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COMPLETE SPECIFICATION Derivatives of α -Aminopenicillins

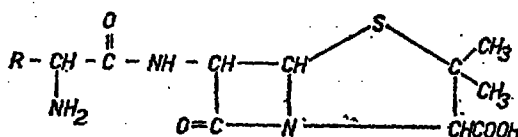
We, BRISTOL-MYERS COMPANY, a Corporation of the State of Delaware, United States of America, of Thompson Road, East Syracuse, State of New York, United States of America do hereby declare the invention for which we pray that a patent may be granted to us and the method by which it is to be performed 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 bacteria and, more particularly, this invention relates to novel derivatives of α -aminopenicillins.

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 aureus* (*Micrococcus pyogenes* var. *aureus*). The compounds of the present invention possess potent antibacterial activity against both Gram-negative and Gram-positive bacteria. The compounds of the present invention exhibit resistance to destruction by acid and are easily isolated from the reaction mixture in which they are prepared.

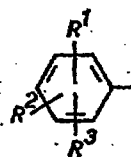
There is provided according to the practice of this invention novel antibacterial compounds which are prepared by the reaction of (A) and α -aminopenicillin having the general formula

(I)



wherein R is selected from hydrogen, alkyl, cycloalkyl, indolylalkyl, naphthyl, benzyl, furyl thienyl, and radicals having the formula

(II)



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Ex. 25

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wherein R¹, R² and R³ are selected from hydrogen, nitro, di(lower)alkylamino, (lower)-alkanoylamino, (lower)alkanoyloxy, (lower)alkyl, (lower)alkoxy, sulfamyl, chloro, iodo, bromo, fluoro, trifluoromethyl, (lower)alkylthio, (lower)alkylsulfonyl, carbo(lower)alkoxy, benzyl, phenethyl, cycloheptyl, cyclohexyl and cyclopentyl, and the salts of said penicillins, with (B) a carbonyl compound selected from aldehydes, cyclopentanone, cyclohexanone and methyl(lower)alkyl ketones at a pH within the range of from 5.5 to 9.5.

The term "(lower)alkyl" as used herein and in the claims means both straight and branched chain aliphatic hydrocarbon radicals having from 1 to 6 carbon atoms for example methyl, ethyl, propyl, isopropyl, butyl, isobutyl, t-butyl, amyl, or hexyl. Similarly, where the term "(lower)" is used as part of the description of another group such as "(lower)alkoxy", it refers to the alkyl portion of such group which is therefore as described above in connection with "(lower)alkyl".

The α -aminopenicillins which may be used in the practice of this invention may be prepared by a variety of methods which are well known to those skilled in the art. Examples of suitable α -aminopenicillins include

aminomethylpenicillin,
 α -aminopentylpenicillin,
 α -aminopropylpenicillin,
 α -aminoethylpenicillin,
 α -aminoisobutylpenicillin,
 α -amino-n-butylpenicillin,
 α -amino- α -methylbutylpenicillin,
 α -amino- β -methylbutylpenicillin,
 α -aminononylpenicillin,
 α -aminoundecylpenicillin,
 α -aminoheptadecylpenicillin,
 α -aminocyclohexylmethylpenicillin,
 α -amino- β -phenylethylpenicillin,
 α -amino- β -3-indolyethylpenicillin,
 α -amino-1-naphthylmethylpenicillin,
 α -amino-2-furylmethylpenicillin,
 α -thienyl- α -aminomethylpenicillin,
 α -amino-p-chlorobenzylpenicillin,
 α -amino-p-methoxybenzylpenicillin,
 α -amino-4-diethylaminobenzylpenicillin,
 α -amino-4-trifluoromethylbenzylpenicillin,
 α -amino-2,4-dibromobenzylpenicillin,
 α -amino-2-nitrobenzylpenicillin,
 α -amino-3-methylbenzylpenicillin,
 α -amino-4-sulfamylbenzylpenicillin,
 α -amino-2-iodobenzylpenicillin,
 α -amino-4-t-butylbenzylpenicillin and
 α -amino-2-acetamidobenzylpenicillin.

In the preferred embodiment of this invention, the α -aminopenicillin is α -aminobenzylpenicillin, an α -thienyl- α -aminomethylpenicillin, or an α -amino-substituted-benzylpenicillin.

The carbonyl compounds which may be used in the practice of this invention are

those compounds having the formula $R^4-\overset{\text{O}}{\parallel}{C}-R^5$ in which R⁴ may be hydrogen or methyl. When R⁴ is methyl, R⁵ may be (lower)alkyl and when R⁴ is hydrogen, R⁵ may be any group which may be attached to the carbonyl group of an aldehyde, for example, aryl, arylalkyl, or alkyl. The groups R⁴ and R⁵ taken together may be an alkylene radical containing 4 or 5 carbon atoms, such as cyclopentanone and cyclohexanone. Examples of suitable carbonyl compounds include acetone, cyclopentanone, cyclohexanone, methyl ethyl ketone, methyl butyl ketone, and methyl pentyl ketone; formaldehyde, acetaldehyde, chloral, glyoxal, propionaldehyde, butyraldehyde, valeraldehyde, isovaleraldehyde, furfural, acrolein phenylacetaldehyde, α -tolualdehyde, β -tolualdehyde, dimethylbenzaldehyde, naphthaldehyde, malonaldehyde, succinaldehyde, terephthaldehyde, propargylaldehyde, β -chloropropionaldehyde, p-chlorobenzaldehyde, p-trifluoromethylbenzaldehyde, o-methoxybenzaldehyde, and 3,4-dimethoxybenzaldehyde.

In performing the reaction of (A) the recited α -amino-penicillin and (B) the recited carbonyl compound, the proportions of each reactant are not critical. Thus, some reaction will occur regardless of the proportions of reactants. It is preferred, however, in order to obtain maximum yields, to use a one mole excess of the carbonyl compound.

Large molar excesses of the carbonyl compound may be used and the carbonyl compound may comprise the reaction medium as well as a reactant if it is a liquid at the reaction temperature. If the carbonyl compound is a solid at the temperature of reaction, the reaction may be conducted in an inert solvent such as methylene chloride. The reaction medium may be anhydrous or aqueous. The reaction of the α -aminopenicillin and the carbonyl compound is an equilibrium reaction during which water is split off. Therefore, it is preferred not to have a major amount of water present in the reaction medium.

The pH of the reaction mixture during the formation of the novel penicillin derivatives should be from about 5.5 to about 9.5 and is preferably from about 7.5 to 9.0. The pH may be adjusted to within this range, if necessary, by the addition of an alkaline material, for example, sodium hydroxide, sodium carbonate, potassium hydroxide, potassium carbonate, ammonium hydroxide, ammonium carbonate, or organic amines (e.g., triethylamine).

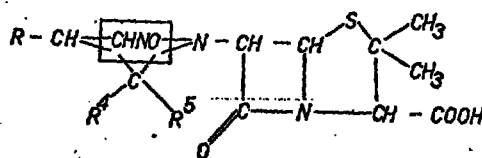
The temperature during the reaction is not critical. The reaction will proceed satisfactorily at room temperature and may be hastened by heating.

After the α -aminopenicillin has reacted with the carbonyl compound, the resultant product, in salt form, may be recovered by evaporation of the liquid reaction medium. Thus, if the reaction medium is a liquid carbonyl compound, such as acetone, the α -aminopenicillin derivative product, in salt form, may be recovered by removing the acetone by evaporation.

In a preferred embodiment of this invention, the derivative product is obtained by adding water to the reaction medium and quickly adjusting the pH of the mixture to within the range of from 1 to 3. The penicillin derivatives of this invention are insoluble in water at this pH and, therefore, they are precipitated from the aqueous mixture. Since the α -aminopenicillins *per se* are soluble in water at a pH of from 1 to 3, any unreacted α -aminopenicillin remains in solution. Thus, this procedure provides an excellent means for isolating the compounds of this invention.

The penicillin derivatives produced in accordance with the practice of this invention are much more water insoluble and more stable in an acid environment than the corresponding α -aminopenicillins from which they are derived.

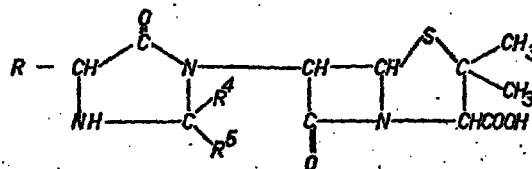
The compounds of this invention have the structure:



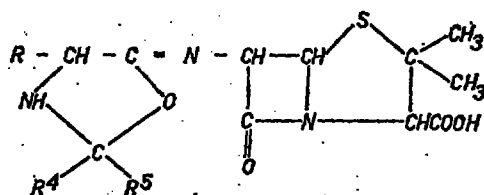
wherein R, R⁴ and R⁵ have the meaning set forth above, except that when R⁴ is hydrogen R⁵ is any group other than hydrogen which may be attached to the carbonyl group of an aldehyde, and the exact connection of the single atoms of carbon, hydrogen, nitrogen and oxygen shown in the rectangle to each other and to the atoms with valences shown extending into the rectangle is not known except in the cases where R⁴ is methyl and R⁵ is (lower)alkyl.

These products each have one of the structures represented by the following formulae:

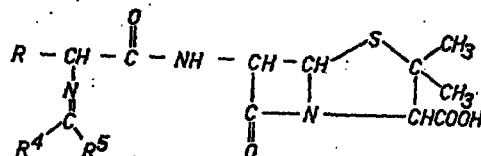
(III)



(IV)

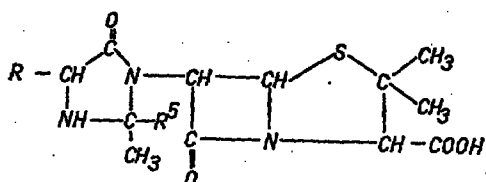


or (V)

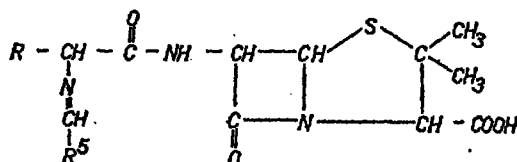


in which R is the same as defined above and R^4 and R^5 are the substituents on the carbonyl group of the carbonyl compound.

In those cases where R^4 is methyl and R^5 is (lower)alkyl, the compounds having the following structure:

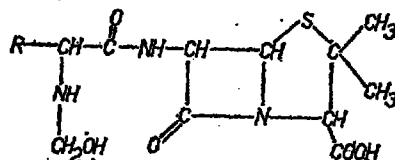


wherein R has the meaning set forth above. When R^4 is hydrogen and R^5 is phenyl or substituted phenyl, it is believed, but not yet definitely established, that the compounds have the structure:



wherein R has the meaning set forth above.

In the case where R^4 is hydrogen and R^5 is hydrogen i.e. when the carbonyl compound reactant is formaldehyde, the compound in accordance with the invention has the structure:



wherein R has the meaning set forth above.

Also included within the scope of this invention are the non-toxic, pharmaceutically acceptable salts of these novel penicillin derivatives. Such salts include for example, (1) the nontoxic pharmaceutically acceptable carboxylic acid salts such as the sodium, potassium, calcium, aluminium and ammonium salts and the non-toxic substituted ammonium salts with an amine selected from trialkylamines, procaine, dibenzylamine, N-benzyl- β -phenethylamine, 1-phenamine, N,N'-dibenzylethylenediamine, dehydroabietylamine, N,N'-bis-dehydroabietylthylenediamine, and N-(lower)alkylpiperidine such as N-ethylpiperidine, (2) the nontoxic pharmaceutically acceptable acid addition salts (the amine

(3)

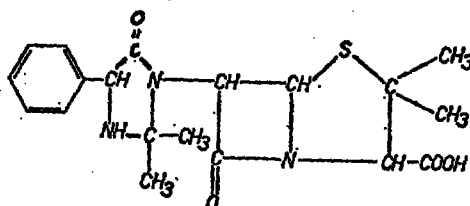
salts) for example (a) the mineral acid addition salts such as the hydrochloride, hydrobromide, hydroiodide, sulfate, sulfamate, sulfonate, or phosphate, and (b) the organic acid addition salts for example the maleate, acetate, citrate, tartrate, oxalate, succinate, benzoate, fumarate, maleate, mandelate, scorbate, β -naphthalene sulfonate, and toluene-sulfonate.

It will be understood that the antibacterial compounds of the invention may be prepared as salts either by reacting the compounds with a suitable reagent, or by the use of a starting material in salt form. For example, the sodium, potassium, calcium, aluminum or tri-substituted ammonium salt of the starting material may be employed.

The acetone- α -aminobenzylpenicillin derivative may be best prepared by mixing the sodium salt of α -aminobenzylpenicillin with acetone at 24–35°C. until a solution results (1–5 hours). The acetone solution is concentrated *in vacuo* to about 1/4 volume and is added to a large volume of cold water. The pH is adjusted to about 2–3 and a nicely crystalline white solid precipitates. The rectangular plates melt at about 180–190°C. with decomposition (varies slightly depending on water content) and are soluble in methanol and dimethylformamide, slightly soluble in acetone, and insoluble in water and most organic solvents. The infrared spectrum of the product contains absorption bands at 5.6 μ (β -lactam), 5.8–5.9 μ (carbon-oxygen or carbon-nitrogen double bond), and at 8.0 μ (ether linkage). According to elemental analysis and Karl Fischer determinations on a purified sample, this compound has the empirical formula $C_{19}H_{23}N_5O_4S$. Calculated for $C_{19}H_{23}N_5O_4S$: C, 58.6; H, 5.94; M.W., 389.

Found: C, 58.8; H, 6.25; M.W. 389, 393. The specific rotation in pH 7.0 buffer is +266°, and the isoelectric point, determined by electrophoresis, is 2.5. It does not react with 2,4-dinitrophenylhydrazine reagent in aqueous alcoholic, sulfuric acid at room temperature. Iodometric assays give results of 700–800 mcg./mg. and biological assays are in the 900–1000 mcg./mg. range when compared to α -aminobenzylpenicillin which has been assigned an arbitrary activity of 1000 mcg./mg.

The acetone- α -aminobenzylpenicillin derivative has the formula:



The following examples illustrate the methods for carrying out this invention.

EXAMPLE 1

α -Aminobenzylpenicillin (8.4 g.; 0.023 mole) is dissolved in a mixture of water (100 ml.) and acetone (400 ml.) at a pH of 8.8 and at room temperature. This solution is then stirred at 36°C. for 2 hours during which time the pH drops to 7.9. The pH of the solution is adjusted to 7.0 and the acetone is removed in *vacuo*. The resultant pale yellow solution (100 ml.) is extracted with ether. The yellow color remains. The aqueous layer, which has a pH of 8.0, is filtered. The filtrate is then acidified by the addition of HCl. A cream colored crystalline solid starts to separate at pH 4.5. This precipitate is still insoluble at pH 1.0. The pH is then adjusted to 2.0, the slurry is cooled for about 1 hour and filtered. The crystalline precipitate is washed with cold water and dried. The product (1.4 g.) is found to contain the β -lactam structure as shown by infra-red analysis, to melt at 189.2–191.0°C. with decomposition, to be soluble in a pH 6.9 buffer, in methyl alcohol and in dimethylformamide and to be only slightly soluble in acetone. The product (the acetone derivative of α -aminobenzylpenicillin) is determined by bioassay to have an activity of 920 mcg./mg. versus α -aminobenzylpenicillin. It inhibits *Staph. aureus* Smith at concentrations of 0.001 per cent by weight.

EXAMPLE 2

The sodium salt of α -aminobenzylpenicillin (3.9 g.; 0.01 mole) is slurried in 200 ml. of acetone for 21 hours at room temperature. A turbid solution results after 16 hours. Water (50 ml.) is then added and the acetone is removed in *vacuo*. The resultant yellow aqueous solution is filtered and the filtrate is acidified with 20% HCl. At a pH of 4.0, an amorphous solid begins to separate which crystallizes upon scratching. The slurry is stirred cold at pH 2.9–3.0 for 1 3/4 hours. It is filtered, the product washed with cold water and dried. The product, the acetone derivative of α -aminobenzylpenicillin, (1.4 g.)

melts at 183.1—185.0°C. with decomposition and is found by bioassay to have an activity of 960 mcg./mg. and to contain the β -lactam structure as shown by infrared analysis. It inhibits *Staph. aureus* Smith at a concentration of 0.05 mcg./ml., *Diplococcus pneumoniae* at a concentration of 0.025 mcg./ml., and *Salmonella enteritidis* at a concentration of 0.4 mcg./ml., and upon intramuscular injection in mice, exhibits versus *Staph. aureus* Smith a CD_{50} of 3.8 mg./kg.

EXAMPLE 3

The sodium salt of α -aminobenzylpenicillin (58.4 g.; 0.15 mole) is slurried at 35—40°C. for 19 hours in 750 ml. of acetone. The yellow color of the slurry appears to be slightly fluorescent. The composition is diluted with 250 ml. of water and the acetone is removed in vacuo. The remaining yellow aqueous portion (750 ml.) is filtered and the pH is adjusted from 8.5 to 3.0. During the pH adjustment, a white crystalline solid begins to form. The mixture is then diluted to a volume of 800 ml. with water. The resulting slurry is cooled in an ice bath and stirred for 1.0 hour. It is then filtered and the recovered precipitate is washed and dried in a vacuum oven at 50°C. The product (16.5 g.) is found to melt at 184.0—189.0°C. with decomposition. This product is dissolved in 150 ml. of water at pH 8.0, the solution is filtered and the pH of the filtrate is adjusted to 3.0 by the addition of 70% HCl. A crystalline solid separates which is slurried with cold water, filtered, washed and dried. The product, which is the condensation product of acetone with α -aminobenzylpenicillin, (8.3 g.) has a melting point of 184.7—186.0°C. with decomposition. Analysis for $C_{18}H_{23}N_3O_4S \cdot 1/2 H_2O$: Found: C, 57.2, 57.4; H, 5.80, 5.96; Calculated: C, 57.4%; H, 6.08%. The product inhibits *Staph. aureus* Smith at a concentration of 0.1 mcg./ml. The compound retained approximately 50% of its activity after 24 hours in an aqueous medium at a pH of 1.0. By contrast, α -aminobenzylpenicillin retained only 10% of its activity under the same conditions.

EXAMPLE 4

The sodium salt of α -aminobenzylpenicillin (8.1 g.; 0.0208 mole) is slurried in 160 ml. of isobutyraldehyde. The resultant solution is stirred for 22 hours. Subsequently, 100 ml. of water is added to the yellow solution. Two layers are thereby formed. The mixture is then concentrated in vacuo to a volume of 100 ml. Another 40 ml. of water is added and the volume is again concentrated to 100 ml. The small amount of yellow oil which has been formed remains on the bottom of the flask. The mixture is then filtered to separate the oil and the yellow aqueous filtrate is adjusted from a pH of 5.0 to 3.0. A semicrystalline cream-colored solid separates and is slurried cold for a few minutes after which it is filtered, washed and dried. The product (1.4 g.) is found to have a melting point of 162.5—175.5°C. with decomposition. This product is then dissolved in about 15 ml. of water at a pH of 8.0 and the pH of the solution is readjusted to 3.0. A semicrystalline cream-colored solid separates. The mixture is stirred cold for a few minutes, filtered, washed and dried. The product, the isobutyraldehyde derivatives of α -aminobenzylpenicillin, (0.6 g.) is determined to have a melting point of 169.5—172.5°C. with decomposition. It is found by bioassay to have an activity of 175 mcg./mg. versus α -aminobenzylpenicillin and to contain the β -lactam structure as shown by infra-red analysis. The product, the condensation product of α -aminobenzylpenicillin with isobutyraldehyde, has an empirical formula of $C_{26}H_{25}N_3O_4S \cdot H_2O$. Analysis for this formula: Calculated: C, 57.1; H, 6.45; N, 9.98; S, 7.61; H_2O , 4.3. Found: C, 57.3; H, 6.12; N, 10.37; S, 7.57; H_2O , 3.8. The product inhibits *Staph. aureus* Smith at a concentration of 0.001% by weight.

EXAMPLE 5

A slurry of α -aminobenzylpenicillin (36.7 g.; 0.1 mole) in 600 ml. of methylene chloride and 25 ml. of triethylamine is prepared whereby the triethylamine salt of α -aminobenzylpenicillin is formed. To this slurry there is added distilled benzaldehyde (10.6 g.; 10.1 ml.; 0.1 mole). The slurry becomes thicker and after 18 1/2 hours a complete solution results. This solution is filtered and concentrated in vacuo to a yellow gum which is water soluble. The gum is dissolved in 2 liters of cold water and the pH is adjusted to 2.9 to form a precipitate. This precipitate is collected and dissolved in water at a pH of 6.5. This aqueous solution is extracted with ether and the pH of the aqueous solution is adjusted to 3.0 whereby a crystalline solid precipitate is formed. The precipitate is collected by filtration, washed with water and dried. The product, which is the benzaldehyde derivative of α -aminobenzylpenicillin, (4.2 g.) is found to have a melting point of 168.3—171.6°C. with decomposition and is determined by bioassay to have an activity of 340 mcg./mg. versus α -aminobenzylpenicillin. It inhibits *Staph. aureus* Smith at concentrations of 0.001% by weight. Analysis for $C_{23}H_{23}N_3O_4S \cdot 1/2 H_2O$

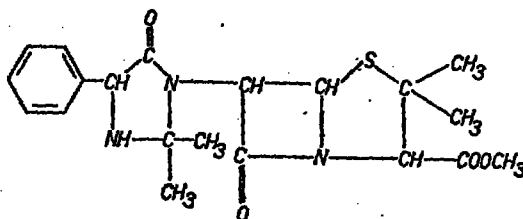
(molecular weight 446.5): Calculated: C, 61.8; H, 5.41; H₂O, 2.0. Found: C, 61.4; H, 5.50; H₂O, 1.4.

EXAMPLE 6

The sodium salt of α -aminobenzylpenicillin (37.1 g.; 0.1 mole) is slurried in 600 ml. of methylene chloride. Subsequently 10.4 ml. of cyclohexanone (0.1 mole) is added in one portion. Following this, 80 additional milliliters of cyclohexanone is added in incremental portions over a period of 24 1/2 hours. The yellow slurry is added to 2 liters of cold water. This mixture is agitated, filtered, and the filtrate emulsion is broken by centrifugation. The aqueous layer is adjusted to a pH of 2.5 and the crystalline solid which is thus formed is collected by filtration, washed with water and dried. The product (3.2 g.) is determined to have a melting point of 185.4—187.1°C. with decomposition. The product, the cyclohexanone derivative of α -aminobenzylpenicillin, is found by bioassay to have an activity of 440 mcg./mg. and to inhibit *Staph. aureus* Smith at concentrations of 0.001% by weight.

EXAMPLE 7

Diazomethane is prepared by the dropwise addition of a 50% aqueous potassium hydroxide solution (6 ml.) to a cold stirred slurry of N-nitroso-N-methylurea (3.1 g.; 0.03 mole) in 60 ml. of ether. The resultant yellow solution is decanted into a cold slurry of the reaction product of acetone and α -aminobenzylpenicillin (which is obtained by the process of Example 3) (3.9 g.; 0.01 mole) in 160 ml. of ether and 40 ml. of methyl alcohol. Reaction is instantaneous and is accompanied by the evolution of N₂ gas. A complete solution results. About 1/2 of the ether solution of diazomethane is added before a permanent yellow colour persists in the reaction vessel. Most of this yellow colour is destroyed by the addition of acetic acid and the resulting solution is dried over Na₂SO₄. The filtrate is then concentrated in vacuo to a clear yellow gum which weighs 3.7g. This is crystallized twice from a mixture of carbon tetrachloride and Skellysolve B to give a white crystalline solid product (0.2g) which is soluble in organic solvents and melts at 101.5—102°C. (corrected). Its infrared spectrum indicated that it contains a β -lactam group. The product is the methyl ester of the acetone- α -aminobenzylpenicillin derivative and has an empirical formula of C₂₀H₂₅O₄N₃S, and the structural formula (as determined by X-ray diffraction analysis)



Analysis calculated for C₂₀H₂₅O₄N₃S: C, 59.54; H, 6.25; N, 10.42; S, 7.92; molecular weight 403.4. Found: C, 59.00; H, 6.03; N, 10.47; S, 8.05; molecular weight 402.

EXAMPLE 8

To 100 gm. of α -aminobenzylpenicillin slurried in 2500 ml. of acetone is added 200 ml. of a methyl isobutyl ketone solution of potassium ethylhexanoate (22% solution) and the mixture is warmed to 45°C. whereupon the acid dissolves. After the mixture is agitated for one hour at 40° to 45°C., the product begins to crystallize out. Agitation is continued for 4 hours at 45°C. after which the product, the potassium salt of the acetone derivative of α -aminobenzylpenicillin, is collected by filtration washed with 500 ml. of dry acetone, dried for 17 hours at 40°C. and found to weigh 70.0 grams. It inhibits *staph. aureus* Smith at a concentration of 0.001% by weight.

EXAMPLE 9

α -Aminobenzylpenicillin trihydrate (270 gm.) is slurried in 1350 ml. of acetone. Triethylamine (189 ml.) is added to the slurry which is then heated to 40°C. whereupon the acid is dissolved. The solution is stirred for 5 hours at 40° to 45°C., polish-filtered through filter aid and then slowly poured into 1500 ml. of water at 5°C., the pH of the water being maintained at 2.5 by intermittent addition of 6 N hydrochloric acid. After addition of all the rich acetone solution to the ice water, the resulting slurry (containing a crystalline precipitate) is stirred for 1 hour in an ice bath. The product, the acetone derivative of α -aminobenzylpenicillin, is collected by filtration washed with 500 ml. of cold water, dried for 17 hours at 40°C., is found to weigh 127.4 gms. and to inhibit *Staph. aureus* Smith at a concentration of 0.001% by weight.

EXAMPLE 10

Sixty gms. of the product of Example 9 above, is mixed in 1200 ml. of water at 20°C. To the resulting slurry is added 300 ml. of a 40% aqueous solution of β -naphthalene sulfonic acid. The slurry is stirred 4 hours at room temperature whereupon the product, the β -naphthalene sulfonic acid salt of the acetone derivative of α -aminobenzylpenicillin, crystallizes out of solution as long needles, is collected by filtration, washed with 1200 ml. of cold water, dried for 20 hours at 40°C., and is found to weigh 76.5 gms. It inhibits *Staph. aureus* Smith at a concentration of 0.001% by weight.

EXAMPLE 11

Part A: Three hundred gms. of α -aminobenzylpenicillin trihydrate are mixed in 1500 ml. of acetone. Triethylamine (210 ml.) is added to the slurry which is then heated to and maintained at 30°C. for 20 hours. The solution is then polish-filtered through filter aid and the filter washed with 200 ml. acetone. The mixed filtrate and wash acetone is then added slowly to 1500 ml. ice water maintained at pH 2.5 by intermittent addition of 6 N hydrochloric acid. The resulting solution is stirred for 2 hours at 0° to 10°C. while crystallization of the acetone derivative of α -aminobenzylpenicillin occurs. The crystalline product is then collected by filtration washed on the filter, with 600 ml. of ice water, dried and found to weigh 134.0 gms.

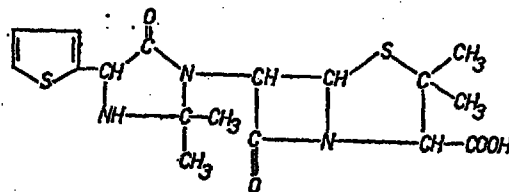
Part B: 120 gms. of the product of Part A above are slurried in 2200 ml. of water at 15°C. The slurry is agitated while 200 ml. concentrated hydrochloric acid are added and the solids dissolve. The strongly acidic solution is stirred for 4 hours at 27°C. during which time crystallization occurs. The crystalline product, the hydrochloric acid salt of the acetone derivative of α -aminobenzylpenicillin, is collected by filtration, quickly washed sequentially on the filter with 200 ml. of ice cold water and several portions of dry acetone, dried 18 hours at 40°C. and found to weigh 75 gms. The product inhibits *Staph. aureus* Smith at a concentration of 0.001% by weight.

EXAMPLE 12

(-)- α -Amino-2-thienylmethylpenicillin (2 g. chemical assay 780 mcg./mgm. versus α -aminobenzylpenicillin) was slurried in 20 ml. acetone, 1.5 ml. triethylamine was added, and the mixture was stirred for 16 hours at 25°C. The suspension was filtered, the solid was washed with 5 ml. acetone and the combined acetone-wash and filtrate was added slowly to 25 ml. cold water (5—10°C.) maintained at pH 2.5—3.0 with 6 N HCl. The mixture was then stirred three hours in an ice bath as the product, the condensation product of acetone and (-)- α -amino-2-thienylmethylpenicillin precipitated and was collected by filtration. The precipitate was washed with 25 ml. cold water and then with dry acetone, dried 20 hours at 45°C. and found to weigh 0.38 g. to have a specific rotation of +307° in 1% solution in dimethylformamide, to give a chemical assay of 900 mcg./mgm. versus α -aminobenzylpenicillin and a bio-assay of 980 mcg./mgm. versus α -amino-2-thienylmethylpenicillin, to darken on heating at 165—170°C, to melt with decomposition at 195—200°C, and to have an isoelectric point of 2.5 determined by electrophoresis.

Anal: Calc'd. for $C_{17}H_{21}N_3S_2O_4$: C, 51.7; H, 5.37; N, 10.63; S, 16.20. Found C, 50.65; H, 5.48; N, 10.75; S, 16.5.

The compound produced as described above has the formula:



The present invention includes a therapeutic composition comprising an antibacterial compound in accordance with the invention, an antibacterial compound prepared by processes in accordance with the invention, or a non-toxic pharmaceutically acceptable salt thereof and a pharmaceutically acceptable diluent or carrier.

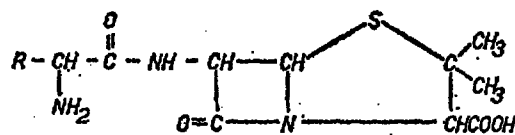
The invention also includes a method for the treatment of infectious diseases caused by gram-positive or gram-negative bacteria in warm blooded animals excluding man, which method comprises administering to the animal an antibacterial compound, or salt in accordance with the invention, or a therapeutic composition as described above.

The invention further provides an animal feed including an antibacterial compound, or a salt in accordance with the invention, or a therapeutic composition as described above.

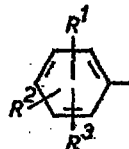
The invention still further includes a method for the treatment of mastitis in cattle, which method comprises administering to an infected animal of the cattle class an antibacterial compound, or salt in accordance with the invention, or therapeutic composition as described above.

WHAT WE CLAIM IS:—

1. A process for the preparation of a novel antibacterial compound wherein an α -aminopenicillin having the general formula



wherein R is selected from hydrogen, alkyl, cycloalkyl, indolylalkyl, naphthyl, benzyl, furyl and radicals having the formula



wherein R^1 , R^2 and R^3 are selected from hydrogen, nitro, di(lower)alkylamino, (lower)alkanoylamino, (lower)alkanoyloxy, (lower)alkyl, (lower)alkoxy, sulfamyl, chloro, iodo, bromo, fluoro, trifluoromethyl, (lower)alkylthio, (lower)alkylsulfonyl, carbo(lower)alkoxy, benzyl, phenethyl, cycloheptyl, cyclohexyl and cyclopentyl; is reacted with a carbonyl compound selected from aldehydes, cyclopentanone, cyclohexanone and methyl-(lower)alkyl ketones at a pH within the range of from about 5.5 to about 9.5; and subsequently recovering the reaction product from the reaction mixture.

2. A process as claimed in claim 1, wherein the pH is within the range of 7.5 to 9.0.

3. A process as claimed in either claim 1 or claim 2 wherein the reaction product is recovered by adding water to the reaction mixture, thereafter adjusting the pH of the mixture as soon as possible to within the range of from 1 to 3, and recovering the resultant precipitated product.

4. A process as claimed in any one of claims 1—3, wherein the α -aminopenicillin is α -aminobenzylpenicillin.

5. A process as claimed in any one of claims 1—3 wherein the α -aminopenicillin is

aminomethylpenicillin,
 α -aminopentylpenicillin,
 α -aminopropylpenicillin,
 α -aminoethylpenicillin,
 α -aminoisobutylpenicillin,
 α -amino-n-butylpenicillin,
 α -amino- α -methylbutylpenicillin,
 α -amino- β -methylbutylpenicillin,
 α -aminononylpenicillin,
 α -aminoundecylpenicillin,
 α -aminoheptadecylpenicillin,
 α -aminocyclohexylmethylpenicillin,
 α -amino- β -phenylethylpenicillin,
 α -amino- β -3-indolyethylpenicillin,
 α -amino-1-naphthylmethylpenicillin,
 α -amino-2-furylmethylpenicillin,
 α -amino-p-chlorobenzylpenicillin,
 α -amino-p-methoxybenzylpenicillin,
 α -amino-4-diethylaminobenzylpenicillin,
 α -amino-4-trifluoromethylbenzylpenicillin,
 α -amino-2,4-dibromobenzylpenicillin,



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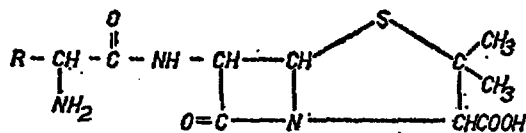
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The invention further provides an animal feed including an antibacterial compound, or a salt in accordance with the invention, or a therapeutic composition as described above.

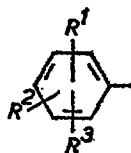
The invention still further includes a method for the treatment of mastitis in cattle, which method comprises administering to an infected animal of the cattle class an antibacterial compound, or salt in accordance with the invention, or therapeutic composition as described above.

WHAT WE CLAIM IS:—

1. A process for the preparation of a novel antibacterial compound wherein an α -aminopenicillin having the general formula



wherein R is selected from hydrogen, alkyl, cycloalkyl, indolylalkyl, naphthyl, benzyl, furyl and radicals having the formula



wherein R^1 , R^2 and R^3 are selected from hydrogen, nitro, di(lower)alkylamino, (lower)alkanoylamino, (lower)alkanoyloxy, (lower)alkyl, (lower)alkoxy, sulfamyl, chloro, iodo, bromo, fluoro, trifluoromethyl, (lower)alkylthio, (lower)alkylsulfonyl, carbo(lower)alkoxy, benzyl, phenethyl, cycloheptyl, cyclohexyl and cyclopentyl; is reacted with a carbonyl compound selected from aldehydes, cyclopentanone, cyclohexanone and methyl-(lower)alkyl ketones at a pH within the range of from about 5.5 to about 9.5; and subsequently recovering the reaction product from the reaction mixture.

2. A process as claimed in claim 1, wherein the pH is within the range of 7.5 to 9.0.

3. A process as claimed in either claim 1 or claim 2 wherein the reaction product is recovered by adding water to the reaction mixture, thereafter adjusting the pH of the mixture as soon as possible to within the range of from 1 to 3, and recovering the resultant precipitated product.

4. A process as claimed in any one of claims 1—3, wherein the α -aminopenicillin is α -aminobenzylpenicillin.

5. A process as claimed in any one of claims 1—3 wherein the α -aminopenicillin is

aminomethylpenicillin,
 α -aminopentylpenicillin,
 α -aminopropylpenicillin,
 α -aminoethylpenicillin,
 α -aminoisobutylpenicillin,
 α -amino-n-butylpenicillin,
 α -amino- α -methylbutylpenicillin,
 α -amino- β -methylbutylpenicillin,
 α -aminononylpenicillin,
 α -aminoundecylpenicillin,
 α -aminoheptadecylpenicillin,
 α -aminocyclohexylmethylpenicillin,
 α -amino- β -phenylethylpenicillin,
 α -amino- β -3-indolylethylpenicillin,
 α -amino-1-naphthylmethylpenicillin,
 α -amino-2-furylmethylpenicillin,
 α -amino-p-chlorobenzylpenicillin,
 α -amino-p-methoxybenzylpenicillin,
 α -amino-4-diethylaminobenzylpenicillin,
 α -amino-4-trifluoromethylbenzylpenicillin,
 α -amino-2,4-dibromobenzylpenicillin,

α -amino-2-nitrobenzylpenicillin,
 α -amino-3-methylbenzylpenicillin,
 α -amino-4-sulfamylbenzylpenicillin,
 α -amino-2-iodobenzylpenicillin,
 α -amino-4-*t*-butylbenzylpenicillin, or α -amino-2-acetamidobenzylpenicillin.

6. A process as claimed in any one of claims 1 to 5, wherein the carbonyl compound is acetone.

7. A process as claimed in any one of claims 1 to 5, wherein the carbonyl compound is isobutyraldehyde.

8. A process as claimed in any one of claims 1 to 5, wherein the carbonyl compound is benzaldehyde.

9. A process as claimed in any one of claims 1 to 5, wherein the carbonyl compound is cyclohexanone.

10. A process as claimed in any one of claims 1 to 5, wherein the carbonyl compound is methyl ethyl ketone, methyl butyl ketone, methyl pentyl ketone, formaldehyde, acetaldehyde, chloral, glyoxal, propionaldehyde, butyraldehyde, valeraldehyde, isovaleraldehyde, furfural, acrolein phenylacetaldehyde, α -tolualdehyde, β -tolualdehyde, dimethylbenzaldehyde, naphthaldehyde, malonaldehyde, succinaldehyde, terephthaldehyde, propargylaldehyde, β -chloropropionaldehyde, β -chlorobenzaldehyde, *p*-trifluoromethylbenzaldehyde, α -methoxybenzaldehyde, or 3,4-dimethoxybenzaldehyde.

11. A process as claimed in any one of claims 1 to 3, wherein R is thienyl, the α -aminopenicillin being α -thienyl- α -aminomethylpenicillin.

12. A process as claimed in any one of claims 1 to 10, wherein a salt of the penicillin is reacted with the carbonyl compound.

13. A process as claimed in claim 12, wherein a sodium, potassium, calcium, aluminium, or tri-substituted ammonium salt is employed.

14. A process as claimed in claim 11 wherein a salt of α -thienyl- α -aminomethylpenicillin is reacted with the carbonyl compound.

15. A process as claimed in claim 14, wherein the salt is as defined in claim 13.

16. A process for the preparation of an antibacterial compound as claimed in claim 1 and substantially as hereinbefore described with reference to any one of Examples 1-7.

17. A process for the preparation of an antibacterial compound as claimed in claim 1 and substantially as hereinbefore described with reference to any one of Examples 8-12.

18. A process as claimed in any one of claims 1-10 wherein the reaction product is recovered as a non-toxic pharmaceutically acceptable salt.

19. A process as claimed in claim 11 wherein the reaction product is recovered as a non-toxic pharmaceutically acceptable salt.

20. An antibacterial compound whenever prepared by a process as claimed in any one of claims 1-10 or 13.

21. An antibacterial compound whenever prepared by a process as claimed in any one of claims 11, 14 or 15.

22. An antibacterial compound whenever prepared by a process as claimed in claim 12.

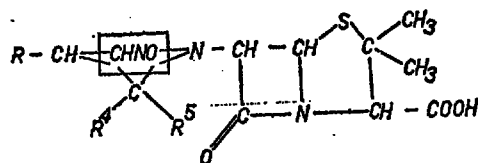
23. An antibacterial compound whenever prepared by a process as claimed in claim 16.

24. An antibacterial compound whenever prepared by a process as claimed in claim 17.

25. An antibacterial compound whenever prepared by a process as claimed in claim 18.

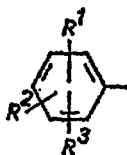
26. An antibacterial compound whenever prepared by a process as claimed in claim 19.

27. A novel antibacterial compound having the general formula:



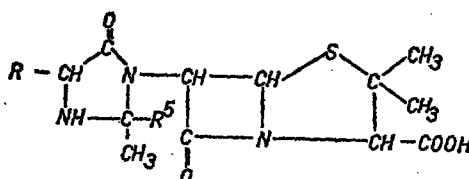
wherein R is hydrogen, alkyl, cycloalkyl, indolylalkyl, naphthyl, benzyl, furyl or a radical having the formula





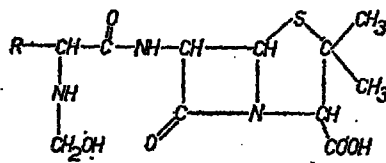
wherein R^1 , R^2 and R^3 are hydrogen, nitro, di(lower)alkylamino, (lower)alkanoylamino, (lower) alkanoyloxy, (lower) alkyl, (lower)alkoxy, sulfamyl, chloro, iodo, bromo, fluoro, trifluoromethyl, (lower)alkylthio, (lower)alkylsulfonyl, carbo(lower)alkoxy, benzyl, phenethyl, cycloheptyl, cyclohexyl and cyclopentyl; R^4 is hydrogen or methyl, R^5 is (lower)alkyl when R^4 is methyl, and, when R^4 is hydrogen, R^5 is any group other than hydrogen which may be attached to the carbonyl group of an aldehyde, and wherein R^4 and R^5 , when taken together with the carbon atom to which they are attached, represent a cyclopentyl or cyclohexyl radical.

28. A compound of the formula



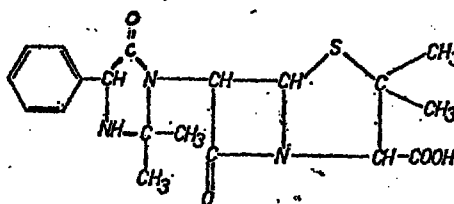
wherein R is as defined in claim 27 and R^5 is (lower)alkyl.

29. A compound of the formula



wherein R is as defined in claim 27.

30. The acid having the formula



31. A non-toxic pharmaceutically acceptable salt of a compound as claimed in any one of claims 27 to 30.

32. The sodium, potassium, calcium, aluminium, or ammonium salt of a compound as claimed in any one of claims 27 to 30.

33. The trialkylamine, procaine, dibenzylamine, N-benzyl- β -phenethylamine, 1-phenylamine, N,N'-dibenzylethylenediamine, dehydroabietylamine, N,N'-bis-dehydroabietylthylenediamine, or N-ethylpiperidine salt of a compound as claimed in any one of claims 27 to 30.

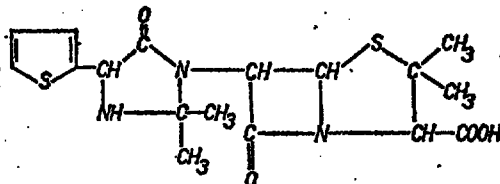
34. The hydrochloride, hydrobromide, hydroiodide, sulfate, sulfamate, sulfonate, or phosphate salt of a compound as claimed in any one of claims 27 to 30.

35. The maleate, acetate, citrate, tartrate, oxalate, succinate, benzoate, fumarate, malate, mandelate, ascorbate, β -naphthalene sulfonate, or toluenesulfonate salt of a compound as claimed in any one of claims 27 to 30.

36. A compound of the formula set forth in in any one of claims 27 to 29 wherein R is thienyl.



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38. A non-toxic pharmaceutically acceptable salt of a compound as claimed in claim 36 or claim 37.

39. The sodium, potassium, calcium, aluminium, or ammonium salt of a compound as claimed in claim 36 or claim 37.

40. The trialkylamine, procaine, dibenzylamine, N-benzyl- β -phenethylamine, 1-ephenamine, N,N'-dibenzylethylenediamine, dehydroabietylamine, N,N'-bis-dehydroabietylthylenediamine, or N-ethylpiperidine salt of a compound as claimed in claim 36 or claim 37.

41. The hydrochloride, hydrobromide, hydroiodide, sulfate, sulfonate, sulfamate, or phosphate salt of a compound as claimed in claim 36 or claim 37.

42. The maleate, acetate, citrate, tartrate, oxalate, succinate, benzoate, fumarate, malate, mandelate, ascorbate, β -naphthalene sulfonate, or toluenesulfonate salt of a compound as claimed in claim 36 or claim 37.

43. A therapeutic composition comprising an antibacterial compound as claimed in any one of claims 27 to 30 or a salt as claimed in any one of claims 31 to 35, and a pharmaceutically acceptable diluent or carrier.

44. A therapeutic composition comprising an antibacterial compound as claimed in claim 36 or claim 37 or a salt as claimed in any one of claims 38 to 42, and a pharmaceutically acceptable diluent or carrier.

45. A therapeutic composition comprising an antibacterial compound as claimed in claims 20 or 23 and a pharmaceutically acceptable diluent or carrier.

46. A therapeutic composition comprising an antibacterial compound as claimed in any one of claims 21, 22 or 24 or a salt as claimed in claim 25 or claim 26 and a pharmaceutically acceptable diluent or carrier.

47. An animal feed including a compound as claimed in any one of claims 27 to 30 or a salt as claimed in any one of claims 31 to 35.

48. An animal feed including a compound as claimed in claim 36 or claim 37 or a salt as claimed in any one of claims 38 to 42.

49. An animal feed including an antibacterial compound as claimed in claim 20 or 23.

50. An animal feed including an antibacterial agent as claimed in any one of claims 21, 22 or 24 or a salt as claimed in claim 25 or claim 26.

51. A method for the treatment of infectious diseases caused by gram-positive bacteria or gram-negative bacteria in warm-blooded animals, excluding man, which method comprises administering to the animal a compound as claimed in any one of claims 27 to 30, a salt as claimed in any one of claims 31 to 35, or a composition as claimed in claim 43.

52. A method for the treatment of infectious diseases caused by gram-positive bacteria or gram-negative bacteria in warm-blooded animals, excluding man, which method comprises administering to the animal a compound as claimed in claim 36 or claim 37, a salt as claimed in any one of claims 38 to 42, or a composition as claimed in claim 44.

53. A method for the treatment of infectious diseases caused by gram-positive bacteria or gram-negative bacteria in warm-blooded animals, excluding man, which method comprises administering to the animal a compound as claimed in claim 20 or 23 or a therapeutic composition as claimed in claim 45.

54. A method for the treatment of infectious diseases caused by gram-positive bacteria or gram-negative bacteria in warm-blooded animals, excluding man, which method comprises administering to the animal, a compound as claimed in any one of claims 21, 22 or 24 or a salt as claimed in claim 25 or claim 26 or a therapeutic composition as claimed in claim 46.

55. A method for the treatment of mastitis in cattle which method comprises administering to an infected animal of the cattle class a compound as claimed in any one of claims 27 to 30, a salt as claimed in any one of claims 31 to 35, or a composition as claimed in claim 43.

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56. A method for the treatment of mastitis in cattle which method comprises administering to an infected animal of the cattle class a compound as claimed in claim 36 or claim 37, a salt as claimed in any one of claims 38 to 42 or a composition as claimed in claim 44.

57. A method for the treatment of mastitis in cattle which method comprises administering to an infected animal of the cattle class a compound as claimed in claim 20 or 23 or a therapeutic composition as claimed in claim 45.

58. A method for the treatment of mastitis in cattle which method comprises administering to an infected animal of the cattle class a compound as claimed in any one of claims 21, 22 or 24 or a salt as claimed in claim 25 or claim 26 or a therapeutic composition as claimed in claim 46.

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Chartered Patent Agents
Agents for the Applicants

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ERRATA

SPECIFICATION No. 1,057,654

Page 5, line 4, for "scorbate" read "ascorbate"
Page 5, line 19, for "analysis" read "analysis"
Page 5, line 30, for "carying" read "carrying"
Page 5, line 32, for "a-Aminobenzylpenicilin"
read "a-Aminobenzylpenicillin"
Page 7, line 52, after "is" insert "then"
Page 7, line 53, before "cold" delete "of"
Page 11, line 31, after "in" delete "in"
(second occurrence)

THE PATENT OFFICE

PATENTS ACT 1949

SPECIFICATION NO. 1,057,654

In accordance with the Decision of the Superintending Examiner, acting for the Comptroller-General, dated 4 November 1970 this Specification has been amended under Section 14 in the following manner:-

Page 10 line 32, for "1-7" read "1-8"

Reference has been directed in pursuance of Section 9, subsection (1) of the Patents Act 1949, to patent Nos. 873,049 and 902,703

THE PATENT OFFICE
15 December 1970

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