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PATENT SPECIFICATION

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COMPLETE SPECIFICATION

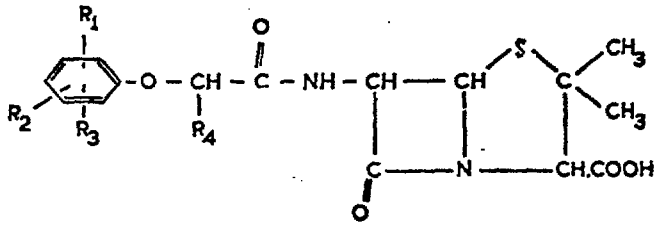
Antibacterial Agents

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 the preparation of new synthetic compounds of value as antibacterial agents, and, more particularly, to substituted 6 - (2 - phenoxypropionamido) - penicillanic acids and non-toxic salts thereof.

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 the serious drawbacks 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 aureaus (Micrococcus pyogenes* var. *aureus)*, which produce penicillinase. Many of the compounds of the present invention, in addition to their potent antibacterial activity, exhibit resistance to destruction acid or by penicillinase.

The present invention provides acids of the general formula:—



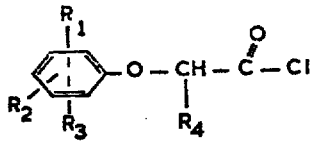
(I)

wherein R_1, R_2 and R_3 are the same or different and each is a hydrogen atom or a nitro, amino, lower alkylamino, di-lower alkylamino, lower acylamino (where the acylating acid is an aliphatic carboxylic acid containing from one to ten carbon atoms inclusive and the substituent may thus also be named lower alkanoylamino), lower alkyl, chloro, bromo, iodo, lower alkoxy, hydroxy, sulphamyl, benzyl, cyclohexyl, phenyl or trifluoromethyl group and R_4 is a lower alkyl (including, both here and above, straight and branched chain saturated aliphatic groups containing from one to ten carbon atoms inclusive), phenyl or phenylalkyl (including benzyl and α- and β-phenethyl and α-, β- and γ-phenylpropyl) group and non-toxic salts thereof, 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 trialkylamine, 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 term "lower" as used herein refers to radicals containing not more than ten carbon atoms.

The present invention also includes a process for the preparation of the compounds of the present invention wherein 6-aminopenicillanic acid, preferably in the form of a neutral salt, such as the sodium salt or the triethylamine salt, is reacted with an acid chloride of the general formula:

[Price 3s. 6d.]



(II)

wherein R_1, R_2, R_3 and R_4 have the meanings given above, or a functional equivalent thereof as an acylating agent for a primary amino group. Such equivalents include the corresponding carboxylic acid bromides, acid anhydrides and mixed anhydrides with other carboxylic acids, including mono-esters, and particularly lower aliphatic esters, of carbonic acid.

In one form of this invention a mixed anhydride is prepared by mixing an acid (whose acid chloride is of the general formula II) with isobutyl chloroformate and a tertiary hydrocarbonyl or aliphatic amine (such as triethylamine) in an anhydrous, inert and preferably water-miscible solvent such as *p*-dioxane and cooling the mixture. A chilled solution of 6-aminopenicillanic acid and a tertiary hydrocarbonyl amine (e.g., triethylamine) in water, for example, is added to the cold mixture and stirred to form the substituted ammonium salt of the desired product. The unreacted starting materials are extracted, leaving the desired product in an aqueous phase. The aqueous phase is cooled and acidified by the addition of dilute mineral acid. The product is extracted as the free acid into a water immiscible, neutral organic solvent such as ether, and recovered.

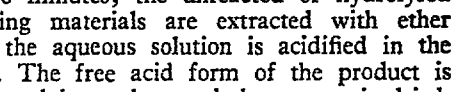
In another form of the invention an aqueous solution of 6-aminopenicillanic acid is treated directly with an acid chloride of the general formula II, and the mixture is shaken vigorously at room temperature for about 20 to 60 minutes; the unreacted or hydrolysed starting materials are extracted with ether and the aqueous solution is acidified in the cold. The free acid form of the product is extracted into ether, and the extract is dried. The product can be obtained from the dry ethereal solution in the form of an ether-insoluble salt such as the potassium salt. This direct 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 an equimolecular amount of the corresponding acid bromide or acid anhydride.

The reaction conditions used in carrying out the process of the present invention will depend upon the reactivity of the reagent being used. It will be understood that a compromise usually has to be made between the use of very mild conditions for a long period and the use of more vigorous conditions for a shorter time with the attendant possibility of decomposing some of the antibiotic substance.

The preferable reaction conditions for the present invention include temperatures close to ambient temperature, generally not above 30°C; and a pH range of from 6 to 9, which can conveniently be controlled by using a buffer such as sodium bicarbonate or a sodium phosphate. The reaction may be carried out in an aqueous medium or in organic solvents such as dimethylformamide, dimethylacetamide, chloroform, acetone, methyl isobutyl ketone and dioxane.

The products prepared according to this invention can be isolated if desired by the techniques used with benzyl-penicillin and phenoxymethylpenicillin. Such techniques include, for example, extraction into a solvent at acid pH followed by recovery by lyophilisation; or precipitation from aqueous solution as a water-insoluble amine salt; or direct recovery from aqueous solution by lyophilisation. A particularly elegant 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 solution of potassium 2-ethylhexanoate (e.g., 0.373 gm/ml.,) in dry *n*-butanol. The potassium salt precipitates, usually in crystalline form, and is collected by filtration or decantation.

One method of preparing the starting material, 6-aminopenicillanic acid is according to Batchelor *et al* (Nature *183*, 257—258, January 24, 1959). It may be used in the processes of the present invention as a metal salt or a tertiary hydrocarbonyl amine salt or as an ester of a hydrocarbonyl alcohol. The tertiary hydrocarbonyl amines and the hydrocarbonyl alcohols are compounds having the general formulae:—

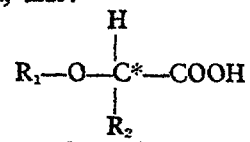


where the R groups contain only the elements carbon and hydrogen.

When an acid chloride, an acid bromide or an acid anhydride is used in the process of the present invention, it is prepared from the corresponding acid according to the techniques set forth in the literature for acids such as phenylacetic acid and phenoxyacetic acid. In any instances where the substituted α-phenoxyalkanoic acid has not been described, it is prepared from the appropriately substituted phenol and the appropriate α-chloro- or α-bromo-acid by the methods described in the art for phenoxyacetic acid or substituted phenoxyacetic acids.

The compounds of the present invention may be viewed, broadly speaking, as the result of combining the single, naturally

occurring optical isomer, 6-aminopenicillanic acid, with an acid which contains at least one asymmetric carbon atom as indicated by the asterisk, thus:



Thus, the product when a racemic acid is used will be a mixture of two diastereoisomers. Both are biologically active and both of these isomers and mixtures thereof are included within the scope of the present invention. The individual isomers are prepared in pure form by starting with the pure dextro or laevo form of the acid or by physical separation of the mixture produced from the racemic acid.

The following examples illustrate the invention.

EXAMPLE 1

Triethylamine (1.5 ml.) was added to a cold solution (10°C.) of α-phenoxypropionic acid (1.66 gm., 0.01 mole) in 15 ml. of pure dioxane, with stirring and cooling to 5—10°C. while isobutyl chloroformate (1.36 gm., 0.01 mole) in 5 ml. of dioxane was added dropwise. Then the mixture was stirred for ten minutes at 5 to 8°C. A solution of 6-aminopenicillanic acid (2.16 gm., 0.01 mole) in 15 ml. of water and 2 ml. of triethylamine was then added dropwise while the temperature was maintained below 10°C. The resulting mixture was stirred in the cold for 15 minutes then at room temperature for 30 minutes, diluted with 30 ml. of cold water and extracted with ether which was discarded. The cold aqueous solution was then covered with 75 ml. of ether and acidified to pH 2 with 5N H_2SO_4. After shaking, the ether layer containing the product 6-(α-phenoxypropionamido)penicillanic acid, was dried for ten minutes over anhydrous sodium sulphate and filtered. Addition of 6 ml. of dry *n*-butanol containing 0.373 gm./ml. of potassium 2-ethylhexanoate precipitated the potassium salt of the product as a colourless oil which crystallised on stirring and scratching and was collected, dried *in vacuo* and found to weigh 2.75 gm., to melt at 217—219°C. to be very soluble in water, to contain the β-lactam structure as shown by infra-red analysis and to inhibit *Staph. aureus* Smith at a concentration of 0.07 mcg./ml.

EXAMPLE 2

α-(2,4-Dichlorophenoxy)propionic acid (0.01 mole), triethylamine (0.011 mole) and isobutyl chloroformate (0.01 mole) were stirred in 20 ml. of pure, dry dioxane and 2 ml. of dry acetone for about thirty minutes at about 4°C. To this solution there was then added a chilled solution of 6-aminopenicillanic acid (0.01 mole) and triethylamine (0.01 mole) in 20 ml. of water and the mixture was stirred for about an hour in the cold. After the addition of 1.0 gm. of sodium bicarbonate in 30 ml. of cold water, the solution was extracted twice with 75 ml. portions of ether and the ethereal extract was discarded. The aqueous solution was cooled and stirred in an ice bath, covered with 75 ml. of ether and adjusted to pH 2 with 5N H_2SO_4. The ether was separated and the aqueous solution was again extracted with 75 ml. of ether. The combined ethereal extracts containing the product, 6 - [α - (2,4 - dichlorophenoxy)propionamido] - penicillanic acid, were dried rapidly over anhydrous sodium sulphate and filtered. The addition of 6 ml. of dry *n*-butanol containing 0.373 gm./ml. of potassium 2-ethylhexanoate followed by additional dry ether precipitated the potassium salt of the product. After trituration with ether, this potassium salt of the product was dried *in vacuo* over phosphorus pentoxide and recovered as a water-soluble powder which inhibited the growth of *Staph. aureus* Smith at a concentration of 0.001 per cent by weight.

EXAMPLE 3

The potassium salt of 6 - (α - phenoxyphenylacetamido)penicillanic acid was prepared according to the procedure of Example 1 using α-phenoxyphenylacetic acid (6.8 g., 0.03 mole). The product precipitated as an amorphous white solid which was collected, dried *in vacuo* over phosphorus pentoxide and found to weigh 11.0 g., m.p. 88—95°C. decomposed at 120—125°C. to contain the β-lactam ring as shown by infra-red analysis and to inhibit *Staph.* aureus Smith at a concentration of 0.2 mcg./ml.

EXAMPLE 4

Following the procedure of Example 2, the potassium salt of 6 - [α - (4 - benzylphenoxy)-propionamido] - penicillanic acid was prepared using α-(4-benzylphenoxy)propionic acid (0.02 mole). The product was recovered as a water-soluble powder which inhibits the growth of *Staph. aureus* Smith at a concentration of 0.001 per cent by weight.

EXAMPLE 5

The potassium salt of 6 - [α - (*p* - tertiary - butylphenoxy) - propionamido] - penicillanic acid was prepared by the procedure of Example 2 using α - (*p* - tertiary - butylphenoxy)-propionic acid (0.03 mole, 6.68 g.). The product was precipitated as a white, crystalline solid upon dilution with anhydrous ether, collected by filtration, dried overnight *in vacuo* over phosphorus pentoxide and found to weigh 6.2 g., to melt at 219—220°C. with decomposition, to contain the β-lactam ring as shown in infra-red analysis and to inhibit *Staph. aureus* Smith at a concentration of 0.1 mcg./ml.

EXAMPLE 6

The procedure of Example 2 was followed except that the α - (2,4 - dichlorophenoxy)-propionic acid was replaced by α - (*p* -

tertiary - amylphenoxy) - *n* - butyric acid (7.53 g., 0.03 mole) also called α - [*p* - (1,1 - dimethylpropyl)phenoxy] - *n* - butyric acid) to produce the final product, potassium 6 - [α - (*p* - tertiary - amylphenoxy) - *n* - butyramido] - penicillinate as a solid gum (after trituration with ether) which was converted to a fine powder on drying in a vacuum dessicator over phosphorus pentoxide. This salt weighed 4.7 g., on heating slowly decomposed above 125°C., contained the β-lactam ring as shown by infra-red analysis and was found to inhibit *Staph.* aureus Smith at a concentration of less than 10 mcg./ml.

EXAMPLE 7

Following the procedure of Example 2, α - (2,4 - diisoamylphenoxy) - *n* - butyric acid (0.2 mole) was employed to produce the potassium salt of 6 - [α - (2,4 - diisoamylphenoxy) - *n* - butyramido] - penicillanic acid. The product was recovered as a water-soluble powder which inhibited the growth of *Staph. aureus* Smith at a concentration of 0.001 per cent by weight.

EXAMPLE 8

The procedure of Example 2 was followed except that the α - (2,4 - dichlorophenoxy)-propionic acid was replaced by α - (2,4 - dichlorophenoxy) - *n* - butyric acid (0.04 mole). The product, the potassium salt of 6 - [α - (2,4 - dichlorophenoxy) - *n* - butyramido] - penicillanic acid, was recovered as a water-soluble powder which inhibited the growth of *Staph. aureus* Smith at a concentration of 0.001 per cent by weight.

EXAMPLE 9

The procedure of Example 2 was followed except that the α - (2,4 - dichlorophenoxy)-propionic acid was replaced by α - (4 - trifluoromethylphenoxy) - *n* - butyric acid (0.01 mole). The product, the potassium salt of 6 - [α - (4 - trifluoromethylphenoxy) - *n* - butyramido] - penicillanic acid, was recovered as a water-soluble powder which inhibited the growth of *Staph. aureus* Smith at a concentration of 0.001 per cent by weight.

EXAMPLE 10

In the procedure of Example 1, the α-phenoxypropionic acid was replaced by 0.10 mole of α - (2 - chlorophenoxy)propionic acid, α - (*p* - sulphonamylphenoxy) - *n* -butyric acid, α - (3,4 - dimethoxyphenoxy) - *n* - pentanoic acid, α - (3 - methylphenoxy) - iso - valeric acid, α - (4 - dimethylaminophenoxy) - *n* - hexanoic acid, α - (2 - methoxyphenoxy) - *n* - decanoic acid, α - (2,4 - dichlorophenoxy)-phenylacetic acid, α - (2 - nitrophenoxy) - β - phenylpropionic acid, α - (2 - acetamidophenoxy) - γ - phenylbutyric acid, α - (2,4 - dimethylphenoxy) - *n* - butyric acid, α - (4 - isopropylphenoxy)propionic acid, α - (3 - bromophenoxy) - *n* - butyric acid, α - (2 - iodophenoxy) - phenylacetic acid, α - (2 - ethylaminophenoxy) - iso - valeric acid, α - (2,5 - dihydroxyphenoxy) - isohexanoic acid, α - (4 - hydroxyphenoxy)propionic acid, α - phenoxy - iso - valeric acid, α - phenoxy - *n* - decanoic acid, α - phenoxy - γ - phenylbutyric acid, α - (2 - benzylphenoxy) - *n* - butyric acid, α - (2 - trifluoromethylphenoxy) - propionic acid and α - (4 - aminophenoxy)-propionic acid, respectively, to produce the acids 6 - [α - (2 - chlorophenoxy)propionamido] - penicillanic acid, 6 - [α - (4 - sulphamylphenoxy) - *n* - butyramido] - penicillanic acid, 6 - [α - (3,4 - dimethoxyphenoxy) - *n* - pentanoamido] - penicillanic acid, 6 - [α - (3 - methylphenoxy) - iso - valeramido] - penicillanic acid, 6 - [α - (4 - dimethylaminophenoxy) - *n* - hexanoamido] - penicillanic acid, 6 - [α - (2 - methoxyphenoxy) - *n* - decanoamido] - penicillanic acid, 6 - [α - (2,4 - dichlorophenoxy)-phenylacetamido] - penicillanic acid, 6 - [α - (2 - nitrophenoxy) - β - phenylpropionamido] - penicillanic acid, 6 - [α - (2 - acetamidophenoxy) - γ - phenylbutyramido] - penicillanic acid, 6 - [α - (2,4 - dimethylphenoxy) - *n* - butyramido] - penicillanic acid, 6 - [α - (4 - isopropylphenoxy) - propionamido] - penicillanic acid, 6 - [α - (3 - bromophenoxy) - *n* - butyramido] - penicillanic acid, 6 - [α - (2 - iodophenoxy)-phenylacetamido] - penicillanic acid, 6 - [α - (2 - ethylaminophenoxy) - iso - valeramido] - penicillanic acid, 6 - [α - (2,5 - dihydroxyphenoxy) - iso - hexanoamido] - penicillanic acid, 6 - [α - (4 - hydroxyphenoxy)propionamido] - penicillanic acid, 6 - [α - phenoxy - iso - valeramido] - penicillanic acid, 6 - [α - phenoxy - *n* - decanoamido] - penicillanic acid, 6 - [α - phenoxy - γ - phenylbutyramido] - penicillanic acid, 6 - [α - (2 - benzylphenoxy) - n - butyramido] - penicillanic acid, 6 - [α - (2 - trifluoromethylphenoxy)propionamido] - penicillanic acid and 6 - [α - (4 - aminophenoxy)propionamido] - penicillanic acid, respectively, which were isolated as their solid water-soluble potassium salts and found to inhibit *Staph. aureus* Smith at concentration of 0.001 per cent by weight.

EXAMPLE 11

In the procedure of Example 1, the α-phenoxypropionic acid was replaced by 0.10 mole of α - (2 - chloro - 4 - phenylphenoxy) - propionic acid, α - (4 - chlorophenoxy) - propionic acid, α - (4 - aminophenoxy)-propionic acid, α - (4 - amino - 3 - trifluoromethylphenoxy - propionic acid, α - (*o* - methoxyphenoxy)propionic acid and α - (*m* - methoxyphenoxy)propionic acid, respectively, to produce the acids 6 - [α - (2 - chloro - 4 - phenylphenoxy) - propionamido] - penicillanic acid, 6 - [α - (4 - chlorophenoxy) - propionamido] - penicillanic acid, 6 - [α - (4 - aminophenoxy) - propionamido] - penicillanic acid, 6 - [α - (4 - amino - 3 - trifluoromethylphenoxy) - propionamido] - penicillanic acid, 6 - [α - (*o* - methoxyphenoxy) - propionamido] - penicillanic acid, and

6 - [α - (*m* - methoxyphenoxy) - propionamido] - penicillanic acid, respectively, which were isolated as their solid, water-soluble potassium salts and found to inhibit *Staph. aureus* Smith at concentration of 0.001 per cent by weight.

EXAMPLE 12

6 - (α - Phenoxypropionamido) - penicillanic acid was prepared by the direct acylation of fermentation broth (beer) followed by isolation therefrom of the penicillin as its potassium salt. Thus, a submerged aerobic fermentation of *Penicillin chrysogenum* was carried out according to the general methods for the production of penicillin G except for the omission of the usual addition of phenylacetic acid as the precursor. At the end of the fermentation the broth was filtered and its pH was adjusted to 7.5 with 10% sodium hydroxide. After cooling the filtered broth to about 10—20°C. there was added with agitation 5 moles of α-phenoxypropionyl chloride to the filtered broth for each mole of 6-aminopenicillanic acid therein as determined by analysis. The phenoxypropionyl chloride was added as a 5% solution in acetone, i.e., approximately 0.00031 moles of acid chloride per ml. The pH fell during the addition of α-phenoxypropionyl chloride and, therefore, the rate of addition was kept slow enough to enable the pH to be maintained at 7.5 by the addition of 10% sodium hydroxide. The reaction mixture was then agitated at 10—20°C. for an additional 30 minutes. The reaction was presumed to be complete when the pH remained constant; this required about 10—15 minutes. To avoid contamination with acid-labile penicillins present in the broth, these were destroyed by next lowering the pH of the raction mixture to pH 2 for 30 minutes before subsequent solvent extraction (the same result could also be obtained by originally filtering the broth at pH 2 and holding it there for 30 minutes). The acidified reaction mixture was extracted with 1/2 volume of methyl isobutyl ketone for 20 minutes and the methyl isobutyl ketone containing the desired 6 - (α - phenoxypropionamido) - penicillanic acid was separated and filtered. This acid penicillin in the solvent was then converted to its potassium salt by stirring the methyl isobutyl ketone solution vigorously with 5% by volume of aqueous potassium acetate having a specific gravity of 1.30 while cooling to 5 to 10°C. Crystallisation of the product, potassium 6 - (α - phenoxypropionamido) - penicillanate started almost immediately and after one hour the crystalline product was collected by filtration, washed successively with methyl isobutyl ketone, dry butanol and acetone and found to be a potent, white crystalline solid.

In numerous runs by this procedure the efficiency of the activation step was generally about 80% with the remaining 6-amino-penicillanic acid accounted for in the spent broth. From activation broth to crude penicillin the activity yield was 77%. On a molar basis 0.64 moles of the new penicillin was produced per mole of 6-aminopenicillanic acid in the broth. Thus, in one series of runs 11.9 kg. of crude penicillin was produced from 5500 gallons of broth.

EXAMPLE 13

To a mixture of one litre of water and 100 ml. of acetone there was added 105 g. (1.25 mole) of sodium bicarbonate. After stirring for one hour in an ice bath there was added 54 g. (0.25 mole) of 6-aminopenicillanic acid. This slurry was stirred for thirty minutes in an ice bath and then there was added dropwise over thirty minutes with vigorous stirring at a maximum temperature of 10°C. a solution in 100 ml. of acetone of 2-phenoxypropionyl chloride (68.8 g., 0.375 mole). Methyl isobutyl ketone (400 ml.) was added and the vigorous stirring was continued for five minutes. After separating and discarding the methyl isobutyl ketone, the aqueous layer was extracted with 250 ml. portions of methyl isobutyl ketone which were also discarded. The aqueous layer was cooled, acidified to pH 2 with 40% H_2SO_4 in an ice bath and extracted with a total of 800 ml. of methyl isobutyl ketone. The combined solvent extracts containing the product, 6 - (α - phenoxypropionamido) - penicillanic acid, were dried over anhydrous sodium sulphate for two hours in an ice bath and filtered. The product was then converted to its potassium salt by the addition of 100 ml. of 50% potassium 2-ethylhexanoate in *n*-butanol to precipitate 67 g. of crystalline potassium salt of the product.

EXAMPLE 14

Potassium 6 - (α - phenoxybutyramido) - penicillanate (the potassium salt of 6 - (α - phenoxybutyramido) - penicillanic acid) was prepared according to the procedure of Example 1 using 6-aminopenicillanic acid (21.6 g., 0.1 m.) in 100 ml. of water and sufficient triethylamine to dissolve, 2-phenoxybutyric acid (18 g., 0.1 m.) in 80 ml. of *p*-dioxane and 20 ml. of pure acetone and 13.7 ml. of isobutyl chloroformate. There was obtained 10.3 g. of product, m.p. 175—197°C. with decomposition (darkened at 170°C.) which was very soluble in water, contained the β-lactam structure as shown by infra-red analysis, inhibited *Staph. aureus* Smith at a concentration of 0.05 mcg./ml. and exhibited versus *Staph. aureus* Smith upon intramuscular injection in mice a CD_{50} of 1.1 mgm/kg. ($C_{18}H_{21}N_2O_5SK$: Calculated C, 51.8; H, 5.1. Found: C, 51.1; H, 5.49).

EXAMPLE 15

Potassium 6 - [α - (2,5 - dichlorophenoxy)-propionamido] - penicillanate was prepared according to the general procedure of Example 1 using α - (2,5 - dichlorophenoxy)propionic

acid (0.1 m., 23.5 g.) in 160 ml. of dimethylformamide and 14 ml. of triethylamine, 6 - aminopenicillanic acid (0.1 m., 21.6 g.) in 160 ml. of water and 14 ml. of triethylamine and isobutyl chloroformate (0.1 m., 13.7 g.). There was obtained 21.5 g. product, m.p. 200—204°C. with decomposition (darkens above 190°C.) as a solid which was found to be very soluble in water, to contain the β-lactam structure as shown by infra-red analysis, to inhibit *Staph. aureus* Smith at a concentration of 0.05 mcg./ml., and exhibited versus *Staph. aureus* Smith upon intramuscular injection in mice a CD_{50} of 2.5 mgm/kg.

EXAMPLE 16

Potassium 6 - [α - (*p* - acetamidophenoxy)-propionamido] - penicillanate was prepared according to the procedure of Example 1 using 31 g., (0.15 mole) of α - (*p* - acetamidophenoxy) - propionic acid and obtained as 27.7 g. of solid m.p. 211—215°C. with decomposition when put on a rapidly heating stage at 200°C. and found to be very soluble in water, to contain the β-lactam structure as shown by infra-red analysis, to inhibit *Staph. aureus* Smith at a concentration of 0.4 mcg./ml. and to exhibit versus *Staph. aureus* Smith upon intramuscular injection in mice a CD_{50} of 6.4 mgm/kg. ($C_{19}H_{22}N_3O_6SK$: Calculated C, 50.95; H, 4.70. Found: C, 50.12; H, 5.21). An additional yield of 15.9 g. was obtained by adding more acetone to the mixture of methyl isobutyl ketone and 50% potassium 2-ethylhexanoate in butanol. This fraction melted at 223—226°C. with decomposition but some melting was observed starting at about 214°C.

EXAMPLE 17

Thionyl chloride (30 ml.) was added fairly rapidly at room temperature to a cool solution of 200 ml. of benzene, 1 ml. of pyridine and 21 g. (0.0725 mole) of α - (2 - benzyl - 4 - chlorophenoxy)propionic acid and the mixture was refluxed for two hours and then the solvents were removed by distillation *in vacuo* up to 100°C. at 20 mm. The residual acid chloride was cooled and dissolved in 80 ml. of acetone and added slowly to a mixture of 13 g. (0.06 mole) of 6-aminopenicillanic acid in 100 ml. of water containing 12.6 g. (0.15 mole) of sodium bicarbonate and 20 ml. of acetone. Proceeding according to Example 12, there was obtained 13.4 g. of solid potassium 6 - [α - (2 - benzyl - 4 - chlorophenoxy)-propionamido] - penicillanate, melting at 230—231°C. with decomposition, which was soluble in water, contained the β-lactam structure as shown by infra-red analysis, inhibited *Staph. aureus* Smith at a concentration of 0.4 mcg./ml. exhibited versus *Staph. aureus* Smith upon intramuscular injection in mice a preliminary CD_{50} of 9 mgm/kg., and was only 26% inactivated by pH 2.5 acid under conditions which decomposed benzylpenicillin to the extent of greater than 96%, i.e. in 0.75 molar citric acid for one hour at 37°C.

EXAMPLE 18

The procedure of Example 2 was followed using α-phenoxy caproic acid (m.p. 70—72°C., 13.70 g., 0.0659 m.) in place of the α-(2,4-dichlorophenoxy)propionic acid. The product, the potassium salt of 6 - (α - phenoxycaproamido) - penicillanic acid, was recovered to give an amorphous solid salt which was very soluble in water, decomposed on heating at about 160°C., contained the β-lactam structure as shown by infra-red analysis, inhibited *Staph. aureus* Smith at a concentration of 0.4 mcg./ml. exhibited versus *Staph. aureus* Smith upon intramuscular injection in mice a preliminary CD_{50} of 4 mgm./kg. and was only 36% inactivated by pH 2.5 acid under conditions which decomposed benzylpenicillin to the extent of greater than 96% i.e. in 0.75 molar aqueous citric acid for one hour at 37°C.

EXAMPLE 19

α - (4 - Chloro - 3,5 - dimethylphenoxy)-caproic acid (0.65 m., 17.6 g.) was used in the procedure of Example 2 to produce 6 - [α - (4 - chloro - 3,5 - dimethylphenoxy)-caproamido] - penicillanic acid which was isolated as its potassium salt in the form of a fluffy, amorphous, hygroscopic solid which weighed 10.0 g., melted at 105—108°C. with decomposition, was soluble in water, contained the β-lactam structure as shown by infra-red analysis, inhibited *Staph. aureus* Smith at concentration of 0.4 mcg./ml., exhibited versus *Staph. aureus* Smith upon intramuscular injection in mice a preliminary CD_{50} of 9 mgm./kg., was only 29% inactivated by 1 μ./ml. penicillinase under conditions which inactivated benzylpenicillin to the extent of 73% and was only 55% inactivated by pH 2.5 acid under conditions which decomposed benzylpenicillin to the extent of greater than 96% i.e. in 0.75 molar aqueous citric acid for one hour at 37°C.

EXAMPLE 20

α - Phenoxy - *n* - valeric acid (0.1 m., 19.4 ml.) was used in the procedure of Example 2 on a 0.1 mole scale and methyl isobutyl ketone was used as the extraction solvent to produce 6 - (α - phenoxyvaleramido) - penicillanic acid which was isolated as its potassium salt in the form of solid weighing 17.7 g. melting at 170—175°C. with decomposition. This salt was soluble in water, contained the β-lactam structure as shown in infra-red analysis, inhibited *Staph. aureus* Smith at a concentration of 0.4 mcg./ml., exhibited versus *Staph. aureus* Smith upon intramuscular injection in mice a CD_{50} of 3.7 mgm./kg., was only 30% inactivated by 1 μ./ml. penicillinase under conditions which inactivated benzylpenicillin to the extent of 73% and was only 13% inactivated by pH 2.5 acid under conditions

which decomposed benzylpenicillin to the extent of greater than 96% i.e., in 0.75 molar aqueous citric acid for one hour at 37°C.

EXAMPLE 21

Potassium 6 - [α - (2,4,6 - trichlorophenoxy)propionamido] - penicillanate was prepared from α - (2,4,6 - trichlorophenoxy) - propionic acid (0.1 m., 27 g.) according to the procedure of Example 1 and recovered as a crystalline solid, 4.9 g., m.p. 163—165°C. with decomposition (darkens slowly above 140°C.). The product was soluble in water, contained the β-lactam structure as shown by infra-red analysis, inhibited *Staph. aureus* Smith at a concentration of 0.4 mcg./ml., exhibited versus *Staph. aureus* Smith upon intramuscular injection in mice a preliminary CD_{50} of 9 mgm./kg., was only 37% inactivated by 1 μ./ml. penicillinase under conditions which inactivated benzylpenicillin to the extent of 58% and was only 27% inactivated by pH 2.5 acid under conditions which decomposed benzylpenicillin to the extent of greater than 96%, i.e. in 0.75 molar aqueous citric acid for one hour at 37°C. ($C_{17}H_{16}Cl_3KN_2O_5S$; Calculated: C, 40.3; H, 3.19. Found: C, 40.8; H, 3.55).

EXAMPLE 22

Potassium 6 - [α - (*p* - methoxyphenoxy)-propionamido] - penicillanate was prepared from 19.6 g. (0.1 mole) α - (*p* - methoxyphenoxy)propionic acid according to the procedure of Example 1 and recovered as a white crystalline solid, 14.6 g. m.p. 211—214°C. with decomposition with darkening above 208°C. The product was soluble in water, contained the β-lactam structure as shown by infra-red analysis, inhibited *Staph. aureus* Smith at a concentration of 0.1 mcg./ml., exhibited versus *Staph. aureus* Smith upon intramuscular injection in mice a preliminary CD_{50} of 1.9 mgm./kg. was only 23% inactivated by 1 μ./ml. penicillinase under conditions which inactivated benzylpenicillin to the extent of 58% and was only 2% inactivated by pH 2.5 acid under conditions which decomposed benzylpenicillin at the extent of greater than 96%, i.e., in 0.75 molar citric acid for one hour at 37°C. ($C_{18}H_{21}KN_2O_6S$: Calculated C, 49.9; H, 4.90. Found: C, 49.66; H, 5.10.).

EXAMPLE 23

α - (*m* - Trifluoromethylphenoxy)propionic acid (0.1 m., 23.4 g.) was used in the procedure of Example 2 on a 0.1 mole scale to produce 6 - [α - (3 - trifluoromethylphenoxy)propionamido] - penicillanic acid which was isolated as its potassium salt in the form of a white solid which weighed 11.0 g., melted at 188—190°C. with decomposition, contained the β-lactam structure as shown by infra-red analysis, inhibited *Staph. aureus* Smith at a concentration of 0.1 mcg./ml. and exhibited versus *Staph. aureus* Smith upon intramuscular injection in mice a preliminary CD_{50} of 1.8 mgm./kg.

EXAMPLE 24

α - (*p* - Nitrophenoxy)propionic acid (0.1 m., 22.1 g.) was used in the procedure of Example 2 on a 0.1 mole scale to produce 6 - [α - (4 - nitrophenoxy)propionamido] - penicillanic acid which was isolated as its potassium salt in the form of a solid which weighed 31.8 g., melted at 202—203°C. with decomposition, contained the β-lactam structure as shown by infra-red analysis and inhibited *Staph. aureus* Smith at a concentration of 0.8 mcg./ml.

EXAMPLE 25

Potassium 6 - [α - (4 - chloro - 3,5 - dimethylphenoxy)propionamido] - penicillanate was prepared from 22.9 g. (0.1 mole) of α - (4 - chloro - 3,5 - dimethylphenoxy)-propionic acid according to the procedure of Example 1 and recovered as a white crystalline solid, 14.8 g., m.p. 210—213°C. with decomposition, (darkening above 200°C.). The product was soluble in water, contained the β-lactam structure as shown by infra-red analysis and inhibited *Staph. aureus* Smith at a concentration of 1.6 mcg./ml. ($C_{19}H_{22}ClKN_2O_5S$: Calculated: C, 49.0; H, 4.77. Found: C, 48.78; H, 4.90.).

EXAMPLE 26

The potassium salts of 6 - [α - (2,4 - dibromophenoxy)propionamido] - penicillanic acid, 6 - [α - (2, - *n* - pentylphenoxy) - propionamido] - penicillanic acid, 6 - [α - (2,6 - dimethoxyphenoxy) - propionamido] - penicillanic acid, 6 - [α - (2,3,4,5,6 - pentachlorophenoxy)propionamido] - penicillanic acid, and 6 - [α - (4 - nitro - 3 - trifluoromethylphenoxy)propionamido] - penicillanic acid were prepared according to the methods described in Example 1, isolated as colourless solids and found to be soluble in water and to inhibit *Staph. aureus* Smith at the following concentrations in mcg./ml. respectively: 0.05, 0.4, 3.1, 0.1 and 0.2.

EXAMPLE 27

Potassium 6 - [α - (*p* - cyclohexylphenoxy)-propionamido] - penicillanate was prepared from α - (*p* - cyclohexylphenoxy)propionic acid (30 g., 0.121 mole) according to the procedure of Example 16 and found to weigh 37 g., to melt at 230—231°C. with decomposition, to contain the β-lactam structure as shown by infra-red analysis and to inhibit *Staph. aureus* Smith at a concentration of 0.4 mcg./ml.

EXAMPLE 28

Potassium 6 - [α - (3,5 - dimethylphenoxy)-propionamido] - penicillanate was prepared according to Example 2 from 19.4 g. (0.1 mole) α - (3,5 - dimethylphenoxy)propionic acid and recovered as a white crystalline material, 21.5 g. m.p. 220—222°C., which contained the β-lactam structure as shown by infra-red analysis and inhibited *Staph. aureus* Smith at a concentration of 0.1 mcg./ml.

EXAMPLE 29

The two diastereoisomers of 6 - (α - phenoxypropionamido) - penicillanic acid were prepared in the form of their potassium salts by resolving racemic α-phenoxypropionic acid using yohimbine, converting the dextro and laevo isomers of the acid to the corresponding acid chlorides and then reacting each isomer with 6-aminopenicillanic acid obtained from a fermentation broth. This resolution was accomplished from the method of E. Fourneau and G. Sandulesco, Bull. Soc., Chim., Ser. 4, *31*, 988—990 (1922). The final products were arbitrarily named as the alpha and beta isomers, the former being derived from dextro-α-phenoxypropionic acid and the latter from laevo-α-phenoxypropionic acid.

EXAMPLE 30

Dextro - α - phenoxypropionic acid (83.31 g., 0.5 mole, $[\alpha]_D^{24°} = +40°$, c = 1, C_2H_5OH) in 500 ml. of pure dioxane and 200 ml. of acetone was converted by reaction with 70 g. of isobutyl chloroformate and 57 ml. of triethylamine and 108 g. (0.5 mole) of 6-aminopenicillanic acid in 700 ml. of water and sufficient triethylamine to dissolve, to the alpha isomer of potassium 6 - (α - phenoxypropionamido) - penicillanate, 134 g., $[\alpha]_D^{24°} = +250°$ (1% in water), m.p. 240—241°C. with decomposition. Recrystallisation of 100 g. from 1 litre of *n*-butanol and 200 ml. of water gave 61 g. of material having $[\alpha]_D^{24°} = +251°$, 1% in water, and decomposing on heating at 236—237°C.

An additional crop of 22 g. of crystalline salt had $[\alpha]_D^{24°} = +251°$ (1% in water) and decomposed at 230—231°C. Both crops were active against *S. lutea* and *Staph. aureus*.

EXAMPLE 31

By the procedure of Example 29 on a 0.026 mole scale, laevo α-phenoxypropionic acid (4.3 g., $[\alpha]_D^{23.5°} = -39.5°$, c = 1 in C_2H_5OH) was converted to the beta isomer of potassium 6-(α-phenoxypropionamido)-penicillanate, and recrystallised (6 g. from 100 ml. of *n*-butanol and 20 ml. of water) to give 4.75 g. of salt exhibiting $[\alpha]_D = +218°$, 1% in water.

The beta isomer could also be isolated in pure form from solids prepared by broth acylation in which it was present in a ratio of 70:30 by repeated recrystallisations from *n*-butanol-water. Thus, 500 g. was recrystallised from 3.1 litres of *n*-butanol and 900 ml. of water to give 200 g. ($[\alpha]_D^{25°} = +218°$, 1% in water, decomposition point 242—243°C.). A second recrystallisation gave 125 g. ($[\alpha]_D = +218—221°$, 1% in water, decomposition point 245—245°C. ($C_{17}H_{19}N_2O_5SK$: Calculated, C, 50.73; H, 4.76; N, 6.96. Found: C, 50.65; H, 4.83; N, 6.82; H_2O, none). A third recrystallisation gave 73 g. ($[\alpha]_D^{24°} = +220°$, 1% in water, decomposition point 239—240°C.).

Studies were carried out on the diastereoisomers obtained from the reactions of dextro and laevo-α-phenoxypropionic acid with 6-aminopenicillanic acid as well as on a mixture of the two diastereoisomers representing a typical lot produced by broth acylation with racemic acid chloride. The separated diastereoisomers are designated as "alpha isomer" and "beta isomer" respectively, and the mixture of isomers designated as "mixture". Comparisons of these materials were made with penicillin V. All were in the form of potassium salts.

(1) The rates of inactivation of potassium 6 -(α - phenoxypropionamido)penicillanate (mixture) (the potassium salt of 6 - (α - phenoxypropionamido)penicillanic acid), penicillin G, and penicillin V by *Bacillus cereus* penicillinase were determined and it was found that potassium 6 - (α - phenoxypropionamido)penicillanate (mixture) was considerably more resistant to *B. cereus* penicillinase then penicillin V or G.

(2) The stability of potassium 6 - (α - phenoxypropionamido) - penicillanate (mixture) was compared with penicillin V and G at three temperatures. The penicillins were dissolved in 002M citrate buffer at pH 2 and pH 3 and the percentage decrease in activity of samples kept at 5°C., 25°C. and 37°C., was determined by plate assays. It was found that potassium 6 - (α - phenoxypropionamido) - penicillanate (mixture) and penicillin V have essentially equivalent acid stability, both being considerably more stable than penicillin G.

(3) Comparative animal protection tests were carried out using *Staphylococcus aureus* (Smith) as the infecting organism. The antibiotics were given intramuscularly at the time of infection and the amount of antibiotic necessary to cure half the animals was determined (CD_{50}). The results were as follows:—

Penicillin	CD_{50}(mg./kg.)
Alpha isomer	0.85
Beta isomer	0.35
"Mixture"	0.18
Penicillin V	0.64
Penicillin G	0.60

WHAT WE CLAIM IS:—

1. Acids of the general formula:—

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(3) Comparative animal protection tests were carried out using *Staphylococcus aureus* (Smith) as the infecting organism. The antibiotics were given intramuscularly at the time of infection and the amount of antibiotic necessary to cure half the animals was determined (CD_{50}). The results were as follows:—

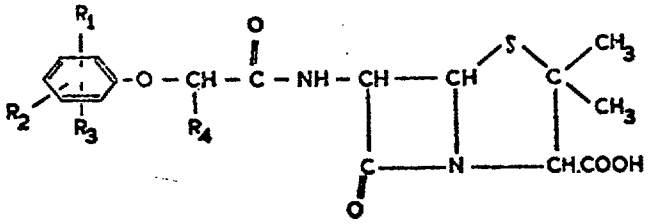
Penicillin	CD_{50}(mg./kg.)
Alpha isomer	0.85
Beta isomer	0.35
"Mixture"	0.18
Penicillin V	0.64
Penicillin G	0.60

WHAT WE CLAIM IS:—

1. Acids of the general formula:—

* C L M S *

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and non-toxic salts thereof, where R_1, R_2 and R_3 are the same or different and each is a hydrogen atom or a nitro, amino, lower alkylamino, lower dialkylamino, lower alkanoylamino, lower alkyl, chloro, bromo, iodo, lower alkoxy, hydroxy, sulphamyl, benzyl, cyclohexyl, phenyl or trifluoromethyl group and R_4 is a lower alkyl or phenyl group or phenylalkyl group containing not more than ten carbon atoms.

2. Acids as claimed in claim 1, wherein R_1, R_2 and R_3 are hydrogen atoms and R_4 is a lower alkyl group containing from 1 to 10 carbon atoms, and non-toxic salts thereof.

3. 6 - (α - Phenoxypropionamido) - penicillanic acid and non-toxic salts thereof.

4. 6 - (α - Phenoxybutyramido) - penicillanic acid and non-toxic salts thereof.

5. 6 - (α - Phenoxyvaleramido) - penicillanic acid and non-toxic salts thereof.

6. 6 - [α - (2,4 - Dichlorophenoxy)propionamido] - penicillanic acid and non-toxic salts thereof.

7. 6 - [α - (2,4 - Diisoamylphenoxy) - *n* - butyramido] - penicillanic acid and non-toxic salts thereof.

8. 6 - [α - (4 - Trifluoromethylphenoxy) - *n* - butyramido] - penicillanic acid and non-toxic salts thereof.

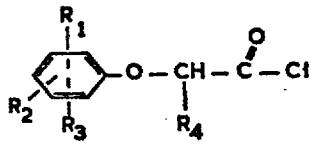
9. 6 - [α - Phenoxy - γ - phenylbutyramido] - penicillanic acid and non-toxic salts thereof.

10. 6 - [α - (2,4 - Dichlorophenoxy) - *n* - butyramido] - penicillanic acid and non-toxic salts thereof.

11. 6 - (α - phenoxyphenylacetamido) - penicillanic acid and non-toxic salts thereof.

12. 6 - [α - (*p* - tertiary - butylphenoxy)-propionamido] - penicillanic acid and non-toxic salts thereof.

13. A process for the preparation of the compounds claimed in claim 1 wherein 6-aminopenicillanic acid, or a neutral salt thereof, is reacted with an acid chloride of the general formula:—



wherein R_1, R_2, R_3 and R_4 have the meanings given in claim 1 or with a functional equivalent thereof as an acylating agent for a primary amino group.

14. A process as claimed in claim 13 wherein the functional equivalent of the acid chloride is obtained by mixing the corresponding acid with isobutyl chloroformate and a tertiary amine in an inert anhydrous water-miscible solvent.

15. A process as claimed in claim 13 wherein the acid chloride or its functional equivalent is reacted directly in an aqueous solution with the 6-aminopenicillanic acid.

16. A process for the preparation of the compounds claimed in claim 1 substantially as described with reference to any one of Examples 1 to 10.

17. A process for the preparation of the compounds claimed in claim 1 substantially as described with reference to any one of Examples 11 to 31.

18. The compounds claimed in claim 1 when produced by the process claimed in any one of the preceding claims 13 to 17.

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

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