl - 3 - methyl - 4-isoxazolylpenicillin and non-toxic salts thereof.

11. 3 - Ethoxy - 2 - methyl - 4 - quinolylpenicillin and non-toxic salts thereof.

12. A process for the preparation of the new penicillins and non-toxic salts thereof claimed in Claim 1, wherein 6-aminopenicillanic acid, or a liquor containing 6-aminopenicillanic acid is reacted with an acid chloride, bromide, anhydride or mixed anhydride derived from a carboxylic acid of the general formula:

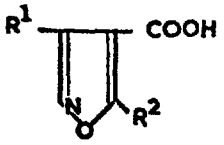


(III)

where Z, R^1 and R^2 are as defined in Claim 1.

13. A process as claimed in Claim 12 wherein the 6-aminopenicillanic acid is in the form of a neutral salt.

14. A process as claimed in Claim 12 wherein 6-aminopenicillanic acid or a liquor containing 6-aminopenicillanic acid is reacted with an acid chloride, bromide, anhydride or mixed anhydride derived from a carboxylic acid of the general formula:



(IV)

where R^1 and R^2 are the same or different and are each an alkyl, aryl, acyl, aralkyl, cycloalkyl, heterocyclic, alkoxy, aryloxy, aralkoxy, alkylthio, arylthio or aralkylthio group which may be substituted or a halogen atom.

15. A process as claimed in any one of claims 12 to 14 wherein the reaction takes place in the presence of an anhydrous organic solvent and an organic base.

16. A process as claimed in any one of claims 12 to 15 wherein the penicillin is subsequently converted to a non-toxic salt.

17. A process for the preparation of new penicillins and non-toxic salts thereof substantially as described with reference to any one of the Examples.

18. New penicillins and non-toxic salts thereof when prepared by the process claimed in any one of Claims 12 to 17.

19. A composition comprising a pharmaceutical carrier and a new penicillin or a non-toxic salt thereof as claimed in any one of Claims 1 to 11 and 18.

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

PROVISIONAL SPECIFICATION
No. 11412. A.D. 1960

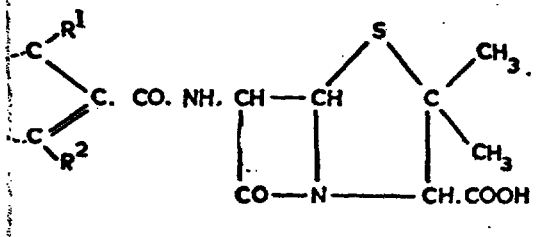
Improvements in or relating to Penicillins

We, BEECHAM RESEARCH LABORATORIES LIMITED of Great West Road, Brentford, Middlesex, do hereby declare this invention to be described in the following statement:—

This invention relates to new penicillins, and is concerned with an improvement in or modification of the invention of our Specification No. 24607/57 (Serial No. 870,395).

In the said Specification No. 24607/57 (Serial No. 870,395) there is described and claimed a process for the preparation of derivatives of 6-aminopenicillanic acid in which 6-aminopenicillanic acid or a salt thereof is reacted with a carboxylic acid chloride or bromide, a sulphonic acid chloride, an ester of chlorocarbonic acid, an acid anhydride of a carboxylic acid or a mixed acid anhydride derived from a carboxylic acid.

The new penicillins of the present invention are compounds of the general formula:

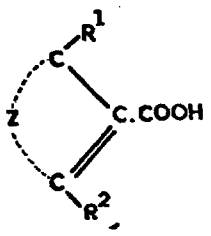


(I)

and non-toxic salts thereof, where Z is the residue of a substituted or unsubstituted heterocyclic ring system and R^1 and R^2 are the same or different and are each an alkyl, aryl, acyl, aralkyl, cycloalkyl, heterocyclic, hydroxy, alkoxy, aryloxy, aralkoxy, mercapto, alkylthio, arylthio, aralkylthio, acyloxy, acylthio, acylamino, alkoxycarbonyl, alkylsulphonyl or nitro group or a halogen atom. Alternatively, R^1 or R^2 may form part of a further substituted or unsubstituted cyclic system fused to the ring containing Z.

The salts are non-toxic salts including non-toxic metallic salts such as sodium, potassium, calcium and aluminium, ammonium 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 provides a process for the preparation of the new penicillins of the general formula (I) and non-toxic salts thereof, wherein 6-aminopenicillanic acid or a liquor containing 6-aminopenicillanic acid is reacted with an acid chloride, bromide, anhydride or mixed anhydride derived from a carboxylic acid of the general formula:



(II)

where Z, R^1 and R^2 are as defined above.

The following are examples of carboxylic acids of the general formula (II):

2:4 - Dimethylfuran - 3 - carboxylic acid
2:4 - Dimethylthiophene - 3 - carboxylic acid
2:4 - Dimethylpyrrole - 3 - carboxylic acid
4:6 - Dimethyl - α - pyrone - 5 - carboxylic acid
2:4 - Dimethylpyridine - 3 - carboxylic acid
4 - Chloro - 2 - hydroxypyridine - 3 - carboxylic acid
4 - Chloro - 2:6 - dimethylpyridine - 3-carboxylic acid
3:5 - Dimethylpyrazole - 4 - carboxylic acid
3:5 - Dimethylisooxazole - 4 - carboxylic acid
4 - Hydroxy - 2 - methylquinoline - 3-carboxylic acid
2 - Methylindole - 3 - carboxylic acid
2:4 - Dichloroquinoline - 3 - carboxylic acid
2:3 - (Trimethylene)quinoline - 4 - carboxylic acid
Acridine - 4 - carboxylic acid
2 - Ethylbenzofuran - 3 - carboxylic acid.

Some of the new penicillins of the present invention have shown a particular advantage in that they are less readily destroyed by penicillinase than the conventional Penicillins G and V.

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 conditions which are sufficiently moderate to avoid their decomposition. The reaction conditions chosen will, of course, depend largely upon the reactivity of the chemical reagent being used. In most instances a compromise has to be made between the use of very mild conditions for a lengthy period and the use of more vigorous conditions for a shorter time with the possibility of decomposing some of the antibiotic substance.

The temperature chosen for the process of the present invention should in general not exceed 30°C. and in many cases a suitable temperature is ambient temperature. Since the use of strongly acid or alkaline conditions in the process of this invention should be avoided, it has been found preferable to perform the process at a pH of from 6 to 9, and this can conveniently be achieved by using a buffer, for example, a solution of sodium bicarbonate, or a sodium phosphate buffer.

Aqueous acetone is often a convenient solvent for the reaction, but anhydrous organic solvents together with an organic base may also be employed. Both solvent and base should be chosen so as not themselves to react preferentially with the reagent being employed. Acetone and triethylamine or chloroform and triethylamine have been found to be particularly useful.

It is sometimes preferred to prepare the new penicillins from a fermentation liquor, containing 6-aminopenicillanic acid, or one of its intermediate concentrates.

The following Examples illustrate the invention:

EXAMPLE 1

4:6-Dimethyl-α-pyron-5-yl penicillin (sodium salt)

A solution of isodehydroacetyl chloride (Salemink, Rec. Trav. chim., 1959, *78*, 364) (7.5 g.) in dry chloroform (60 ml.) was slowly added to a stirred ice-cold mixture of 6-aminopenicillanic acid (8.64 g.), triethylamine (11 ml.) and dry chloroform (60 ml.), then the mixture was stirred at room temperature for 3 hours. After concentration *in vacuo* at room temperature, the mixture was shaken with water (120 ml.) and the layers were separated. The aqueous phase was brought to pH 7 by the addition of sodium bicarbonate and washed with ether (2 × 100 ml.) followed by butanol (2 × 30 ml.). It was then covered with butanol (30 ml.) and acidified to pH 3 with N hydrochloric acid. The layers were separated and the aqueous phase was extracted with two further 20 ml. ~~portion~~ of butanol. The combined butanol extracts (which at this stage contained the free penicillin acid) ~~where~~ washed with water (25 ml.) and then neutralised by adding 8% sodium bicarbonate solution (20 ml.) with vigorous shaking. The layers were separated and the aqueous phase was evaporated at low temperature and pressure to leave the impure sodium salt of 4:6-dimethyl-α-pyron-5-yl penicillin as a hygroscopic brown solid which was finally dried in a vacuum desiccator. Yield, 3.55 g.

The product inhibited *Staph. Oxford* at a concentration of 5 mcg./ml.

EXAMPLE 2

2:4:5-Triphenyl-3-furylpenicillin (sodium salt)

A solution of 2:4:5 - triphenyl - 3 - furoyl chloride (5.77 g.) in dry acetone (95 ml.) was added during 15 minutes to a stirred

solution of 6-aminopenicillanic acid (3.46 g.) in ½ aqueous sodium bicarbonate (135 ml.) and acetone (40 ml.). The mixture was stirred for 4 hours and then filtered. The filtrate was extracted with ether (2 × 150 ml.). The aqueous phase was then covered with ether (75 ml.) and brought to pH 2 by adding dilute hydrochloric acid. The layers were separated and the aqueous phase was extracted with two further 50 ml. portions of ether. The combined ether extracts (which at this stage contained the free penicillan acid) were washed with water (3 × 25 ml.) and then shaken with sufficient 8% sodium bicarbonate solution to give a neutral aqueous phase (pH 7). The neutral aqueous layer was separated, washed with ether, and evaporated at low temperature and pressure. The residue was dried *in vacuo* over phosphorous pentoxide to leave the crude sodium salt of 2:4:5 - triphenyl - 3 - furyl - penicillin as a white powder (7.42 g.). Colorimetric assay with hydroxylamine against a benzylpenicillin standard indicated a purity of 36%.

The product inhibited *Staph. Oxford* at 5 mcg./ml., *Staph. 1* at 25 mcg./ml., and *Staph. 2* at 25 mcg./ml.

The 2:4:5-triphenyl-3-furoyl chloride used in this Example was prepared by warming the corresponding acid with thionyl chloride and a trace of pyridine, removing excess reagent *in vacuo*, and crystallising the residue from light petroleum to give colourless needles, m.p. 97—98°C.

EXAMPLE 3

1:2-Dimethyl-3-indolylpenicillin

A solution of 1:2 - dimethylindole - 3-carboxylic acid (3.78 g.) in dry benzene (50 ml.) was treated with thionyl chloride (3 ml.) and pyridine (3 drops), refluxed for 4 hours and evaporated under reduced pressure. The residual crude 1:2 - dimethylindole - 3 - carbonyl chloride was dissolved in dry chloroform (33 ml.) and added during 10 minutes to a stirred suspension of 6-aminopenicillanic acid (4.33 g.) in dry chloroform (100 ml.) and triethylamine (8.4 ml.). The mixture was stirred at room temperature for 4 hours and then filtered. The orange-brown filtrate was shaken with sufficient N hydrochloric acid to give an aqueous phase of pH 2. The layers were separated and the chloroform solution was washed with water (2 × 50 ml.) and then shaken with sufficient 8% aqueous sodium bicarbonate solution to give a neutral aqueous phase (pH 7). This final aqueous solution was separated and evaporated at low temperature and pressure to leave the crude sodium salt of 1:2 - dimethyl - 3 - indolylpenicillin as a yellow-brown solid, which was finally dried over phosphorus pentoxide *in vacuo*. Yield, 4.47 g.

Colorimetric assay with hydroxylamine against a benzylpenicillin standard indicated it to be about 70% pure.

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

PROVISIONAL SPECIFICATION

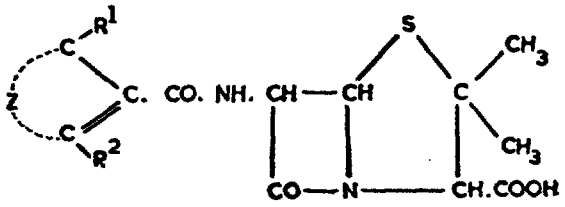
No. 25049. A.D. 1960

Improvements in or relating to Penicillins

We, BEECHAM RESEARCH LABORATORIES LIMITED of Great West Road, Brentford, Middlesex, a British Company, do hereby declare the invention to be described in the following statement:—

This invention relates to new penicillins and is concerned with an improvement in or relating to the invention of our Specification No. 24607/57 (Serial No. 870,395) and is more particularly concerned with a development of the invention of our Specification No. 11412/60.

In our said co-pending Application No. 11412/60 we have described new penicillins of the general formula:



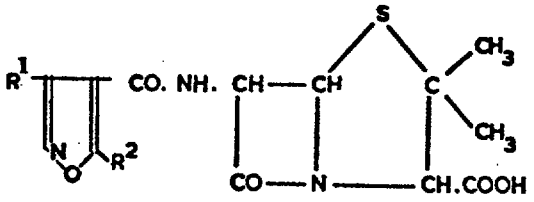
(I)

and non-toxic salts thereof, where Z is the residue of a substituted or unsubstituted heterocyclic ring system and R^1 and R^2 are the same or different and are each an alkyl, aryl, acyl, aralkyl, heterocyclic, hydroxy, alkoxy, aryloxy, aralkoxy, mercapto, alkylthio, arylthio, aralkylthio, acyloxy, acylthio, acylamino, alkoxycarbonyl, alkylsulphonyl or

nitro group or a halogen atom. Alternatively, R^1 or R^2 may form part of a further substituted or unsubstituted cyclic system fused to the ring containing Z.

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

Accordingly, the present invention provides new penicillins of the general formula:—



(II)

and non-toxic salts thereof, where R^1 and R^2 are as defined above.

The compounds of the present invention may be prepared in the manner described in Application No. 11412/60. Examples of carboxylic acids which may be employed to prepare the new penicillins are:—

5 - methyl - 3 - phenylisoxazole - 4 - carboxylic acid

3:5 - diphenylisoxazole - 4 - carboxylic acid

3:5 - dimethylisoxazole - 4 - carboxylic acid

3 - *p* - chlorophenyl - 5 - methylisoxazole-4 - carboxylic acid

5 - methyl - 3 - styrylisoxazole - 4 - carboxylic acid

5 - *t* - butyl - 3 - phenylisoxazole - 4 - carboxylic acid

5 - ethoxy - 3 - methylisoxazole - 4 - carboxylic acid

3 - methyl - 5 - methylthioisoxazole - 4-carboxylic acid.

The following Examples illustrate the invention.

EXAMPLE 1

5-Methyl-3-phenyl-4-isoxazolylpenicillin (AB. 1400)

A solution of 5 - methyl - 3 - phenylisoxazole-4-carbonyl chloride (4.43 g.) in acetone (120 ml.) was added gradually to a stirred solution of 6-aminopenicillanic acid (4.32 g.) in 3% aqueous sodium bicarbonate (168 ml.) and acetone (50 ml.). When addition was complete the mixture was stirred at room temperature for 4 hours and then extracted with ether (2 × 200 ml.), only the aqueous phase being retained. This aqueous solution was covered with ether (50 ml.) and adjusted to pH 2 by the addition of N. hydrochloric acid. After separating the layers, the aqueous phase was extracted with two further 50 ml. portions of ether. The combined ether solutions (which at this stage contained the free penicillin acid) were washed with water and then neutralised by shaking with N. sodium bicarbonate solution (20 ml.). The aqueous phase was separated, washed with ether, and evaporated at low temperature and pressure to leave the crude sodium salt of 5 - methyl - 3 - phenyl - 4 - isoxazolylpenicillin as a white solid, which was finally dried *in vacuo* over phosphorus pentoxide. The product (7.34 g.) was estimated by colourimetric assay with hydroxylamine against a benzylpenicillin standard to be about 66% pure.

It inhibited *Staph. Oxford* at a concentration of 0.25 mcg./ml., the benzylpenicillin-resistant *Staph. 1* at 1.25 mcg./ml., and the benzylpenicillin-resistant *Staph. 2* at 1.25 mcg./ml.

EXAMPLE 2

3:5-Diphenyl-4-isoxazolylpenicillin (AB. 1413)

A solution of 3:5 - diphenylisoxazole - 4-carbonyl chloride (750 mg.) in acetone (16 ml.) was added dropwise to a stirred solution of 6-aminopenicillanic acid (575 mg.) in 3% aqueous sodium bicarbonate solution (23 ml.) and acetone (7 ml.). The mixture was stirred for 3 hours at room temperature and then worked up as described in Example 1, to give the sodium salt of 3:5 - diphenyl - 4-isoxazolyl - penicillin as a cream - coloured solid (890 mg.). Colourimetric assay with hydroxylamine against a benzylpenicillin standard indicated a purity of 51%.

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