

1017.617



PATENT SPECIFICATION

NO DRAWINGS

1017.615

Date of Application and filing Complete Specification: April 25, 1963.
No. 16397/63.

Two Applications made in United States of America (Nos. 190,776 and 190,781)
on April 27, 1962.

Complete Specification Published: Jan. 19, 1966.

© Crown Copyright 1966.

Index at acceptance:—C2 A(1C2A, 1C2B, 1C2C, 2C1); C2 C(1F4C3, 1F4D3, 1F4F5, 2A3, 2A7, 2B2A4, 2B2F, 2B2G1B, 2B2G2, 2B2G6, 2B2G11, 2B40D, 2B40J1, 2S16, 3A14B3A, 3A14B5, 3A14B7C)

Int. Cl.:—C 07 d // C 07 c

COMPLETE SPECIFICATION

Penicillins

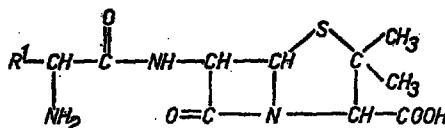
We, BRISTOL-MYERS COMPANY, a Corporation of the State of Delaware, United States of America, of Thompson Road, East Syracuse, State of New York, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed to be particularly described in and by the following statement:—

This invention relates to a process for the preparation of new synthetic compounds of value as antibacterial agents, as nutritional supplements in animal feeds, 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 and Gram-negative bacteria and, more particularly, relates to 6-[α -amino- α -(heterocyclyl)acetamido]penicillanic acids and nontoxic 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 strains of bacteria, e.g., most Gram-negative bacteria. The compounds prepared by the process of the present invention are particularly useful in that they possess potent antibacterial activity against both Gram-positive and Gram-negative bacteria upon either parenteral or oral administration and also exhibit resistance to destruction by acid and penicillinase.

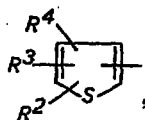
There is provided, according to the present invention, a process for the preparation of penicillanic acids having the general formula

(I)

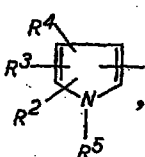


wherein R¹ is

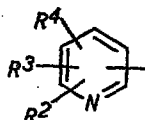
(II)



(III)



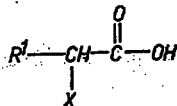
(IV)



wherein R⁵ is hydrogen, alkyl, alkanoyl, alkylsulfonyl, phenyl, chlorophenyl, phenethyl or a cycloalkyl group and R², R³ and R⁴ are selected from hydrogen, chloro, bromo, fluoro, iodo, or nitro, alkyl, alkoxy, alkylthio, dialkylamino, alkanoylamino, alkanoyl, alkylsulfonyl, cycloalkyl groups, cycloalkoxy groups, or substituted or unsubstituted aryl, aralkyl, aryloxy or aralkyloxy groups, and non toxic salts of said penicillanic

[Price 4s. 6d.]

acids which process comprises reacting 6-aminopenicillanic acid or a salt thereof with an acid, having the general formula



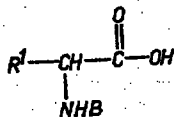
or functional equivalent thereof, wherein R^1 is as defined above and X is $-\text{NH}_2$, $-\text{N}_3$, or $-\text{NHB}$ where B is a blocking group removable by hydrogenation, in a suitable solvent; and, if X is not $-\text{NH}_2$, hydrogenating in the presence of catalyst to produce the desired penicillin.

The process of the invention is usually carried out employing about an equimolar amount of the acid, or functional equivalent thereof, having the formula



and at a temperature of from about -50°C . to 40°C .

The acid having the formula



may be one in which R^1 is as defined above, and wherein the blocking group B is the

trityl group or a group of the general formula $\text{R}^6 - \text{O} - \text{C}(=\text{O}) -$ wherein R^6 is an alkyl, benzyl, substituted benzyl, phenyl, or substituted phenyl group. The acid may be in the form of its acid chloride, acid bromide, anhydride, or mixed anhydride.

In the above formula when R^2 , R^3 , R^4 or R^5 is alkyl the (lower) alkyl radical is preferred. Similarly where R^2 , R^3 , R^4 or R^5 is another group containing an alkyl portion, e.g. alkylsulfonyl, the (lower) groups are preferred. When R^2 , R^3 , R^4 or R^5 is a cycloalkyl group, those having from 5 to 7 carbon atoms inclusive are preferred. When R^2 , R^3 or R^4 is a cycloalkoxy group those having from 5 to 7 carbon atoms inclusive are preferred.

Included within the scope of this invention are the compounds of the formula-I and their pharmaceutically acceptable non toxic carboxylic acid salts, including non toxic metallic salts such as sodium, potassium, calcium and aluminium, the ammonium salt and substituted ammonium salts, e.g., salts of such non toxic amines as trialkylamines, including triethylamine procaine, dibenzylamine, N-benzyl-beta-phenethylamine, 1-phenamine, N,N'-dibenzylethylenediamine, dehydroabietylamine, N,N'-bis-dehydroabietylthylenediamine, N-(lower)alkylpiperidine (e.g., N-ethylpiperidine) and other amines which have been used to form salts with benzylpenicillin. Also included within the scope of this invention are the non toxic acid addition salts thereof (i.e. the amine salts) including the mineral acid addition salts such as the hydrochloride, hydrobromide, hydroiodide, sulfate, sulfamate and phosphate and the organic acid addition salts such as the maleate, acetate, citrate, oxalate, succinate, benzoate, tartrate, fumarate, malate, mandelate and ascorbate.

Among the preferred compounds of this invention are 6-[($-$)- α -Amino-(4-pyridyl)acetamido] penicillanic acid, 6-[($-$)- α -Amino-(3-pyridyl)acetamido] penicillanic acid, D,L-6-[($-$)- α -Amino-(2-pyridyl)acetamido] penicillanic acid, 6-[($-$)- α -Amino-(2-pyridyl)acetamido] penicillanic acid, 6-[($-$)- α -Amino-(2-pyrrolyl)acetamido] penicillanic acid, D,L-6-[($-$)- α -Amino-(3-pyrrolyl)acetamido] penicillanic acid, and their non-toxic acids. The invention includes therapeutic compositions and animal foodstuffs containing the novel penicillins described herein. The compositions and foodstuffs are formulated in a similar way to those containing known penicillins.

The term "(lower) alkyl" as used herein means both straight and branched chain aliphatic hydrocarbon radicals having from 1 to 6 carbon atoms such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, t-butyl, amyl, and hexyl. Similarly, where the term "(lower)" is used as part of the description of another group, e.g. "(lower)alkoxy",

it refers to the alkyl portion of such group which is therefore as described above in connection with "(lower)alkyl".

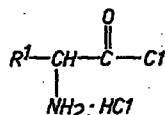
To illustrate groups including (lower)alkyl groups, it is pointed out that (lower)-alkoxy includes such radicals as methoxy, ethoxy, and isopropoxy; (lower)alkylthio includes methylthio, ethylthio, and butylthio; (lower)alkanoyl includes acetyl, propionyl, and butyryl; (lower)alkylsulfonyl includes methylsulfonyl, ethylsulfonyl, and hexylsulfonyl; di(lower)alkylamino includes dimethylamino, diethylamino, and ethylmethylamino.

The α -carbon atom of the acyl group (to which the α -amino group is attached) is an asymmetric carbon atom and the compounds of this invention can therefore exist in two optically active isomeric forms (the D- and L-diastereoisomers), as well as in a mixture of the two optically active forms. Although the D-isomer is the preferred form, all such isomeric forms of the compounds are included within the scope of the present invention. Also included within the scope of the present invention are easily hydrolyzed esters which are converted to the free acid form by chemical or enzymatic hydrolysis.

It should be noted in connection with the foregoing consideration of the diastereoisomers of this invention that many isomers other than the two caused by the asymmetric carbon of the side chain are possible due to the presence of asymmetric carbon atoms in the 6-aminopenicillanic acid nucleus. Such additional isomers, however, are not presently significant since commercially available 6-amino-penicillanic acid which is the product of fermentation processes is consistently of one configuration; such 6-aminopenicillanic acid is presently used in the production of the compounds of this invention.

One preferred embodiment of this invention, by which the penicillins of formula I may be prepared, is the reaction of 6-aminopenicillanic acid, or a neutral salt thereof, such as the sodium, potassium or triethylamine salt, with a chloride hydrochloride of the appropriate α -(heterocyclyl) glycine having the general formula

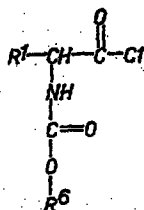
(V)



wherein R^1 is as defined above. The α -(heterocyclyl) glycyl chloride hydrochloride may be formed by reacting the corresponding α -(heterocyclyl) glycine, suspended in a suitable solvent and cooled to about 0°C ., with about an equimolar amount of phosphorus pentachloride. The α -(heterocyclyl) glycyl chloride hydrochloride forms as a precipitate and is recovered by filtration. This product is slowly added to a solution of about an equimolar amount of 6-aminopenicillanic acid or salt thereof, in water or an aqueous liquid medium, e.g. acetone at a low pH, preferably at or below 3, and maintained at a temperature of about 0°C . The solution is stirred for a few minutes after completion of the addition of the chloride hydrochloride, the pH is adjusted to about 4—6, and the acetone is removed by distillation *in vacuo*. The remaining aqueous solution of the desired 6-[α -amino- α -(heterocyclyl)acetamido]penicillanic acid is filtered to remove any insoluble material, the filtrate is cooled to about 5°C ., layered with about one-fourth volume of methyl isobutyl ketone and acidified to about pH 2 while stirring the mixture. The methyl isobutyl ketone layer is separated and discarded. The aqueous layer is then cooled to about 0°C . and extracted with a solution of dioctyl sodium sulfosuccinate in methyl isobutyl ketone. The extract is filtered and neutralized to a pH of about 5—6 at which point a precipitate of the desired 6-substituted penicillanic acid is formed.

Another preferred embodiment of this invention, by which the penicillins of formula I may be prepared, is the reaction of 6-aminopenicillanic acid, preferably in the form of a neutral salt such as the sodium salt or the triethylamine salt, with an α -(heterocyclyl)- α -(substituted amino)acetyl chloride having the general formula

(VI)



wherein R^1 is as described above and $\text{R}^6\text{OCO}-$ is a blocking group to protect the amino radical, or the functional equivalent of said acid chloride which may act as an acylating agent for the primary amino group of 6-aminopenicillanic acid, and subsequently removing the blocking group from the amino radical by hydrogenation under sufficiently mild conditions as to avoid destruction of the penicillin nucleus. Such functional equivalents include the corresponding acid bromides, acid anhydrides and mixed anhydrides with other carboxylic acids, including monoesters and particularly lower aliphatic esters, of carbonic acid.

In the blocking group referred to above, R^6 may be allyl, tertiary butyl, phenyl or substituted phenyl, benzyl or substituted benzyl, or $\text{R}^6\text{OCO}-$ may be the trityl group $(\text{Ph})_3\text{C}-$. As used here, the abbreviation Ph is understood to mean the phenyl group.

The blocking group $\text{R}^6\text{OCO}-$ may be removed by hydrogenation of the protected aminoacyl derivative of 6-aminopenicillanic acid in the presence of a catalyst such as palladium, platinum or rhodium on an inert support such as carbon, barium carbonate, strontium carbonate, or diatomaceous earth. Hydrogenation is preferably carried out at room temperature and at atmospheric pressure in a solvent such as water, non-reducible organic solvent such as ethanol or dioxane, or aqueous solutions of such organic solvents, the pH of the reaction mixture being essentially neutral, e.g. 5 to 9.

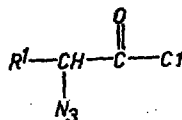
An additional preferred embodiment of this invention, by which the compounds of formula I may be prepared, is the reaction of an appropriate α -(heterocyclyl)- α -aminoacetic acid with 6-aminopenicillanic acid or a salt thereof, in the presence of a carbodiimide reagent, which comprises adding a solution of a carbodiimide such as $\text{N,N}'$ -dicyclohexyldiimide or $\text{N,N}'$ -diisopropyldiimide, in dioxane or other solvents such as tetrahydrofuran, methylene chloride, etc., to a solution of about an equimolar amount of a neutral salt of 6-aminopenicillanic acid in an aqueous solution of dioxane, tetrahydrofuran, methylene chloride, etc. A solution of about an equimolar amount of the desired α -(heterocyclyl)- α -aminoacetic acid is slowly added, and the mixture is stirred, with cooling, for about 1—2 hours. Dicyclohexylurea, which forms as a precipitate, is removed by filtration. The filtrate is lyophilized, and the solid thus obtained is dissolved in a small amount of water. The pH is adjusted to about 6—7, and the solution is filtered to remove any insoluble material. The resulting aqueous solution of the desired 6-[α -amino- α -(heterocyclyl)acetamido]penicillanic acid may be treated as described above to isolate the product.

The substituted α -(heterocyclyl)- α -amino acetic acids and the corresponding acetyl chlorides which may be used in the preparation of the compounds of the present invention may be prepared by a variety of synthetic methods which are common in the art. Most of these starting compounds are described in the prior art and many of them are commercially available. Detailed discussions of methods for the preparation of these starting materials are found in such reference works as the "Chemistry of Carbon Compounds", E. H. Rodd, Editor, Vol. IV^A, Elsevier Publishing Company, (1957). The preparations of α -(2-thienyl)-glycine and α -(3-thienyl)glycine are illustrative of such methods of preparation. These acids may be prepared according to the procedures described respectively in Ber., 19, 2115 (1886) and C. A. 53, 4249b.

The protected α -(heterocyclyl)- α -(substituted-amino) acetyl halides of formula VI above may be prepared by reacting the corresponding α -heterocyclyl glycine, or salt thereof, with a suitable ester of chlorocarbonic acid. Thus, the reaction of equivalent quantities of an α -thienyl glycine with benzyl chloro-carbonate yields an α -(carbobenzoyloxyamino)thienyl acetic acid. This may be converted into an acid chloride corresponding to formula VI above, in which R^6 is benzyl. Alternatively, this acid may be converted into the functionally equivalent acylating agents such as the acid anhydrides and mixed anhydrides with other carboxylic acids, or monoesters, and particularly lower aliphatic esters, of carbonic acid.

Still another preferred embodiment of this invention, by which the penicillins of formula I may be prepared, is the reaction of 6-aminopenicillanic acid with an α -(heterocyclyl)- α -azidoacetyl chloride of the general formula

(VII)



wherein R^1 is as described above, or the functional equivalent of said acid chloride which may act as an acylating agent for the primary amino group of 6-aminopenicillanic acid, and subsequently hydrogenating the azido group under sufficiently mild conditions as to avoid destruction of the penicillin nucleus. Such functionally equivalent acylating agents, and the preparation thereof, have been described above.

The α -(heterocyclyl)- α -azidoacetic acid may be prepared according to the method described by W. Huber in J.A.C.S. 77, 112-116, (1955).

In the process for the preparation of the compounds of formula I above, the compounds are sometimes in aqueous solution in the form of their sodium or potassium salt. These compounds in the aqueous phase can then be converted to the free acid, preferably in the cold under a layer of ether by the addition of dilute mineral acid, e.g. 5N H_2SO_4 to pH 2. The free acid can thus be extracted into a water-immiscible, neutral organic solvent such as ether, the extract washed with water quickly in the cold, if desired, and dried as with anhydrous Na_2SO_4 and the free acid recovered from the ethereal solution. The product in the ethereal extract in its free acid form can then be converted to any desired metal or amine salt, particularly the pharmaceutically acceptable nontoxic amine salts described above, 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.

The following examples will serve to illustrate this invention.

EXAMPLE 1.

Preparation of D,L-6-[α -Amino-(2-Thienyl)Acetamido]Penicillanic Acid

α -(2-Thienyl)glycine [5.0 gm.; 0.032 mole, obtained by the method disclosed in Ber. 19, 2115 (1886)] is suspended in 35 ml. of methylene chloride and cooled to -7°C . with stirring and in the presence of calcium chloride desiccant. Phosphorus pentachloride (10 gm.; 0.048 mole) is added in one portion causing the temperature to rise to -3°C . The mixture is stirred for one-half hour while maintaining the temperature at -5° to 0°C ., 15 ml. of methylene chloride is added, and stirring at -5°C . to 0°C . is continued for another one-half hour. Then, 10 drops of dimethyl formamide are added and another 20 ml. of methylene chloride are added to thin the mixture. The mixture is stirred rapidly and a purple slurry results. The product, D,L- α -(2-thienyl)-glycyl chloride hydrochloride, is collected by filtration under reduced pressure, washed three times with methylene chloride and dried in vacuo over P_2O_5 . The weight of the product is 5.9 g. which represents a 72% yield.

The pH of a solution of 6-aminopenicillanic acid (4.1 gm.; 0.019 mole) in 80 ml. of water is adjusted to 6.3 by the addition of a 10% by weight aqueous solution of NaOH with stirring. The solution is then diluted with 340 ml. of acetone and cooled to -5°C . The pH of the solution is then adjusted quickly to 2.5 by the addition of 6N HCl to the well-stirred mixture. To this solution is added D,L- α -(2-thienyl)-glycyl chloride hydrochloride (5.9 gm.; 0.028 mole) over a 20-minute period, the pH being kept at 2 to 2.5 by intermittent additions of 10% NaOH. The solution is stirred for 5 minutes after completion of the addition of the chloride hydrochloride and the pH is then adjusted to 4.8 by the addition of 10% NaOH. The acetone is removed under reduced pressure at $30-34^\circ\text{C}$. and the solution is filtered to remove insoluble material. The filtrate, having a pH of 4, is cooled to 5°C ., adjusted to a pH of 7 by the addition of 10% NaOH, layered with 20 ml. of methyl isobutyl ketone and acidified to pH 2 with 20% H_2SO_4 . Upon removal of the methyl isobutyl ketone layer, the color of the remaining aqueous layer clarifies. This aqueous layer is extracted again with 20 ml. of methyl isobutyl ketone and the extracts are discarded. The aqueous layer is then cooled to 0°C . and extracted at pH 2 with a 10% solution containing dioctyl sodium sulfosuccinate (5.3 gm.; 0.013 mole) in methyl isobutyl ketone. The methyl isobutyl ketone

extract is separated by decanting, filtered through anhydrous Na_2SO_4 and concentrated slightly by evaporation under reduced pressure at 30°C . It is then neutralized to pH 5.3 by the addition of triethylamine. The solid product which forms is collected by filtration under reduced pressure, slurried with ether, refiltered and dried in vacuo over P_2O_5 . The product, D,L-6-[α -amino(2-thienyl)acetamido]-penicillanic acid, is found to weigh 2.6 gm., to contain the β -lactam structure as shown by infrared analysis, to melt with decomposition at 186° — 190°C ., and to inhibit *Staph. aureus* Smith at a concentration of 0.2 mg./ml.

EXAMPLE 2.

Preparation of D,L-6-[α -Amino-(3-Thienyl)Acetamido]Penicillanic Acid
 α -(3-Thienyl)glycine (5.0 gm.; 0.032 mole, obtained by the method disclosed in C. A., 53, 4249b) is suspended in 35 ml. of methylene chloride and cooled to -7°C . with stirring and in the presence of calcium chloride desiccant. Phosphorus pentachloride (10 gm.; 0.048 mole) is added in one portion causing the temperature to rise to -3°C . The mixture is stirred for one-half hour while maintaining the temperature at -5° to 0°C . 15 ml. of methylene chloride is added, and stirring at -5° to 0°C . is continued for another one-half hour. Then, 10 drops of dimethyl formamide is added and another 20 ml. of methylene chloride is added to thin the mixture. The mixture is stirred rapidly and a purple slurry results. The product, D,L- α -(3-thienyl)glycyl chloride hydrochloride is collected by filtration under reduced pressure, washed three times with methylene chloride and dried in vacuo over P_2O_5 . The weight of the product is 5.9 g. which represents a 72% yield.

The pH of a solution of 6-aminopenicillanic acid (4.1 gm.; 0.019 mole) in 80 ml. of water is adjusted to 6.3 by the addition of a 10% by weight solution of NaOH with stirring. The solution is then diluted with 340 ml. of acetone and cooled to -5°C . The pH of the solution is then adjusted quickly to 2.5 by the addition of 6N HCl to the well-stirred mixture. To this solution is added D,L- α -(3-thienyl)glycyl chloride hydrochloride (5.9 gm.; 0.028 mole) over a 20 minute period, the pH being kept at 2 to 2.5 by intermittent additions of 10% NaOH. The solution is stirred for 5 minutes after completion of the addition of the chloride hydrochloride and the pH is then adjusted to 4.8 by the addition of 10% NaOH. The acetone is removed under reduced pressure at 30 — 34°C . and the solution is filtered to remove insoluble material. The filtrate, having a pH of 4, is cooled to 5°C ., adjusted to a pH of 7 by the addition of 10% NaOH, layered with 20 ml. of methyl isobutyl ketone and acidified to pH 2 with 20% H_2SO_4 . Upon removal of the methyl isobutyl ketone layer, the color of the remaining aqueous layer clarifies. This aqueous layer is extracted again with 20 ml. of methyl isobutyl ketone and the extracts are discarded. The aqueous layer is then cooled to 0°C . and extracted to pH 2 with a 10% solution of dioctyl sodium sulfosuccinate (5.3 gm.; 0.013 mole) in methyl isobutyl ketone. The methyl isobutyl ketone extract is separated by decanting, filtered through anhydrous Na_2SO_4 and concentrated slightly by evaporation under reduced pressure at 30°C . It is then neutralized to pH 5.3 by the addition of triethylamine. The solid product which forms is collected by filtration under reduced pressure, slurried with ether, refiltered and dried in vacuo over P_2O_5 . The product, D,L-6-[α -amino(3-thienyl)acetamido]-penicillanic acid, is found to weigh 2.6 gm., to contain the β -lactam structure as shown by infrared analysis, and to inhibit *Staph. aureus* Smith at a concentration of 0.001% by weight.

EXAMPLE 3.

Preparation of D,L-6-[α -Amino-(3-Thienyl)Acetamido]Penicillanic Acid
 α -(3-Thienyl)glycine (4.8 gm.; 0.03 mole) is suspended in 55 ml. of methylene chloride and cooled to -10°C . with stirring and in the presence of calcium chloride desiccant. Phosphorus pentachloride (9.4 gm.; 0.045 mole) is added in one portion causing the temperature to rise to -3°C . The mixture is stirred for forty-five minutes while maintaining the temperature at -10° to -5°C . Then, 10 drops of dimethyl formamide is added, and stirring is continued for an additional forty-five minutes. The product, D,L- α -(2-thienyl)glycyl chloride hydrochloride is collected by filtration under reduced pressure, washed three times with methylene chloride and dried in vacuo over P_2O_5 . The weight of the product is 3.8 g. which represents a 60% yield.

The pH of a solution of 6-aminopenicillanic acid (2.6 gm.; 0.012 mole) in 50 ml. of water is adjusted to 7 by the addition of a 10% by weight solution of NaOH, with stirring. The solution is then diluted with 200 ml. of acetone and cooled to 7°C . The pH of the solution is then adjusted quickly to 3 by the addition of 6N HCl to the well-stirred mixture. To this solution is added D,L- α -(3-thienyl)glycyl chloride

hydrochloride (3.8 gm.; 0.018 mole) over a 20-minute period, the pH being kept at 3 to 3.5 by intermittent additions of 10% NaOH. The solution is stirred for 15 minutes after completion of the addition of the chloride hydrochloride and the pH is then adjusted to 7 by the addition of 10% NaOH. The acetone is removed under reduced pressure at 37°C. and the solution is filtered to remove insoluble material. The filtrate is adjusted to pH 2 by the addition of 20% H₂SO₄, extracted twice with methyl isobutyl ketone, and the extracts are discarded. The aqueous layer is then cooled to 0°C. and extracted with a 10% solution of dioctyl sodium sulfosuccinate (3.3 gm.; 0.0075 mole) in methyl isobutyl ketone. The methyl isobutyl ketone extract is separated by decanting, filtered through anhydrous Na₂SO₄ and concentrated slightly by evaporation under reduced pressure at 30°C. It is then neutralized to pH 6.5 by the addition of triethylamine. The solid product which forms is collected by filtration under reduced pressure, slurried with ether, refiltered and dried *in vacuo* over P₂O₅. The product, D,L-6-[α -amino(3-thienyl)acetamido]penicillanic acid, is found to weigh 0.9 gm., to melt with decomposition at 177–180°C., to contain the β -lactam structure as shown by infrared analysis, and to inhibit *Staph. aureus* Smith at a concentration of 0.001% by weight.

EXAMPLE 4.

Preparation of 6-[α -Amino(5-Ethyl-2-Thienyl)-Acetamido]Penicillanic Acid

The procedure of Example 1 is repeated except that α -(5-ethyl-2-thienyl)glycine, prepared as described in C. A., 49, 8244c, is substituted for α -(2-thienyl)glycine. The product is found to contain the β -lactam structure as shown by infrared analysis and to inhibit *Staph. aureus* Smith at a concentration of 0.001% by weight.

EXAMPLE 5.

Preparation of 6-[α -Amino(5-Methyl-2-Thienyl)-Acetamido]Penicillanic Acid

The procedure of Example 1 is repeated except that α -(5-methyl-2-thienyl)glycine, prepared as described in C. A., 49, 8244d, is substituted for α -(2-thienyl)glycine. The product is found to contain the β -lactam structure as shown by infrared analysis and to inhibit *Staph. aureus* Smith at a concentration of 0.001% by weight.

EXAMPLE 6.

Preparation of 6-[α -Amino(5-t-Butyl-2-Thienyl)Acetamido]Penicillanic Acid

The procedure of Example 1 is repeated except that α -(5-t-butyl-2-thienyl)glycine, prepared as described in C. A., 51, 4944b, is substituted for α -(2-thienyl)glycine. The product is found to contain the β -lactam structure as shown by infrared analysis and to inhibit *Staph. aureus* Smith at a concentration of 0.001% by weight.

EXAMPLE 7.

Preparation of 6-[α -Amino(2,5-Dimethyl-3-Thienyl)Acetamido]Penicillanic Acid

The procedure of Example 2 is repeated except that α -(2,5-dimethyl-2-thienyl)glycine, prepared as described in C. A., 51, 4944a, (1957), is substituted for α -(2-thienyl)glycine. The product is found to contain the β -lactam structure as shown by infrared analysis and to inhibit *Staph. aureus* Smith at a concentration of 0.001% by weight.

EXAMPLE 8.

Preparation of 6-[(–)- α -Amino-(3-Thienyl)Acetamido]Penicillanic Acid

A sample of D,L- α -(3-thienyl)glycine is reacted, in a manner known to those skilled in the art, to obtain an optically active isomer which is isolated as the hydrochloride salt. The product, (–)- α -(3-thienyl)glycine hydrochloride, is found to melt at 238–242°C. and to have a rotation of $[\alpha]_D^{25} = -92.7$ (C=0.1).

To a solution of 11 gm. (0.068 mole) of (–)- α -(3-thienyl)glycine hydrochloride and 8.2 gm. (0.21 mole) of sodium hydroxide in 80 ml. of water at 5°C., is added 12.8 gm. (0.075 mole) of carbobenzoxychloride. The mixture is stirred vigorously for two hours, extracted with ether, and the aqueous layer is separated, acidified to a pH of 2 with hydrochloric acid, and extracted with ethyl acetate. The organic layer is dried over anhydrous magnesium sulfate and evaporated under reduced pressure to give 13.8 gms. of N-carbenzoxy-(–)- α -(3-thienyl)glycine which melts at 122–124°C.

Ethyl chloroformate (4.0 gms.; 0.037 mole) is added dropwise, over a period of five minutes, to a stirred mixture of 10.9 gms. (0.037 mole) of N-carbenzoxy-(–)- α -(3-thienyl)glycine, 40 gm. (0.037 mole) of 2,6-lutidine, and 100 ml. of dimethylformamide at 5°C., and stirring is continued for an additional 15 minutes. A solution of 8 gm. (0.037 mole) of 6-aminopenicillanic acid in 75 ml. of water and 25 ml. of

2,6-lutidine, at 5°C., is added all at once. The mixture is stirred for 15 minutes, diluted with 300 ml. of water, and acidified to pH 2 with dilute sulfuric acid. The penicillin is extracted with ether, washed with water, and then back extracted into 200 ml. of dilute aqueous sodium carbonate. A few drops of acetic acid are added to adjust the pH to 7. The mixture is hydrogenated at 50 pounds per square inch pressure, over a period of twenty minutes at 25°C. using 7 gms. of 30% palladium on Celite (Registered Trade Mark) as the catalyst. The pH is lowered to 2 with dilute sulfuric acid and the catalyst is removed by filtration. The filtrate is extracted with a solution of 8 gms. of dioctyl sodium sulfosuccinate in 150 ml. of methyl isobutyl ketone, and the organic layer is separated and dried over anhydrous magnesium sulfate. The drying agent is removed by filtration, and the pH of the filtrate is adjusted to 4.5 by the addition of triethylamine. The solid product which forms is slurried with 5 ml. of water, and removed by filtration. It is recrystallized by dissolving in 10 ml. of water with enough ammonium hydroxide present to raise the pH to 8.5, and precipitating by adjusting the pH to 5 with acetic acid. The product, 6-[(α -amino(3-thienyl)-acetamido]-penicillanic acid, is collected by filtration and is found to weigh 750 mg., to melt with decomposition at 205°C., and to inhibit *Staph. aureus* Smith at a concentration of 0.001% by weight.

EXAMPLE 9.

Preparation of D,L-6-[(α -Amino-(3-Thienyl)Acetamido]Penicillanic Acid

α -Azido(3-thienyl)acetic acid is reacted at reflux temperature with an equimolar quantity of thionyl chloride, and the resulting α -azido(3-thienyl)acetyl chloride is separated by distillation under reduced pressure.

α -Azido(3-thienyl)acetyl chloride (24.2 gms.; 0.12 mole) in 15 ml. of acetone is slowly added to a stirred solution of 26.2 gms. (0.12 mole) of 6-aminopenicillanic acid and 29.4 gms. (0.35 mole) of sodium bicarbonate in 500 ml. of a equivolume mixture of water and acetone at 0°C. The reaction mixture is maintained at 0–5°C. and stirred for 3 hours. The pH of the mixture is adjusted to 7 with sodium bicarbonate, it is extracted with ether, in order to remove acetone and then acidified to a pH of 2.5 with dilute hydrochloric acid. The azidopenicillin is extracted with ether, washed with water, and then back extracted into dilute aqueous sodium carbonate. The pH is adjusted to 7 with a few drops of acetic acid, and the mixture is hydrogenated and isolated as shown in example 7. The product, D,L-6-[(α -amino(3-thienyl)acetamido]-penicillanic acid, is found to contain the β -lactam structure as shown by infrared analysis. It melts with decomposition at 177–180°C., and inhibits *Staph. aureus* Smith at a concentration of 0.001% by weight.

EXAMPLE 10.

Preparation of D,L-6-[(α -Amino-(4-Pyridyl)Acetamido]Penicillanic Acid Hydrochloride

4-Pyridylaldehyde (220 gm.; 2.06 mole), ammonium chloride (137 gm.; 2.56 mole) and potassium cyanide (165 gm.; 2.46 mole) are reacted to obtain α -amino-(4-pyridyl)acetonitrile. This product (172 gm.; 1.29 mole) is then placed in concentrated HCl (1700 ml.) and allowed to stand for 5 days at room temperature after which time the HCl is boiled off until the liquid is about 7 N and the liquid is then flashed to remove the rest of the solvent. The product (132.2 gm.), α -amino-(4-pyridyl)acetic acid hydrochloride, is recovered as a solid.

6-Aminopenicillanic acid (10.8 gm.; 0.050 mole) and triethylamine (5.06 gm.; 0.050 mole) are dissolved in 250 ml. of water and 415 ml. of dioxane. The mixture is cooled to 10.5°C. and a solution of dicyclohexylcarbodiimide (10.3 gm.; 0.050 mole) in 125 ml. of dioxane is added, followed, in 45 seconds, by the addition of a solution of α -amino-(4-pyridyl)acetic acid hydrochloride (9.43 gm.; 0.050 mole) and triethylamine (5.06 gm.; 0.050 mole) in 125 ml. of water and 125 ml. of dioxane. The mixture is stirred for 1 1/2 hours at 10°C., and filtered to remove dicyclohexylurea and the filtrate is lyophilized. The dry material resulting from the lyophilization is dissolved in 200 ml. of water, the pH of the solution is adjusted to 8.2 by the addition of triethylamine and the solution is filtered. The pH of the filtrate is adjusted to 4.2 by the addition of 6 N hydrochloric acid and the solution is again lyophilized. The dry material resulting from lyophilization is extracted with 1 liter of a mixture of 95% acetone and 5% water, the extract is filtered and the filtrate flashed several times with ethyl acetate to remove all moisture. The solid material which remains (23.0 gm.) is extracted with two-liter portions of methylene chloride to remove triethylamine hydrochloride leaving an insoluble yellow product, D,L-6-[(α -amino-(4-

pyridyl)acetamido]penicillanic acid hydrochloride. It is found to weigh 8.4 gm., to have a melting point of 160—170°C., to contain the β -lactam structure as shown by infrared analysis and to inhibit the growth of *Staph. aureus* Smith at a concentration of 0.001 per cent by weight, and to have the following elemental analysis for

$C_{15}H_{18}N_4O_4S \cdot HCl \cdot H_2O$: C, 44.6; H, 5.2; N, 13.8
Found: C, 44.4; H, 5.7; N, 13.7.

EXAMPLE 11.

Preparation of D,L-6-[α -Amino-(3-Pyridyl)Acetamido]Penicillanic Acid Hydrochloride

3-Pyridylaldehyde (640 gm.; 5.98 mole) is added to a solution of ammonium chloride (400 gm.; 7.48 mole) and potassium cyanide (480 gm.; 7.17 mole) in a period of 45 minutes. At the end of this time, the solution is made basic by the addition of 448 gm. of potassium carbonate to obtain α -amino-(3-pyridyl)-acetonitrile. The product (512.5 gm.; 3.84 mole) is then placed in 5125 ml. of concentrated HCl and the solution is allowed to stand for 80 hours. The solvent is then flashed off and the 50% mixture of α -amino-(3-pyridyl)acetic acid hydrochloride and the by-product, ammonium chloride (450.3 gm.) is recovered. This mixture is dissolved in 200 ml. of water and the solution is passed through an ion exchange column. (Dowex 50WX8—polystyrene containing sulfonic acid groups) "Dowex" is a Registered Trade Mark. The eluate from the column is flashed to remove the water and the product, α -amino-(3-pyridyl)-acetic acid (148.4 gm.), is dried over P_2O_5 in *vacuo*.

6-Aminopenicillanic acid (4.32 gm.) and triethylamine (2.02 gm.) are dissolved in 100 ml. of water and 150 ml. of dioxane and the solution is cooled in an ice bath. There are then added to the solution N,N' -dicyclohexylcarbodiimide (4.13 gm.) in 50 ml. of dioxane and, after 1 minute, α -amino-(3-pyridyl)-acetic acid (3.2 gm.) in a mixture of 25 ml. of water and 25 ml. of dioxane. The mixture is stirred for 1 1/2 hours, then HCl (0.015 mole) is added and the mixture is stirred for another 45 minutes. Triethylamine (2.02 gm.) is then added and the mixture is filtered to remove dicyclohexylurea. The filtrate is lyophilized overnight and the resulting solid material is treated with 100 ml. of water. The pH of the mixture is adjusted to 8.5 and the mixture is filtered. To the filtrate, there is added sufficient 6 N HCl to adjust the pH to 4.5 and the solution is again lyophilized. The solid material resulting from lyophilization is extracted with 425 ml. of a mixture of 95% acetone—5% water and the extract is filtered. The filtrate is flashed dry, using several portions of ethyl acetate to remove all moisture. The product, D,L-6-[α -amino-(3-pyridyl)acetamido]-penicillanic acid hydrochloride is washed with methylene chloride and dried in *vacuo*. It is found to weigh 2.8 gm., to contain the β -lactam structure as shown by infrared analysis and to inhibit the growth of *Staph. aureus* Smith at a concentration of 0.001 per cent by weight, and to have the following elemental analysis for

$C_{15}H_{18}N_4O_4S \cdot HCl \cdot H_2O$: Calculated: C, 44.6; H, 5.2.
Found: C, 44.5; H, 6.0.

EXAMPLE 12.

Preparation of D,L-6-[α -Amino-(3-Pyridyl)Acetamido]Penicillanic Acid

α -Amino-(3-pyridyl)acetic acid (30.42 gm.; 0.2 mole) is dissolved in 407 ml. of 1N sodium hydroxide solution and 1690 ml. of water. The solution is cooled to 5°C. and 46.8 ml. of carbobenzoxy chloride is added. The solution is shaken vigorously for 25 minutes while maintaining the temperature at from 0 to 5°C. and is then filtered. The pH of the filtrate is adjusted to 3.9 by the addition of 6 N sulfuric acid. A white solid forms as a precipitate and is collected by filtration. The product, α -(N-carbobenzoxycarbonyl)-(3-pyridyl)acetic acid, is washed with ether. It is found to weigh 9.1 gm. and to have a melting point of 156—159°C. with decomposition.

6-Aminopenicillanic acid (7.56 gm.; 0.035 mole) and sodium bicarbonate (2.94 gm.; 0.035 mole) are dissolved in 175 ml. of water. To this solution is added tetrahydrofuran (291 ml.) and the solution is cooled to 10°C. There is then added a solution of N,N' -dicyclohexylcarbodiimide (7.20 gm.; 0.035 mole) in 87.5 ml. of tetrahydrofuran and, after 45 seconds, a solution of α -(N-carbobenzoxycarbonyl)-(3-pyridyl)acetic acid (10 gm.; 0.035 mole) in 87.5 ml. tetrahydrofuran and 40 ml. of water. The mixture is stirred for 45 minutes at 10°C. and filtered to remove dicyclohexylurea. Tetrahydrofuran is flashed off of the filtrate and the aqueous fraction is filtered. The filtrate is extracted with three 175 ml. portions of ethyl acetate, the pH of the solution is adjusted to 2.0 by the addition of 6 N sulfuric acid and the solution is extracted with another three 175 ml. portion of cold ethyl acetate. The pH of the aqueous layer

is adjusted to 3.5 by the addition of sodium bicarbonate and the solution is extracted with another three 175 ml. portion of cold ethyl acetate. The extracts are combined, dried over anhydrous sodium sulfate and filtered. To the filtrate is added potassium 2-ethylhexanoate (12.6 gm.) as a 50% solution in ether. The ethyl acetate is flashed off and 1 liter of dry ether is added to the residual material. A white precipitate is collected by filtration and dried *in vacuo* over P_2O_5 . The product, the potassium salt of 6-[α -(N-carbobenzoxyamino)-(3-pyridyl)acetamido]penicillanic acid, is found to weigh 4.4 gm., to have a melting point of 110—115°C., to contain the β -lactam structure as shown by infrared analysis and to inhibit *Staph. aureus* Smith at a concentration of 3.1 mg./ml.

The potassium salt of 6-[α -(N-carbobenzoxyamino)-(3-pyridyl)acetamido]penicillanic acid (3.2 g.; .00612 mole) is dissolved in 73.5 ml. of water and is hydrogenated under a hydrogen atmosphere of 45 p.s.i.g. for 45 minutes at 25°C., using 3.19 gm. of 30% palladium on diatomaceous earth as a hydrogenation catalyst. Following hydrogenation, the pH of the solution is adjusted to 2.2 and the catalyst removed by filtration. The pH of the filtrate is adjusted to 4.4 and the solution is lyophilized to remove water. The dry material resulting from lyophilization is dissolved in 5 ml. of water and is passed through an ion exchange column (IRC-50, 20—50 mesh, carboxylic acid type in H^+ form). The product is removed from the column with .01 N HCl and the eluate from the column flashed to remove the water. The product, D,L-6-[α -amino-(3-pyridyl)acetamido]penicillanic acid, is found to contain the β -lactam structure as shown by infrared analysis and to inhibit the growth of *Staph. aureus* Smith at a concentration of .001 per cent by weight.

EXAMPLE 13.

Preparation of D,L-6-[α -Amino-(2-Pyrrolyl)Acetamido]Penicillanic Acid

To a stirred solution of 24.5 gms. (0.5 mole) of sodium cyanide and 113 gms. (1.18 mole) of ammonium carbonate in 335 ml. of distilled water under an inert atmosphere of nitrogen, is added, in one portion, 23.8 gms. (0.25 mole) of pyrrole-2-aldehyde in 335 ml. of absolute ethanol. The resulting slurry is stirred and heated at 50—55°C. for four hours, and then stirred for an additional 15 hours at room temperature. The insoluble salts are then removed by filtration and washed with two 50 ml. portions of absolute ethanol. The combined filtrate and washings are concentrated at 30—40°C. *in vacuo* to a volume of about 200 ml., cooled to 5°C., and acidified to pH 1 with 6 N HCl. After several minutes of stirring at 5°C. the product begins to crystallize, and after thirty minutes the solids are removed by filtration, recrystallized from boiling isopropanol (with decolorization by activated charcoal), and air dried. The white crystalline product, 5-(2-pyrrolyl)-hydantoin monoisopropanolate, is found to weigh 25 gms. and to melt at 160—161°C.

Nuclear magnetic resonance analysis shows the product to contain one molecule of isopropanol of crystallization.

Analysis for $C_7H_7N_2O_2 \cdot C_3H_7OH$

	Calculated	Found
C	53.3	53.4
H	6.9	6.7
N	18.9	19.15

A solution of 2.25 gms. (0.01 mole) of 5-(2-pyrrolyl)-hydantoin monoisopropanolate in 8 ml. of 20% aqueous NaOH is refluxed for 16 hours, cooled in an ice bath, and slowly neutralized to pH 7 with 6N HCl. Filtration yields 0.9 gm. of grey solid which is suspended in 10 ml. of water, dissolved by acidifying to pH 1 with 6N HCl, and stirred for three minutes with one gram of decolorizing charcoal. The mixture is filtered, and the cooled filtrate is adjusted to pH 7 with solid sodium bicarbonate. The resulting crystalline solid is recovered by filtration, washed with water and acetone, and air dried. The product, D,L- α -amino-(2-pyrrolyl)acetic acid, is found to weigh 0.130 gm., and to decompose at 202—203°C.

Analysis for $C_6H_6N_2O_2$

	Calculated	Found
C	51.35	50.10
H	5.75	5.67
N	20.00	19.00

Carbobenzoxy chloride (10 gms.; 0.59 mole) is slowly added to a cooled solution of 7 gms. (0.05 mole) of D,L- α -amino-(2-pyrrolyl)acetic acid in 100 ml. (0.1 mole) of 4% aqueous NaOH, with vigorous stirring, while maintaining the temperature at 3–5°C. The slurry is stirred for an additional hour at 5–10°C., and the pH is then adjusted to 8 with a small amount of 50% aqueous NaOH. The resulting solution is extracted with three 100 ml. portions of ethyl ether which are discarded, and the aqueous phase is acidified to pH 2 with 40% H_3PO_4 and again extracted with three 100 ml. portions of ether. The combined ether layers are washed with three 50 ml. portions of saturated aqueous Na_2SO_4 solution, dried over anhydrous sodium sulfate, and the ether is removed at 30°C. by distillation *in vacuo*. The resulting solid is washed with n-pentane, removed by filtration, and air dried. The product, α -(N-carbobenzoxyamino)-(2-pyrrolyl)acetic acid, weighs 14 gms. and decomposes at 170–175°C.

A solution of 6.8 gms. (0.05 mole) of isobutyl chloroformate in 25 ml. of tetrahydrofuran is slowly added with stirring, to a cooled solution of 13.5 gms. (0.05 mole) of α -(N-carbobenzoxy-amino)-(2-pyrrolyl)acetic acid and 5.4 gms. (0.05 mole) of 2,6-lutidine in 100 ml. of tetrahydrofuran, while maintaining the temperature at –10°C. After stirring for an additional ten minutes, an ice-cold slurry of 10.8 gms. (0.05 mole) of 6-amino-penicillanic acid in a mixture of 30 ml. of water and 7 ml. of 2,6-lutidine is added in one portion, whereupon the temperature of the mixture immediately rises to 5°C., and CO_2 is evolved. The cooling bath is removed and the mixture is allowed to slowly rise to room temperature (1 hour) while stirring, and stirring is continued for another hour at room temperature. The pH of the mixture is adjusted to 8 with triethylamine and 500 ml. of water is added. The resulting solution is extracted with two 300 ml. portions of ethyl ether which are discarded, and the aqueous phase is cooled in an ice bath, slowly acidified to pH 2 with 40% aqueous H_3PO_4 and again extracted with two 300 ml. portions of ether. The combined ether extracts are washed with three 100 ml. portions of distilled water and dried over anhydrous sodium sulfate. The resulting ether solution is treated with 25 ml. of a 50% solution of potassium 2-ethylhexanoate in n-butanol. An oily layer separates, and this is allowed to settle for fifteen minutes while cooling in an ice bath. The ether is decanted, and the oily layer is dissolved in 500 ml. of ethyl acetate. This solution is concentrated to 100 ml. by distillation at 30°C. *in vacuo*, and 1 liter of dry ether is added. The resulting solid precipitate is recovered by filtration, washed with dry ether, and dried *in vacuo*. The product, the potassium salt of D,L-6-[α -(N-carbobenzoxyamino)-(2-pyrrolyl)acetamido]penicillanic acid and weighs 18 gms. (72% of theory).

Analysis for $C_{22}H_{23}N_4O_6S \cdot K$

	Calculated	Found
C	51.80	52.00
H	4.53	4.73
N	10.9	10.4

A solution of 15 gms. (0.03 mole) of the potassium salt of D,L-6-[α -(N-carbobenzoxyamino)-(2-pyrrolyl)acetamido]penicillanic acid in 150 ml. of water is hydrogenated for twenty minutes at 50 p.s.i.g., using 15 gms. of 30% palladium on diatomaceous earth as catalyst. The pH of the mixture is adjusted to 2 with a small amount of 40% aqueous H_3PO_4 , and the mixture is filtered to remove catalyst. The filtrate is concentrated *in vacuo* to 25 ml. and is filtered to remove any insoluble material. To the resulting filtrate is added 50 ml. of acetone, and, after shaking, the acetone layer is removed from the nearly saturated aqueous salt solution. The acetone solution is evaporated at 25°C. *in vacuo* to near dryness, to yield an oil. This oil is further dried by two successive treatments with methyl isobutyl ketone, whereby any remaining water is removed by azeotropic distillation *in vacuo*. The resulting semi-solid is washed with dry acetone and filtered to give 7 gms. of product which decomposes at 210°C. The crude product (3.5 gms.) is slurried in 25 ml. of water and is dissolved by adjusting the pH to 2 with 6N HCl. Two gms. of decolorizing charcoal are added and, after stirring for two minutes, the mixture is filtered and the solids are washed with two 5 ml. portions of water. The filtrate and washings are combined, and the pH is adjusted to 5 with a few drops of 50% aqueous NaOH solution. Upon cooling, the desired product crystallizes, is recovered by filtration, and is dried *in vacuo*. The product, D,L-6-[α -amino-(2-pyrrolyl)acetamido]penicillanic acid, weighs

0.52 gms. melts with decomposition at 230°C., and inhibits *Staph. aureus* Smith at a concentration of 0.25 mcg./ml.

Analysis for $C_{14}H_{18}N_4O_4S$

	Calculated	Found
C	49.70	49.20
H	5.36	5.50
N	16.50	15.54

EXAMPLE 14.

Preparation of 6-[(−)-α-Amino(2-Thienyl)Acetamido]Penicillanic Acid

A mixture of 25 gms. (0.157 mole) of D,L-α-amino(2-thienyl)acetic acid and 43.6 gms. (0.188 mole) of d-10-camphorsulfonic acid in 250 ml. of acetone is heated to boiling to bring the solids into solution. The hot solution is filtered to remove any insoluble material, and the filtrate is cooled in an ice bath. The resulting crystalline precipitate is recovered by filtration, washed with acetone, and air-dried to give 27.2 gms. of the diastereoisomeric camphor sulfonate salt of the amino acid. This salt is suspended in a small amount of water, the pH is adjusted to 6.3 with concentrated NH_4OH , and the mixture is cooled in an ice bath. The crystalline product, (−)-α-amino(2-thienyl)acetic acid, is recovered by filtration, washed with water and acetone, recrystallized twice from isopropanol, and air-dried. It is found to weigh 4.5 gms., to melt with decomposition at 189–191°C., and to have an optical rotation of $[\alpha]_D^{25} = -73.7^\circ$ (C=1.0% in water).

Analysis for $C_8H_7NO_2S$

	Calculated	Found
C	45.8	45.5
H	4.49	4.52
N	8.91	8.80

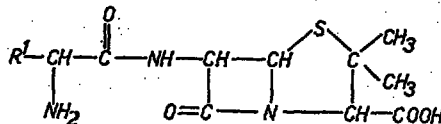
(−)-α-Amino(2-thienyl)acetic acid (10.0 gms.; 0.063 mole) is reacted according to the procedure given in Example 8, to obtain the product 6-[(−)-α-amino(2-thienyl)-acetamido]penicillanic acid. The product is found to weigh 1.9 gms., to darken slowly above 140°C. and decomposes at 205°C., to have a rotation of $[\alpha]_D^{27} = +196^\circ$ (C=0.5% in 0.1N HCl), and to inhibit *Staph. aureus* Smith at a concentration of 0.03 mcg./ml.

Analysis for $C_{14}H_{17}N_3O_4S_2 \cdot 3H_2O$

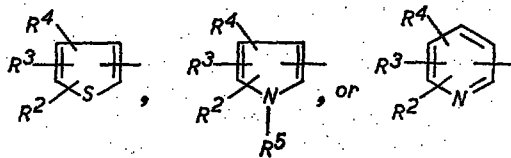
	Calculated	Found
C	41.3	41.6
H	5.68	5.90

WHAT WE CLAIM IS:—

1. A process for the preparation of penicillanic acids of the general formula



wherein R^1 has the formula



in which R^5 is hydrogen, alkyl, alkanoyl, alkylsulfonyl, cycloalkyl, phenyl, chloro-phenyl or phenethyl, and R^2 , R^3 and R^4 are selected from hydrogen, and halogen, nitro, alkyl, alkoxy, alkylthio, dialkylamino, alkanoylamino, alkanoyl, alkylsulfonyl, cyclo-alkyl, cycloalkoxy or substituted or unsubstituted aryl, aralkyl, aryloxy or aralkyloxy

0.52 gms. melts with decomposition at 230°C., and inhibits *Staph. aureus* Smith at a concentration of 0.25 mcg./ml.

Analysis for $C_{14}H_{18}N_4O_4S$

	Calculated	Found
C	49.70	49.20
H	5.36	5.50
N	16.50	15.54

EXAMPLE 14.

Preparation of 6-[(−)-α-Amino(2-Thienyl)Acetamido]Penicillanic Acid

A mixture of 25 gms. (0.157 mole) of D,L-α-amino(2-thienyl)acetic acid and 43.6 gms. (0.188 mole) of d-10-camphorsulfonic acid in 250 ml. of acetone is heated to boiling to bring the solids into solution. The hot solution is filtered to remove any insoluble material, and the filtrate is cooled in an ice bath. The resulting crystalline precipitate is recovered by filtration, washed with acetone, and air-dried to give 27.2 gms. of the diastereoisomeric camphor sulfonate salt of the amino acid. This salt is suspended in a small amount of water, the pH is adjusted to 6.3 with concentrated NH_4OH , and the mixture is cooled in an ice bath. The crystalline product, (−)-α-amino(2-thienyl)acetic acid, is recovered by filtration, washed with water and acetone, recrystallized twice from isopropanol, and air-dried. It is found to weigh 4.5 gms., to melt with decomposition at 189–191°C., and to have an optical rotation of $[\alpha]_D^{25} = -73.7^\circ$ (C=1.0% in water).

Analysis for $C_8H_7NO_2S$

	Calculated	Found
C	45.8	45.5
H	4.49	4.52
N	8.91	8.80

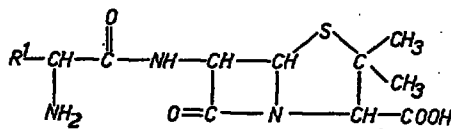
(−)-α-Amino(2-thienyl)acetic acid (10.0 gms.; 0.063 mole) is reacted according to the procedure given in Example 8, to obtain the product 6-[(−)-α-amino(2-thienyl)-acetamido]penicillanic acid. The product is found to weigh 1.9 gms., to darken slowly above 140°C. and decomposes at 205°C., to have a rotation of $[\alpha]_D^{27} = +196^\circ$ (C=0.5% in 0.1N HCl), and to inhibit *Staph. aureus* Smith at a concentration of 0.03 mcg./ml.

Analysis for $C_{14}H_{17}N_3O_4S_2 \cdot 3H_2O$

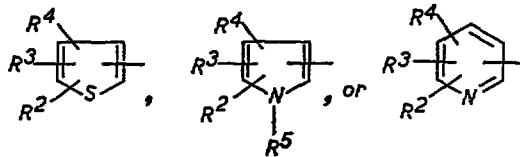
	Calculated	Found
C	41.3	41.6
H	5.68	5.90

WHAT WE CLAIM IS:—

1. A process for the preparation of penicillanic acids of the general formula



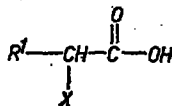
wherein R^1 has the formula



in which R^5 is hydrogen, alkyl, alkanoyl, alkylsulfonyl, cycloalkyl, phenyl, chlorophenyl or phenethyl, and R^2 , R^3 and R^4 are selected from hydrogen, and halogen, nitro, alkyl, alkoxy, alkylthio, dialkylamino, alkanoylamino, alkanoyl, alkylsulfonyl, cycloalkyl, cycloalkoxy or substituted or unsubstituted aryl, aralkyl, aryloxy or aralkyloxy



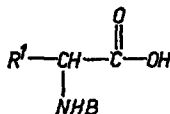
groups, and non toxic salts thereof, which process comprises reacting 6-aminopenicillanic acid or a salt thereof with an acid, having the general formula



or functional equivalent thereof,

wherein R^1 is as defined above and X is $-\text{NH}_2$, $-\text{N}_3$, or $-\text{NHB}$ where B is a blocking group removable by hydrogenation, in a suitable solvent; and, if X is not $-\text{NH}_2$, hydrogenating in the presence of catalyst to produce the desired penicillin.

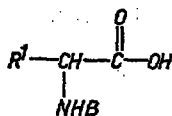
2. A process as claimed in Claim 1 wherein the acid has the formula



in which R^1 is as defined in claim 1, and wherein the blocking group B is the trityl

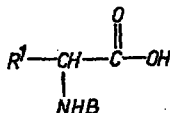
group or a group of the general formula $\text{R}^6-\text{O}-\text{C}(=\text{O})-$ wherein R^6 is an alkyl, benzyl, substituted benzyl, phenyl, or substituted phenyl group, and the reaction product with 6-aminopenicillanic acid or a salt thereof is subsequently hydrogenated to remove the blocking group and produce the desired penicillin.

3. A process as claimed in Claim 1 or Claim 2 wherein the acid



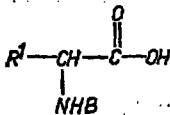
is in the form of the acid chloride, acid bromide, anhydride or mixed anhydride.

4. A process as claimed in Claim 1 or Claim 2 wherein the acid



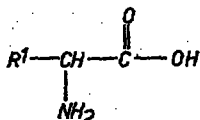
is in the form of the mixed anhydride prepared by reacting the substituted amino carboxylic acid, or salt thereof, with an ester of chlorocarbonic acid.

5. A process as claimed in Claim 1 or Claim 2 wherein the acid



is coupled with 6-aminopenicillanic acid, or a salt thereof, in the presence of a carbodiimide.

6. A process as claimed in Claim 1 wherein the acid



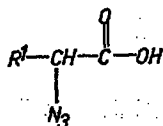
is in the form of its chloride hydrochloride.

7. A process as claimed in Claim 6 wherein the reaction is conducted at a low pH in an aqueous medium.

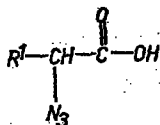
8. A process as claimed in claim 7 in which the aqueous medium is aqueous acetone and the pH is at or below 3.



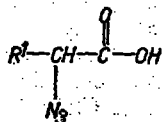
10. A process as claimed in Claim 1 wherein the acid is the α -azido acid having the formula



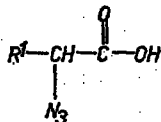
11. A process as claimed in Claim 10 wherein the acid



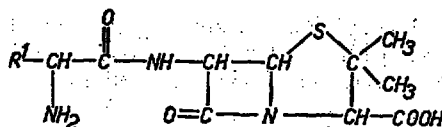
12. A process as claimed in Claim 10 wherein the acid



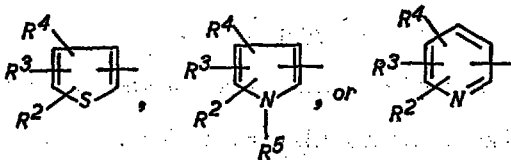
13. A process as claimed in Claim 10 wherein the acid



14. Penicillanic acids having the formula



wherein R¹ has the formula



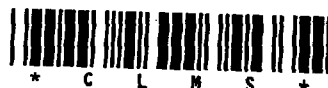
15. D,L-6-[α -Amino-(2-thienyl)acetamido]penicillanic acid.

16: 6-[(-)- α -Amino-(2-thienyl)acetamido]penicillanic acid.

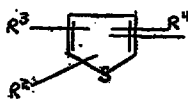
17. D,L-6-[α -Amino-(3-thienyl)acetamido]penicillanic acid

18. 6-[(-)- α -Amino-(3-thienyl)acetamido]penicillanic acid.

19. D,L-6-[α -Amino-(4-pyridyl)acetamido]penicillanic acid.

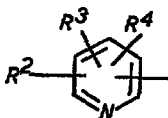


20. 6-[(α -Amino-(4-pyridyl)acetamido]penicillanic acid.
 21. D,L-6-[(α -Amino-(3-pyridyl)acetamido]penicillanic acid.
 22. 6-[(α -Amino-(3-pyridyl)acetamido]penicillanic acid.
 23. D,L-6-[(α -Amino-(2-pyridyl)acetamido]penicillanic acid.
 24. 6-[(α -Amino-(2-pyridyl)acetamido]penicillanic acid.
 25. D,L-6-[(α -Amino-(2-pyrrolyl)acetamido]penicillanic acid, and non-toxic salts thereof.
 26. 6-[(α -Amino-(2-pyrrolyl)acetamido]penicillanic acid, and non-toxic salts thereof.
 27. D,L-6-[(α -Amino-(3-pyrrolyl)acetamido]penicillanic acid, and non-toxic salts thereof.
 28. 6-[(α -Amino-(3-pyrrolyl)acetamido]penicillanic acid, and non-toxic salts thereof.
 29. Penicillanic acids as claimed in Claim 14 wherein R^1 is



in which R^2 , R^3 and R^4 are selected from hydrogen, halogen, nitro, (lower) alkyl, (lower) alkoxy, (lower) alkylthio, di(lower)alkylamino, (lower) alkanoyl amino, (lower) alkanoyl, (lower) alkylsulfonyl, cycloalkyl radicals having from 5 to 7 carbon atoms inclusive, and cycloalkoxy radicals having from 5 to 7 carbon atoms inclusive, and the pharmaceutically acceptable non-toxic carboxylic acid salts thereof.

30. Penicillanic acids as claimed in Claim 14, wherein R^1 is



in which R^2 , R^3 and R^4 are selected from hydrogen, halogen, nitro, (lower) alkyl, (lower) alkoxy, (lower) alkylthio, di(lower)alkylamino, (lower) alkanoyl amino, (lower) alkanoyl, (lower) alkylsulfonyl, cycloalkyl radicals having from 5 to 7 carbon atoms inclusive, and cycloalkoxy radicals having from 5 to 7 carbon atoms inclusive, and the pharmaceutically acceptable non-toxic carboxylic acid salts thereof.

31. Non-toxic salts of a penicillanic acid as claimed in any one of claims 15 to 24.

32. Non-toxic carboxylic acid salts of a penicillanic acid as claimed in any one of claims 15 to 24.

33. A novel penicillin substantially as hereinbefore described with reference to any one of Examples 4 to 7.

34. A process as claimed in Claim 6, wherein R^1 is as defined in Claim 29.

35. A process as claimed in Claim 6, wherein R^1 is as defined in Claim 30.

36. A process for the preparation of the penicillins claimed in claim 14 substantially as hereinbefore described.

37. A process for the preparation of the penicillins claimed in Claim 29 substantially as described in any one of the specific Examples 1, 2 or 4—7.

38. A process for the preparation of the penicillins claimed in Claim 30, substantially as described in any one of the specific Examples 10, 11 or 12.

39. A process for the preparation of the penicillins claimed in Claim 14, substantially as described in any one of the specific Examples 3, 8, 9, 13 or 14.

40. Penicillins whenever prepared by the process claimed in any one of claims 1 to 13, Claim 36 or Claim 39.

41. Penicillins whenever prepared by the process claimed in any one of claims 34, 35, 37 or 38.

42. A therapeutic composition containing a penicillin as claimed in any one of claim 14, 25 to 28, 31 or 40.

43. A therapeutic composition containing a penicillin as claimed in any one of claims 15 to 24, 29, 30, 32, 33, 38 or 41.

44. An animal foodstuff containing a penicillin as claimed in any one of claims 14, 25 to 28, 31 or 40.



45. An animal foodstuff containing a penicillin as claimed in any one of claims 15 to 24, 29, 30, 32, 33, 38 or 41.

BOULT, WADE & TENNANT,
111 & 112, Hatton Garden, London, E.C.1.
Chartered Patent Agents.
Agents for the Applicants.

Leamington Spa: Printed for Her Majesty's Stationery Office, by the Courier Press
(Leamington) Ltd.—1966. Published by The Patent Office, 25 Southampton Buildings,
London, W.C.2, from which copies may be obtained.

