PATENT SPECIFICATION

NO DRAWINGS

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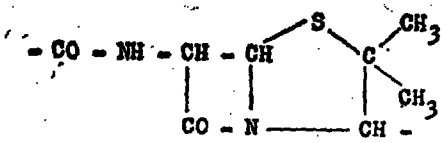
COMPLETE SPECIFICATION

Acyl Derivatives of 6-Aminopenicillanic Acid

We, BEECHAM RESEARCH LABORATORIES LIMITED, a British Company, of Brockham Park, Betchworth, Surrey, 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 acyl derivatives of 6-aminopenicillanic acid.

It is well known that substances having antibiotic activity can be obtained by growing certain moulds, for example moulds of the genus *Penicillium* such as *Penicillium chrysogenum* in the presence of a suitable nutrient medium. One group of these antibiotic substances are known as penicillins and have the general formula: R—Pn—COOH (I) in which Pn is a molecular grouping $C_8H_{10}O_2N_2S$ which is now general agreed to have the following structure:

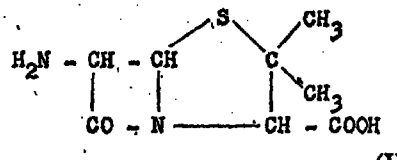


(II)

Previous work on penicillins has shown that the amount of antibiotic material obtained as a metabolite of a penicillin-producing mould was only a minor part of the total products obtained. Later it was found that the yield of penicillins could be increased very considerably by growing the mould in the presence of certain chemical compounds which became known as "precursors".

The term "fermentation liquor" as used herein means the liquid material obtained from a fermentation.

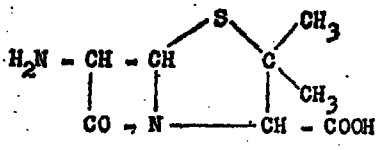
We have found according to our co-pending Application No. 26957/60 (Serial No. 870,396) that this liquor contains a compound of the structural formula:



(III)

which we term 6-aminopenicillanic acid following the nomenclature adopted by other workers in analogous fields.

The invention of our co-pending Application No. 26957/60 (Serial No. 870,396) therefore provides 6-aminopenicillanic acid having a structural formula:



which is capable of reacting with phenylacetyl chloride to produce benzylpenicillin, and which gives a negative Bratten-Marshall test and a negative ninhydrin test, and its salts.

6-Aminopenicillanic acid is a non-hygroscopic compound which in a substantially pure form had a melting point of 209 to 210°C. and analysed as follows:—(Found: C, 44.6; H, 5.7; N, 13.1; S, 14.1%. $C_8H_{12}O_3N_2S$ requires: C, 44.4; H, 5.6; N, 13.0; S, 14.8%).

The invention of our co-pending Application No. 26957/60 (Serial No. 870,396) further includes a process for the preparation of 6-aminopenicillanic acid in which a penicillin-producing mould is grown in a nutrient medium and the 6-aminopenicillanic acid or a salt thereof is isolated from the fermentation liquor obtained by adsorption on an ion-exchange resin and subsequent elution therefrom with an aqueous solvent. This isolation may be effected by first concentrating (and/or

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purifying) the fermentation liquor and thereafter treating it with an ion-exchange resin, the eluate from the resin being concentrated and the 6-aminopenicillanic acid in crystalline form precipitated therefrom by the addition of an acid, e.g. hydrochloric acid. In one form of the invention the eluate from the ion-exchange resin is purified by chromatography on a cellulose column before the 6-aminopenicillanic acid is precipitated therefrom.

The present invention provides a process for the preparation of 6-acylamino derivatives of penicillanic acid in which 6-aminopenicillanic acid having the structural formula III or a salt thereof is reacted with a carboxylic acid chloride or bromide, a sulphonic acid chloride, an ester of chlorocarbonic acid, an acid anhydride of a carboxylic acid or a mixed acid anhydride derived from a carboxylic acid.

The present invention further provides a process for the preparation of derivatives of 6-aminopenicillanic acid wherein 6-aminopenicillanic acid or a salt thereof contained in the fermentation liquor obtained by growing a penicillin-producing mould in a nutrient medium in accordance with the process of our Application No. 26957/60 (Serial No. 870,396) is reacted with a carboxylic chloride or bromide, a sulphonic acid chloride, an ester of chlorocarbonic acid, an acid anhydride of a carboxylic acid or a mixed anhydride derived from a carboxylic acid, whereby a 6-acylamino derivative of penicillanic acid is produced.

If desired the fermentation liquid may be partially concentrated and/or purified before the reaction or the 6-aminopenicillanic acid may be isolated and used in the reaction.

Since some of the antibiotic substances obtained by the process of this invention are relatively unstable compounds which readily undergo chemical changes resulting in the loss of antibiotic activity, it is desirable to choose reaction conditions which are sufficiently moderate to avoid their decomposition. The reaction conditions chosen will, of course, depend largely upon the reactivity of the chemical reagent being used. In most instances, a compromise has to be made between the use of very mild conditions for a lengthy period and the use of more vigorous conditions for a shorter time with the possibility of decomposing some of the antibiotic substance.

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

As already stated, it is sometimes preferred to prepare the antibiotic substances by the use of the isolated 6-aminopenicillanic acid or one of the intermediate concentrates obtained during its isolation. A concentrated solution of 6-aminopenicillanic acid can be prepared by evaporating the clarified harvest brew at reduced temperature and pressure to a small volume, for example in the manner described in Example 1 of our co-pending Application No. 26957/60 (Serial No. 870,396). If desired, the penicillins present in the acidified brew can be largely removed by extraction with an organic solvent therefor such as butyl acetate. After neutralising the liquid, substantial amounts of impurities can then be precipitated by the addition of a water-miscible organic liquid, e.g., acetone, methanol or ethanol. After separating such impurities the clear liquor may then be further concentrated to give a concentrated preparation.

The production by the process of this invention of antibiotic material from fermentation liquor having little or no antibiotic activity is clearly indicated if, before the addition of one of the chemical reagents hereinbefore specified to the fermentation liquor, the penicillins already present as a result of the fermentation reaction by which the fermentation liquor was obtained are removed. This removal can readily be achieved as indicated above by extracting the penicillins from the acidified fermentation liquor by means of an organic solvent, for example, butyl acetate, in which the penicillins are soluble. It will be seen from the Examples below that in some instances a very considerable increase in antibiotic activity was obtained as a result of the chemical modification of the fermentation liquor according to the process of this invention. Where phenylacetyl chloride was used as the chemical reagent, for example, the antibiotic activity obtained after the reaction was 23 times as great as that before the reaction.

The following Examples illustrate the invention:

The procedure to be followed in preparing antibiotic substances from 6-aminopenicillanic acid depends largely upon the extent to which the starting material has itself been purified. Thus 6-aminopenicillanic acid may be used in three different stages of purification, as indicated below:—

(a) From isolated 6-aminopenicillanic acid.

When 6-aminopenicillanic acid is available in relatively pure form it is only necessary to use a small excess (*ca.* 20%) of reagent and the product is, in turn, obtained fairly pure (as indicated by manometric assay using penicillinase).

The reagents used in this way include fifteen different monocarboxylic acid chlorides and also adipyl chloride, propionic anhydride, carbobenzoxyglycine ethoxy-formic anhydride, benzyl chloroformate, and *p*-toluenesulphonyl

chloride.

(b) From 6-aminopenicillanic acid concentrates.

The starting material was a clarified fermentation liquor which had been subjected to an initial concentration procedure and from which the natural penicillins had been substantially removed by solvent extraction at pH 2 to 3. The neutralised aqueous solution usually contained 0.6—1.2 mg./ml. of 6-aminopenicillanic acid, which represented about 1% of the total solids present.

With such material it was necessary to use a much larger excess of reagent (10 to 50 times the theoretical amount) because various impurities (e.g. amino-acids and simple peptides) would also be susceptible to acylation and similar reactions. The products were worked up in essentially the same way as in (a), but the resulting sodium salts were, of course, very much less pure.

The reagents used in this way include phenoxy-acetyl chloride, phenylacetyl chloride, α-chlorophenylacetyl chloride, chloroacetyl chloride, diphenylacetyl chloride, and adipyl chloride (all of which had also been reacted by Method (a)) and also α-naphthylacetyl chloride, β-naphthoxyacetyl chloride and *p*-nitrophenoxyacetyl chloride.

(c) From dilute brew.

The initial material was the original clarified fermentation brew from which natural penicillins had been substantially removed by solvent extraction at pH 2 to 3, but which had not been concentrated. It was thus about ten times more dilute than the solutions used in Method (b). Use of a large excess of reagent was again essential. With this very dilute material no attempt was made to isolate the reaction products, but the formation of antibiotic material was demonstrated by the increased antibacterial activity of the solution after reaction and by paper chromatography, a new zone of biologically active material being detected in every case.

The reagents used successfully in this way include phenoxyacetyl chloride, phenylacetyl chloride, α-naphthylacetyl chloride, α-naphthoxyacetyl chloride, β-naphthoxyacetyl chloride, *p*-nitrophenoxyacetyl chloride, α-chlorophenylacetyl chloride, diphenylacetyl chloride, crotonyl chloride, chloroacetyl chloride, phthalimidoacetyl chloride, benzoyl chloride, hexahydrobenzoyl chloride, *m*-sulphobenzoyl chloride, adipyl chloride, propionic anhydride, and *n*-butyric anhydride.

EXAMPLE 1.

A strain of *Penicillium chrysogenum* (5120C obtained from Professor E. B. Chain, Istituto Superiore di Sanita, Rome) was grown on a glycerol-molasses agar slope for 7 days at 26°C. The spores obtained were removed by washing with sterile distilled water and the suspension of spores obtained was used to inoculate 100 ml. of a culture medium contained in a 500 ml. conical flask. The flask and its contents had previously been sterilised with steam under pressure in an autoclave. The culture medium used was one having the following composition:

	Parts by weight
Water	100
Corn steep liquor	8
"Liquid glucose"	6

and the pH of the medium had been adjusted to a value of 5.2—5.3 by the addition of a solution of sodium hydroxide. The "liquid glucose" used was a mixture of carbohydrates consisting essentially of maltose, glucose and low molecular weight dextrins. The inoculated flask was shaken for 48 hours at a constant temperature of 26°C. on a rotary shaking machine having a throw of 3.4 cms. and operating at 250 r.p.m. At the end of this period of 48 hours a substantial growth of mycelium had been obtained in the flask. The resulting culture was then used to inoculate a synthetic fermentation medium without the addition of an added precursor. The fermentation used had the following composition:

	Parts by weight
Water	100.0
Lactose	4.0
Glucose	2.0
Ammonium lactate	0.5
Ammonium acetates	0.3
KH_2PO_4	0.3
Na_2SO_4	0.05
$FeSo_4.7H_2O$	0.01
$MgSO_4.7H_2O$	0.025
$ZnSO_4.7H_2O$	0.002
$MnSO_4$	0.002
$CaCl_2.2H_2O$	0.005
$CuSO_4.5H_2O$	0.0005
$CaCO_3$	1.0

The pH of the fermentation medium was about 6. The fermentation was carried out in a flask on a shaking machine at 26°C.

At the end of the 96 hours' fermentation period, the mycelium obtained was filtered from the fermentation broth and the fermentation liquor obtained as the filtrate was acidified to pH 3 with phosphoric acid and extracted once with half its volume of butyl acetate at 5°C. which removed most of the penicillins which it contained.

The extracted fermentation liquor was neutralised with sodium hydroxide solution and assayed by means of the cup plate method described by N. G. Heatley in *Biochem. J.*, *38*, 61 (1944), using *B. subtilis* as the bacterium.

A 50 ml. portion of the extracted fermentation liquor was brought to pH 8 by the addition of solid sodium bicarbonate and stirred at 0°C. while a solution of 0.5 gm. of phenoxyacetyl chloride in acetone was added in the course of a few minutes. The mixture was stirred at 0°C. for one hour, filtered, and excess reagent was removed by extraction

with three portions of ether. The ether extracts were themselves washed with water and the washings added to the main aqueous solution which was then readjusted to pH 6 to 7 by the addition of hydrochloric acid.

Assay of the aqueous solution obtained (which had a volume of 65 ml.) using the method referred to above, showed that it contained a material having considerable antibiotic activity which was about 11 times greater for the whole volume of liquid than that of the initial solution. The results obtained from the assay were as follows:

	Activity (International Units)
Extracted fermentation liquid (volume 50 ml.)	650
Reaction product (volume 65 ml.)	7,150

By means of paper chromatography it was shown that the antibiotic material contained in the aqueous solution had an Rf value of the same order as penicillin V and the stability of the aqueous solution at pH 2 also indicated a resemblance to that of penicillin V.

EXAMPLE 2.

Example 1 was repeated using phenylacetyl chloride in place of phenoxyacetyl chloride.

Both the extracted fermentation liquor and the aqueous solution of the reaction product were assayed by the method described in Example 3 with the following results:

	Activity (International Units)
Extracted fermentation liquor (volume 50 ml.)	350
Reaction product (volume 65 ml.)	8,125

It will be seen that the activity was as much as 23 times greater after the reaction with phenylacetyl chloride than before.

By means of paper chromatography it was shown that the antibiotic material contained in the aqueous solution had an Rf value of the same order as penicillin G and the stability of the aqueous solution at pH 2 also indicated a resemblance to that of penicillin G.

EXAMPLE 3.

Example 1 was repeated using benzoyl chloride in place of phenoxyacetyl chloride. Both the extracted fermentation liquor and the aqueous solution of the reaction product were assayed by the method described in Example 3, with the following results:

	Activity (International Units)
Extracted fermentation liquor (volume 50 ml.)	350
Reaction product (volume 70 ml.)	875

It will be seen that the activity was 2.5 times greater after the reaction with benzoyl chloride than before.

EXAMPLE 4.

Example 2 was repeated using a fermentation medium based upon peanut meal instead of upon the synthetic material. The fermentation medium used had the following composition:

	Parts by weight
Water	100.0
Lactose	4.0
Peanut meal	3.0
Na_2SO_4	0.1
$CaCO_3$	1.0.

Both the extracted fermentation liquor and the aqueous solution of the reaction product were assayed by the method described in Examples 1 with the following results:

	Activity (International Units)
Extracted fermentation liquor (volume 50 ml.)	700
Reaction product (volume 56 ml.)	7,840

It will be seen that the activity was 11 times greater after the reaction with phenylacetyl chloride than before.

EXAMPLE 5.

Example 4 was repeated using α-naphthylacetyl chloride in place of phenylacetyl chloride.

Both the extracted fermentation liquor and the aqueous solution of the reaction product were assayed by the method described in Example 1 with the following results:

	Activity (International Units)
Extracted fermentation liquor (volume 50 ml.)	700
Reaction product (volume 56 ml.)	4,032

The results show that the activity was more than 5 times as great after the reaction with α-naphthylacetyl chloride as before the reaction.

EXAMPLE 6.

Example 4 was repeated using β-naphthoxyacetyl chloride in place of phenylacetyl chloride.

The results of assay by the method of Example 3 show that the activity after the reaction with the β-naphthoxyacetyl chloride was more than 5 times greater than before reaction.

	Activity (International Units)
Extracted fermentation liquor (volume 50 ml.)	700
Reaction product (volume 66 ml.)	4,092

EXAMPLE 7.

Example 4 was repeated using α-chlorophenylacetyl chloride in place of phenylacetyl chloride.

Both the extracted fermentation liquor and the aqueous solution of the reaction product were assayed by the method described in Example 1 with the following results:

	Activity (International Units)
Extracted fermentation liquor (volume 50 ml.)	700
Reaction product (volume 62 ml.)	5,332

The results show that the activity was more than 7 times as great after reaction with the α-chlorophenylacetyl chloride as before the reaction.

EXAMPLE 8.

This Example describes the preparation of phenoxymethylpenicillin (penicillin V) and is typical of the reaction of isolated 6-amino-

penicillanic acid with monocarboxylic acid chlorides.

A solution of phenoxyacetyl chloride (360 mgm.) in dry acetone (5 ml.) was added dropwise during 10 minutes to a stirred solution of 6-aminopenicillanic acid (450 mgm., approx. 75% pure) in 3% aqueous sodium bicarbonate (18 ml.) and acetone 12 ml.). When addition was complete the mixture was stirred at room temperature for 30 minutes and then extracted with ether (30 ml. in 3 portions), only the aqueous phase being retained. This aqueous solution was covered with butanol (5 ml.) and adjusted to pH 2 by the addition of N hydrochloric acid. After separating the layers, the aqueous phase was extracted with 2.5 ml. portions of butanol, adjusting to pH 2 each time. The combined butanol solutions (which at this stage contained the free penicillin acid) were washed with water (3 × 2 ml.) and then shaken with water (10 ml.) to which sufficient 3% sodium bicarbonate solution was added to bring the aqueous phase to pH 7. The butanol solution was further extracted with two 5 ml. portions of water to each of which was added enough bicarbonate solution to produce an aqueous phase of pH 7. The combined aqueous solutions were washed with ether (20 ml.) and then evaporated at low temperature and pressure to leave the crude sodium salt of phenoxymethyl penicillin which, after drying in a vacuum desiccator, was obtained as a slightly hygroscopic powder (591 mgm.).

The purity of the product was estimated by the penicillinase assay as 73% and, by bioassay, as 68%. In its chromatographic behaviour and its antibacterial spectrum the product showed no significant difference from authentic phenoxymethyl penicillin. It also exhibited the relative stability towards acids which is characteristic of this particular penicillin. No loss of activity could be detected after 2 hours at pH 2.

EXAMPLE 9.

This Example describes the preparation of δ-carboxybutyl penicillin and is typical of the reaction of isolated 6-aminopenicillanic acid with a dicarboxylic acid chloride. The proportion of reagents and the mode of addition were designed to favour formation of a mono- rather than a bis-derivative of adipic acid.

Two separate solutions, one containing 6-aminopenicillanic acid (500 mgm.) in 3% aqueous sodium bicarbonate solution (6.4 ml.) and the other adipyl chloride (420 mgm.) in dry acetone (6 ml.) were added at equal rates over a period of 10 minutes to a stirred mixture of 3% sodium bicarbonate solution (25 ml.) and acetone (25 ml.). The mixture was stirred at room temperature for 30 minutes, then extracted with ether (60 ml. in 3 portions) and the extracts discarded. The product was isolated by extracting the free penicillin acid into butanol and then re-extracting into water at pH 7 as described in Example 8. The yield of dry sodium salt of δ-carboxybutyl penicillin was 445 mgm. which was estimated by the manometric penicillinase assay to be 52% pure. When subjected to paper chromatography the product gave only a single zone of antibiotic activity. The percentage remaining after 2 hours at pH 2 was less than 25%.

EXAMPLE 10.

This Example describes the preparation of ethyl penicillin and is typical of the reaction of isolated 6-aminopenicillanic acid with a simple carboxylic acid anhydride.

6-Aminopenicillanic acid (400 mgm.) was acylated as described in Example 8 except that the reagent used was propionic anhydride (140 mgm.). After working up as in Example 8 the yield of crude sodium salt of ethyl penicillin was 260 mgm., which was estimated by manometric penicillinase assay to be 53% pure. The percentage remaining after 2 hours at pH 2 was less than 3%.

EXAMPLE 11.

This Example describes the preparation of carbobenzoxyaminomethyl penicillin and is typical of the reaction of isolated 6-aminopenicillanic acid with an unstable mixed anhydride.

A solution of N-carbobenzoxy glycine (450 mgm.) and triethylamine (0.3 ml.) in dry acetone (10 ml.) was stirred and cooled to −5°C. A solution of ethyl chlorocarbonate (0.2 ml.) in dry acetone (2 ml.) was added dropwise with continued cooling and stirring and after 10 minutes the resulting mixture (containing N-carbobenzoxy glycine ethoxyformic anhydride and suspended triethylamine hydrochloride) was cooled to −50°C. and added slowly to a stirred ice-cold mixture prepared from 6-aminopenicillanic acid (430 mgm.), 3% sodium bicarbonate solution (17 ml.) and acetone (5 ml.). The mixture was allowed to attain room temperature and kept thus for 30 minutes with continued stirring. The product was then isolated as described in Example 8, to give the crude sodium salt of carbobenzoxyaminomethyl penicillin (469 mgm.) (purity 63%). The percentage remaining after 2 hours at pH 2 was 94%.

EXAMPLE 12.

This Example describes the preparation of benzyloxy penicillin and is typical of the reaction of isolated 6-aminopenicillanic acid with an ester of chloroformic (chlorocarbonic) acid.

6-Aminopenicillanic acid (500 mgm.) was treated as described in Example 8 except that the reagent was benzyl chloroformate (430 mgm.) and the mixture was stirred for 100 minutes at room temperature before being worked up in the usual way. The yield of the very hygroscopic crude sodium salt of benzyloxypenicillin was 445 mgm. (purity

90%). The percentage remaining after 2 hours was 71%.

EXAMPLE 13.

This Example describes the preparation of 6-*p*-toluenesulphonamidopenicillanic acid and is typical of the reaction of isolated 6-aminopenicillanic acid with a sulphonic acid chloride.

6-Aminopenicillanic acid (400 mgm.) was treated as described in Example 8 except that the reagent was *p*-toluenesulphonyl chloride (0 mgm.). The yield of crude sodium 6-*p*-toluenesulphonamidopenicillanate was 402 mgm. (purity 57%). The percentage remaining after 2 hours at pH 2 was 77%.

EXAMPLE 14.

This Example describes the preparation of α-naphthyl methyl penicillin and is typical of the acylation of a concentrated 6-aminopenicillanic acid brew.

α-Naphthylacetyl chloride (13 gm.) dissolved in acetone (90 ml.) was added over several minutes to a stirred mixture of the concentrate (400 ml., containing about 400 mgm. of 6-aminopenicillanic acid) and sodium bicarbonate (10 gm.). The mixture was stirred for 30 minutes at room temperature and then extracted with ether, the extracts being discarded. The aqueous phase was cooled to 0°C., covered with *n*-butanol (80 ml.) (or butyl acetate could be used) and brought to pH 2 by adding hydrochloric acid with stirring. The layers were separated and the aqueous phase was further extracted with butanol (2 × 40 ml.). The combined butanol extracts were then shaken with water (80 ml.) and sufficient 3% aqueous sodium bicarbonate so that, after shaking, the aqueous phase was at pH 7. The layers were separated and the solvent phase was extracted twice more with water (50 ml.) and sufficient 3% sodium bicarbonate to bring the aqueous phase to pH 7. The combined aqueous solutions were washed with ether (100 ml.) and then evaporated at low temperature and pressure. Final drying in a vacuum desiccator left the very crude sodium salt of α-naphthylmethyl penicillin as a very deliquescent yellow solid (4.8 gm.). It was estimated by manometric penicillinase assay to be 9% pure. The percentage remaining after 2 hours at pH 2 was 31%.

EXAMPLE 15.

The sodium salt of methylpenicillin was obtained by repeating the process of Example 8 but using acetyl chloride instead of phenoxyacetyl chloride. The yield of crude sodium salt (purity 32%) was 27 mgm. per 100 mgm. of 6-aminopenicillanic acid.

EXAMPLE 16.

The sodium salt of 1-propenylpenicillin was obtained by repeating the process of Example 8 but using crotonyl chloride instead of phenoxyacetyl chloride. The yield of crude sodium salt (purity 29%) was 77 mgm. per 100 mgm. of 6-aminopenicillanic acid. The percentage remaining after 2 hours at pH 2 was 24%.

EXAMPLE 17.

The sodium salt of chloromethylpenicillin was obtained by repeating the process of Example 8 but using chloroacetyl chloride instead of phenoxyacetyl chloride. The yield of crude sodium salt (purity 77%) was 79 mgm. per 100 mgm. of 6-aminopenicillanic acid. The percentage remaining after 2 hours at pH 2 was 80%.

EXAMPLE 18.

The sodium salt of dichloromethylpenicillin was obtained by repeating the process of Example 8 but using dichloroacetyl chloride instead of phenoxyacetyl chloride. The yield of crude sodium salt (purity 31%) was 80 mgm. per 100 mgm. of 6-aminopenicillanic acid. The percentage remaining after 2 hours at pH 2 was 100%.

EXAMPLE 19.

The sodium salt of bromomethylpenicillin was obtained by the process of Example 8 but using bromoacetyl chloride instead of phenoxyacetyl chloride. The yield of crude sodium salt (purity 69%) was 107 mgm. per 100 mgm. of 6-aminopenicillanic acid. The percentage remaining after 2 hours at pH 2 was 95%.

EXAMPLE 20.

The sodium salt of cyclohexylpenicillin was obtained by the process of Example 8 but using hexahydrobenzoyl chloride instead of phenoxyacetyl chloride. The yield of crude sodium salt (purity 70%) was 76 mgm. per 100 mgm. of 6-aminopenicillanic acid.

EXAMPLE 21.

The sodium salt of phenylpenicillin was obtained by the process of Example 8 but using benzoyl chloride instead of phenoxyacetyl chloride. The yield of crude sodium salt (purity 69%) was 109 mgm. per 100 mgm. of 6-aminopenicillanic acid. The percentage remaining after 2 hours at pH 2 was less than 15%.

EXAMPLE 22.

The sodium salt of benzylpenicillin was obtained by the process of Example 8 but using phenylacetyl chloride instead of phenoxyacetyl chloride. The yield of crude sodium salt (purity 70%) was 81 mgm. per 100 mgm. of 6-aminopenicillanic acid. The percentage remaining after 2 hours at pH 2 was less than 5%.

EXAMPLE 23.

The sodium salt of benzhydrylpenicillin was obtained by the process of Example 8 but using diphenylacetyl chloride instead of phenoxyacetyl chloride. The yield of crude sodium salt (purity 68%) was 111 mgm. per 100 mgm. of 6-aminopenicillanic acid. The percentage remaining after 2 hours at pH 2 was 65%.

EXAMPLE 24.

The sodium salt of styryl penicillin was obtained by the process of Example 8 using cinnamoyl chloride instead of phenoxyacetyl chloride. The yield of crude sodium salt (purity 24%) was 106 mgm. per 100 mgm. of 6-aminopenicillanic acid. The percentage remaining after 2 hours at pH 2 was less than 4%.

EXAMPLE 25.

The sodium salt of α-chlorobenzylpenicillin was obtained by the process of Example 8 using α-chlorophenylacetyl chloride instead of phenoxyacetyl chloride. The yield of crude sodium salt was 121 mgm. per 100 mgm. of 6-aminopenicillanic acid. The percentage remaining after 2 hours at pH 2 was 98%.

EXAMPLE 26.

The sodium salt of phenylthiomethylpenicillin was obtained by the process of Example 8 using phenylthioacetyl chloride instead of phenoxyacetyl chloride. The yield of crude sodium salt (purity 72%) was 150 mgm. per 100 mgm. of 6-aminopenicillanic acid. The percentage remaining after 2 hours at pH 2 was 82%.

EXAMPLE 27.

The sodium salt of *m*-sulphophenylpenicillin was obtained by the process of Example 8 using *m*-sulphenobenzoyl chloride instead of phenoxyacetyl chloride. The yield of crude sodium salt (purity 42%) was 62 mgm. per 100 mgm. of 6-aminopenicillanic acid. The percentage remaining after 2 hours at pH 2 was 39%.

EXAMPLE 28.

The sodium salt of α-furylpenicillin was obtained by the process of Example 8 using α-furoyl chloride instead of phenoxyacetyl chloride. The yield of crude sodium salt (purity 51%) was 101 mgm. per 100 mgm. of 6-aminopenicillanic acid. The percentage remaining after 2 hours at pH 2 was 34%.

EXAMPLE 29.

The sodium salt of β-naphthoxymethylpenicillin was obtained by the process of Example 14 using β-naphthoxyacetyl chloride instead of α-naphthylacetyl chloride. The yield of crude sodium salt (purity 7%) was 1.9 gm. per 100 ml. of concentrated brew. The percentage remaining after 2 hours at pH 2 was 94%.

EXAMPLE 30.

The sodium salt of *p*-nitrophenoxymethylpenicillin was obtained by the process of Example 14 using *p*-nitrophenoxyacetyl chloride instead of α-naphthylacetyl chloride. The yield of crude sodium salt (purity 6%) was 1.5 gm. per 100 ml. of concentrated brew. The percentage remaining after 2 hours at pH 2 was 78%.

The free penicillins can be obtained from the sodium salts in each case by acidification.

In the foregoing Examples the sodium salt has been prepared but it is to be understood that, if desired, other salts, e.g. potassium and calcium salts, may be obtained by similar means.

The growth-inhibiting properties of some of the products of the Examples are shown in the following Table.

Example	Limiting dilutions for inhibition of bacterial growth* (1 in:—)				
	E. coli	P. vulgaris	S. typhi	Staph. aureus	B. subt.
8	8,000	20,000	16,000	80 M	20 M
9	4,000	8,000	160,000	800,000	800,000
10	40,000	20,000	80,000	4 M	2 M
11	4,000	8,000	16,000	8 M	4 M
12	8,000	16,000	20,000	20 M	400,000
13	4,000	8,000	4,000	800,000	400,000
14	2,000	2,000	2,000	40 M	40 M
15	20,000	8,000	40,000	2 M	800,000
16	16,000	8,000	40,000	2 M	1,600,000
17	20,000	4,000	80,000	4 M	800,000

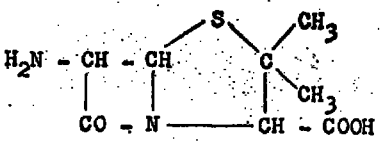
Example	Limiting dilutions for inhibition of bacterial growth* (1 in:—)				
	E. coli	P. vulgaris	S. typhi	Staph. aureus	B. subt.
18	16,000	4,000	80,000	8 M	1,600,000
19	1,600	1,600	40,000	2 M	1,600,000
20	<4,000	4,000	20,000	16 M	2 M
21	<4,000	20,000	4,000	8 M	800,000
22	200,000	200,000	400,000	40 M	40 M
23	8,000	4,000	8,000	40 M	—
24	<4,000	<4,000	<4,000	1,600,000	800,000
25	16,000	8,000	80,000	40 M	8 M
26	16,000	20,000	160,000	160 M	20,000
27	<8,000	<8,000	8,000	320,000	32,000
28	4,000	20,000	8,000	1,600,000	40,000
29	200	2,000	200	10 M	2 M
30	2,000	2,000	2,000	4 M	4 M

* Not corrected for purity.

The penicillins described in Examples 5, 6, 7, 9 to 20, 23, 25, 27 and 28 are new substances.

WHAT WE CLAIM IS:—

1. A process for the preparation of 6-acylamino derivatives of penicillanic acid in which 6-aminopenicillanic acid having the structural formula:



or a salt thereof is reacted with a carboxylic acid chloride or bromide, a sulphonic acid chloride, an ester of chlorocarbonic acid, an acid anhydride of a carboxylic acid or a mixed acid anhydride derived from a carboxylic acid.

2. A process for the preparation of derivatives of 6-aminopenicillanic acid wherein 6-aminopenicillanic acid or a salt thereof contained in the fermentation liquor obtained by growing a penicillin-producing mould in a nutrient medium in accordance with the process of Application No. 26957/60 (Serial No. 870,396) is reacted with a carboxylic acid chloride or bromide, a sulphonic acid chloride, an ester of chlorocarbonic acid, an anhydride of a carboxylic acid or a mixed anhydride derived from a carboxylic acid, whereby a 6-acylamino derivative of penicillanic acid is produced.

3. A process for the preparation of derivatives of 6-aminopenicillanic acid according to claim 2, but in which the 6-aminopenicillanic acid or salt thereof is contained in fermentation liquor which has been partially concentrated and/or purified before the reaction.

4. A process as claimed in claim 1 wherein the 6-aminopenicillanic acid or the salt thereof used is obtained by a process which comprises the isolation of 6-aminopenicillanic acid or a salt thereof from a fermentation liquor of the kind specified in claim 2.

5. A process as claimed in claim 2 or claim 3 wherein any penicillins present in the fermentation liquor are removed prior to the reaction by acidifying the fermentation liquor and extracting with an organic solvent.

6. A process as claimed in any one of the

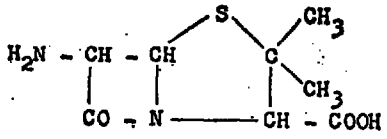
Example	Limiting dilutions for inhibition of bacterial growth* (1 in:—)				
	E. coli	P. vulgaris	S. typhi	Staph. aureus	B. subt.
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19	1,600	1,600	40,000	2 M	1,600,000
20	<4,000	4,000	20,000	16 M	2 M
21	<4,000	20,000	4,000	8 M	800,000
22	200,000	200,000	400,000	40 M	40 M
23	8,000	4,000	8,000	40 M	—
24	<4,000	<4,000	<4,000	1,600,000	800,000
25	16,000	8,000	80,000	40 M	8 M
26	16,000	20,000	160,000	160 M	20,000
27	<8,000	<8,000	8,000	320,000	32,000
28	4,000	20,000	8,000	1,600,000	40,000
29	200	2,000	200	10 M	2 M
30	2,000	2,000	2,000	4 M	4 M

* Not corrected for purity.

The penicillins described in Examples 5, 6, 7, 9 to 20, 23, 25, 27 and 28 are new substances.

WHAT WE CLAIM IS:—

1. A process for the preparation of 6-acylamino derivatives of penicillanic acid in which 6-aminopenicillanic acid having the structural formula:



or a salt thereof is reacted with a carboxylic acid chloride or bromide, a sulphonic acid chloride, an ester of chlorocarbonic acid, an acid anhydride of a carboxylic acid or a mixed acid anhydride derived from a carboxylic acid.

2. A process for the preparation of derivatives of 6-aminopenicillanic acid wherein 6-aminopenicillanic acid or a salt thereof contained in the fermentation liquor obtained by growing a penicillin-producing mould in a nutrient medium in accordance with the process of Application No. 26957/60 (Serial No. 870,396) is reacted with a carboxylic acid chloride or bromide, a sulphonic acid chloride, an ester of chlorocarbonic acid, an anhydride of a carboxylic acid or a mixed anhydride derived from a carboxylic acid, whereby a 6-acylamino derivative of penicillanic acid is produced.

3. A process for the preparation of derivatives of 6-aminopenicillanic acid according to claim 2, but in which the 6-aminopenicillanic acid or salt thereof is contained in fermentation liquor which has been partially concentrated and/or purified before the reaction.

4. A process as claimed in claim 1 wherein the 6-aminopenicillanic acid or the salt thereof used is obtained by a process which comprises the isolation of 6-aminopenicillanic acid or a salt thereof from a fermentation liquor of the kind specified in claim 2.

5. A process as claimed in claim 2 or claim 3 wherein any penicillins present in the fermentation liquor are removed prior to the reaction by acidifying the fermentation liquor and extracting with an organic solvent.

6. A process as claimed in any one of the

preceding claims wherein the reaction is carried out at a pH of from 6 to 9.

7. A process as claimed in any one of the preceding claims wherein the 6-aminopenicillanic acid or a salt thereof is reacted with phenacetyl chloride to produce benzyl penicillin.

8. A process as claimed in any one of claims 1 to 6 wherein the 6-aminopenicillanic acid or a salt thereof is reacted with phenoxyacetyl chloride to produce phenoxymethyl penicillin.

9. A process as claimed in any one of claims 1 to 6 wherein 6-aminopenicillanic acid or a salt thereof is reacted with α-naphthylacetyl chloride to produce α-naphthylmethyl penicillin.

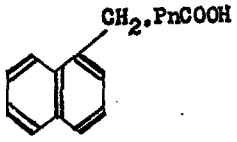
10. A process as claimed in any one of claims 1 to 6 wherein the 6-aminopenicillanic acid or a salt thereof is reacted wtih β-naphthoxyacetyl chloride to produce β-naphthoxymethylpenicillin.

11. A process as claimed in any one of claims 1 to 6 wherein the 6-aminopenicillanic acid or a salt thereof is reacted with α-chlorophenylacetyl chloride to produce α-chlorobenzylpenicillin.

12. A process for the preparation of derivatives of penicillanic acid substantially as described with reference to any one of Examples 1 to 7.

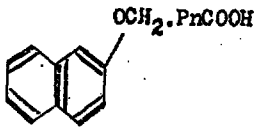
13. A process for the preparation of derivatives of penicillanic acid substantially as described with reference to any one of Examples 8 to 30.

14. α-Naphthylmethylpenicillin of the formula:



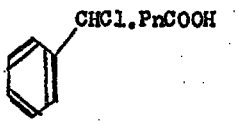
where Pn is as hereinbefore defined and salts thereof.

15. β-Naphthoxymethylpenicillin of the formula:



where Pn is as hereinbefore defined, and salts thereof.

16. α-Chlorobenzylpenicillin of the formula:



where Pn is as hereinbefore defined and salts thereof.

17. δ-Carboxybutylpenicillin of the formula:

$HOOC(CH_2)_4.PnCOOH$

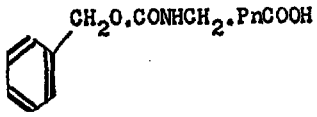
wherein Pn is as herein before defined and salts thereof.

18. Ethylpenicillin of the formula:

$C_2H_5.PnCOOH$

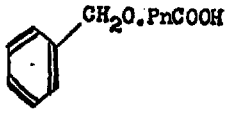
where Pn is as hereinbefore defined and salts thereof.

19. Carbobenzoxyaminomethylpenicillin of the formula:



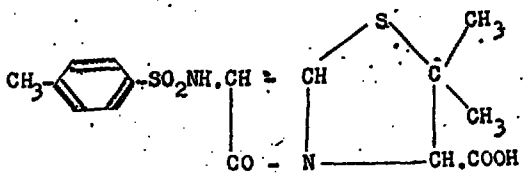
where Pn is as hereinbefore defined and salts thereof.

20. Benzyloxypenicillin of the formula:



where Pn is as hereinbefore defined and salts thereof.

21. 6-*p*-Toluenesulphonamidopenicillanic acid of the formula:



and salts thereof.

22. Methylpenicillin of the formula:

$CH_3.PnCOOH$

where Pn is as hereinbefore defined and salts thereof.

23. 1-Propenylpenicillin of the formula:

$CH_3CH=CH.PnCOOH$

where Pn is as hereinbefore defined and salts thereof.

24. Chloromethylpenicillin of the formula:

$ClCH_2.PnCOOH$

where Pn is as hereinbefore defined and salts thereof.

25. Dichloromethylpenicillin of the formula:

$Cl_2CH.PnCOOH$

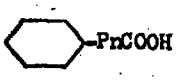
where Pn is as hereinbefore defined and salts thereof.

26. Bromomethylpenicillin of the formula:

$BrCH_2.PnCOOH$

where Pn is as hereinbefore defined and salts thereof.

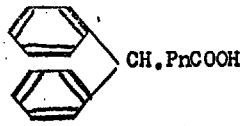
27. Cyclohexylpenicillin of the formula:



where Pn is as hereinbefore defined and salts thereof.

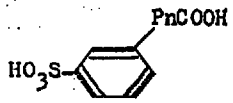
* C L H S *

28. Benzhydrylpenicillin of the formula:



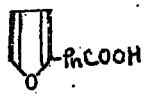
where Pn is as hereinbefore defined and salts thereof.

29. *m*-Sulphophenylpenicillin of the formula:



where Pn is as hereinbefore defined and salts thereof.

30. α-Furylpenicillin of the formula:



where Pn is as hereinbefore defined and salts thereof.

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

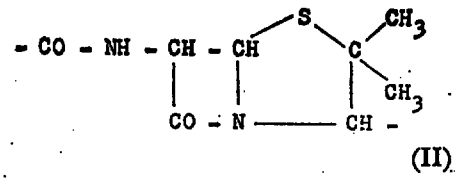
PROVISIONAL SPECIFICATION

Acyl Derivatives of 6-Aminopenicillanic Acid

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

This invention relates to the chemical modification of metabolites obtained from penicillin-producing moulds.

It is well known that substances having antibiotic activity can be obtained by growing certain moulds, for example moulds of the genus *Penicillium* such as *Penicillium chrysogenum* in the presence of a suitable nutrient medium. One group of these antibiotic substances are known as penicillins and have the general formula: R—Pn—COOH (I) in which Pn is a molecular grouping $C_9H_{10}O_2N_2S$ which is now generally agreed to have the following structure:



(II)

Previous work on penicillins had shown that the amount of antibiotic material obtained as a metabolite of a penicillin-producing mould was only a minor part of the total products obtained. Later it was found that the yield of penicillins could be increased very considerably by growing the mould in the presence of certain chemical compounds which became known as "precursors". These compounds appear to function by facilitating the introduction of the group R of formula I into the penicillin molecule and all the penicillins at present produced on a commercial scale are made using a precursor in this way.

The term "precursor" as used herein means a substance whose presence in a fermentation medium containing a penicillin-producing mould leads to an increase in the amount of penicillin produced.

It has now been found according to this invention that substances having antibiotic activity can be obtained by reacting with a suitable chemical reagent, the fermentation liquor obtained by growing a penicillin-producing mould in the absence of an added precursor.

The term "fermentation liquor" as used herein means the liquid material obtained from a fermentation reaction.

The present invention, therefore, provides a process for the preparation of antibiotic substances in which the fermentation liquor obtained by growing a penicillin-producing mould in the absence of an added precursor is reacted with one or more of the chemical reagents hereinafter specified.

Suitable penicillin-producing moulds include species of *Penicillium*, for example *Penicillium chrysogenum* 5120C. The mould is grown preferably under aerobic submerged culture conditions.

In general the chemical reagents which are most convenient for use in the process of this invention are reactive halogen compounds, for example carboxylic acid halides, especially carboxylic acid chlorides. Other reactive halogen compounds which can be used include halides of sulphonic acids and chlorocarbonic esters. Other chemical reagents which can be used include acid anhydrides.

Since some of the antibiotic substances obtained by the process of this invention are relatively unstable compounds which readily undergo chemical changes resulting in the loss of antibiotic activity, it is desirable to choose reaction conditions which are sufficiently moderate to avoid their decomposition. The reaction conditions chosen will, of course, depend largely upon the reactivity of the chemical reagent being used. In most instances, a compromise has to be made between the use of very mild conditions for a lengthy

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period and the use of more vigorous conditions for a shorter time with the possibility of decomposing some of the antibiotic substance.

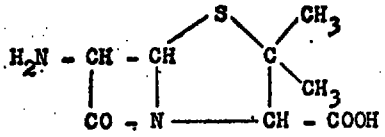
The temperature chosen for the process should in general not exceed 30°C. and the preferred temperature is from about 0°C. to about 15°C. Since the use of strongly acid or alkaline conditions in the process of this invention should be avoided, it has been found preferable to perform the process at a pH of from 6 to 9, and this can conveniently be achieved by using a buffer, for example a solution of sodium bicarbonate, or a sodium phosphate buffer.

The culture medium used to obtain the fermentation liquor used in the process of this invention can be one of the generally accepted media commonly used in the preparation of penicillins. The culture medium usually consists essentially of a carbohydrate nutrient material, for example glucose or lactose; calcium carbonate, sodium sulphate, and a nitrogenous material capable of providing the nitrogen necessary for the growth of the mould. The nitrogenous material can be either a natural substance, for example peanut meal, or it can be one or more chemical compounds containing nitrogen, for example ammonium salts such as ammonium lactate or ammonium acetate. Where one or more chemical compounds are used as the nitrogenous material it is usual to incorporate in the culture medium very small amounts of a number of metals such as calcium, iron, zinc, copper, magnesium and manganese and these are normally introduced in the form of an aqueous solution of their salts. A suitable culture medium containing ammonium salts as the nitrogenous material is described by Jarvis and Johnson, *J.A.C.S.*, *69*, 3010, (1947), and *J. Bact. 59*, 51 (1950). Natural nitrogenous materials such as peanut meal usually contain sufficient amounts of suitable inorganic salts and thus when such materials are used in the culture medium it is usually not necessary to make a separate addition of inorganic salts.

The fermentation conditions used in the preparation of the fermentation liquor used in this invention can vary between wide limits, but it has been found preferable to use conditions similar to those commonly used in the preparation of penicillin G. The temperature employed is preferably one from 20°C. to 35°C. and very satisfactory results have been obtained using a temperature of 25—27°C. The time required for the fermentation depends upon the culture medium and the mould used and the temperature at which the fermentation is carried out. Normal fermentation times are from 48 to 120 hours. The progress of the fermentation can be followed by means of periodic assay.

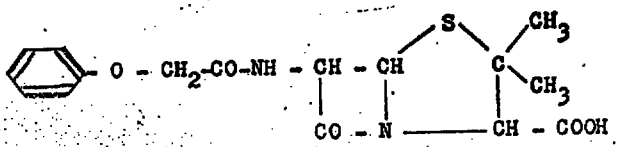
The fermentation liquor is obtained most satisfactorily when the fermentation is carried out under highly aerobic conditions. In the small scale operations referred to in the Examples of this specification, aerobic conditions were achieved by shaking the fermentation mixture on a rotary shaking machine. When working on a larger scale, aerobic conditions can conveniently be obtained either by bubbling air or oxygen through the fermentation mixture, or by rapidly stirring the fermentation mixture. If desired, a combination of stirring and the bubbling ~~or~~ air or oxygen can be used.

The nature of the substance or substances in the fermentation liquor which react with the chemical reagent in the process of this invention is not certain, but it is thought to contain a compound of the following structure:



(III)

which may be present in the form of a water-soluble salt. Evidence that the fermentation liquor contains a compound of this type is provided by Example 1 below which describes the reaction of a fermentation liquor with phenoxyacetyl chloride. The product obtained has antibiotic activity and acid stability similar to that shown by Penicillin V (IV) which is the product which would be expected from the reaction of the compound of formula III with phenoxyacetyl chloride.



(IV)

The production by the process of this invention of antibiotic material from fermentation liquor having little or no antibiotic activity is clearly indicated if, before the addition of the chemical reagent to the fermentation liquor, the penicillins already present as a result of the fermentation reaction by which the fermentation liquor was obtained are removed. This removal can readily be achieved by extracting the penicillins from the acidified fermentation liquor by means of an organic solvent, for example, butyl acetate,

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in which the penicillins are soluble. It will be seen from the Examples below that in some instances a very considerable increase in antibiotic activity was obtained as a result of the chemical modification of the fermentation liquor according to the process of this invention. Where phenylacetyl chloride was used as the chemical reagent, for example, the antibiotic activity obtained after the reaction was 23 times as great as that before the reaction.

The following Examples illustrate the invention. In the Examples, the chemical reagent was added to the fermentation liquor after the mycelium and other solid material had been removed by filtration. However, the process of this invention can, if desired, be carried out without this prior removal of mycelium and other solid material from the fermentation liquor.

EXAMPLE 1.

A strain of *Penicillium chrysogenum* (5120C obtained from Professor E. B. Chain, Istituto Superiore di Sanita, Rome) was grown on a glycerol-molasses agar slope for 7 days at 26°C. The spores obtained were removed by washing with sterile distilled water and the suspension of spores obtained was used to inoculate 100 ml. of a culture medium contained in a 500 ml. conical flask. The flask and its contents had previously been sterilised with steam under pressure in an autoclave. The culture medium used was one having the following composition:

	Parts by weight
Water	100
Corn steep liquor	8
"Liquid glucose"	6

and the pH of the medium had been adjusted to a value of 5.2—5.3 by the addition of a solution of sodium hydroxide. The "liquid glucose" used was a mixture of carbohydrates consisting essentially of maltose, glucose and low molecular weight dextrins. The inoculated flask was shaken for 48 hours at a constant temperature of 26°C. on a rotary shaking machine having a throw of 3.4 cms. and operating at 250 r.p.m. At the end of this period of 48 hours a substantial growth of mycelium had been obtained in the flask. The resulting culture was then used to inoculate a synthetic fermentation medium without the addition of an added precursor. The fermentation medium used had the following composition:

	Parts by weight
Water	100.0
Lactose	4.0
Glucose	2.0
Ammonium lactate	0.5
Ammonium acetate	0.3
KH_2PO_4	0.3
Na_2SO_4	0.05
$FeSO_4.7H_2O$	0.01
$MgSO_4.7H_2O$	0.025
$ZnSO_4.7H_2O$	0.002
$MnSO_4$	0.002
$CaCl_2.2H_2O$	0.005
$CuSO_4.5H_2O$	0.0005
$CaCO_3$	1.0

The pH of the fermentation medium was about 6. The fermentation was carried out in a flask on a shaking machine at 26°C.

At the end of the 96 hours' fermentation period, the mycelium obtained was filtered from the fermentation broth and the fermentation liquor obtained as the filtrate was acidified to pH 3 with phosphoric acid and extracted once with half its volume of butyl acetate at 5°C. which removed most of the penicillins which it contained.

The extracted fermentation liquor was neutralised with sodium hydroxide solution and assayed by means of the cup plate method described by N. G. Heatley in *Biochem. J.*, *38*, 61 (1944), using *B. subtilis* as the bacterium.

A 50 cc. portion of the extracted fermentation liquor was brought to pH 8 by the addition of solid sodium bicarbonate and stirred at 0°C. while a solution of 0.5 g. of phenoxyacetyl chloride in acetone was added in the course of a few minutes. The mixture was stirred at 0°C. for one hour, filtered, and excess reagent was removed by extraction with three portions of ether. The ether extracts were themselves washed with water and the washings added to the main aqueous solution which was then readjusted to pH 6—7 by the addition of hydrochloric acid.

Assay of the aqueous solution obtained (which had a volume of 65 cc.) using the method referred to above showed that it contained a material having considerable antibiotic activity which was about 11 times greater for the whole volume of liquid than that of the initial solution. The results obtained from the assay were as follows:

	Activity (International Units)
Extracted fermentation liquor (volume 50 cc.)	650
Reaction product (volume 65 cc.)	7,150

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By means of paper chromatography it was shown that the antibiotic material contained in the aqueous solution had an Rf value of the same order as penicillin V and the stability of the aqueous solution at pH 2 also indicated a resemblance to that of penicillin V.

EXAMPLE 2.

Example 1 was repeated using phenylacetyl chloride in place of phenoxyacetyl chloride.

Both the extracted fermentation liquor and the aqueous solution of the reaction product were assayed by the method described in Example 1 with the following results:

	Activity (International Units)
Extracted fermentation liquor (volume 50 cc.)	350
Reaction product (volume 65 cc.)	8,125

It will be seen that the activity was as much as 23 times greater after the reaction with phenylacetyl chloride than before.

By means of paper chromatography it was shown that the antibiotic material contained in the aqueous solution had an Rf value of the same order as penicillin G and the stability of the aqueous solution at pH 2 also indicated a resemblance to that of penicillin G.

EXAMPLE 3.

Example 1 was repeated using benzoyl chloride in place of phenoxyacetyl chloride. Both the extracted fermentation liquor and the aqueous solution of the reaction product were assayed by the method described in Example 1, with the following results:

	Activity (International Units)
Extracted fermentation liquor (volume 50 cc.)	350
Reaction product (volume 70 cc.)	875

It will be seen that the activity was 2.5 times greater after the reaction with benzoyl chloride than before.

EXAMPLE 4.

Example 2 was repeated using a fermentation medium based upon peanut meal instead of upon the synthetic material. The fermentation medium used had the following composition:

	Parts by weight
Water	100.0
Lactose	4.0
Peanut meal	3.0
Na_2SO_4	0.1
$CaCO_3$	1.0

Both the extracted fermentation liquor and the aqueous solution of the reaction product were assayed by the method described in Example 1 with the following results:

	Activity (International Units)
Extracted fermentation liquor (volume 50 cc.)	700
Reaction product (volume 56 cc.)	7,840

It will be seen that the activity was 11 times greater after the reaction with phenylacetyl chloride than before.

EXAMPLE 5.

Example 4 was repeated using α-naphthylacetyl chloride in place of phenylacetyl chloride.

But the extracted fermentation liquor and the aqueous solution of the reaction product were assayed by the method described in Example 1 with the following results:

* C L M S *

	Activity (International Units)
Extracted fermentation liquor (volume 50 cc.)	700
Reaction product (volume 56 cc.)	4,032

The results show that the activity was more than 5 times as great after the reaction with α-naphthylacetyl chloride as before the reaction.

EXAMPLE 6.

Example 4 was repeated using β-naphthoxyacetyl chloride in place of phenylacetyl chloride.

The results of assay by the method of Example 1 show that the activity after the reaction with the β-naphthoxyacetyl chloride was more than 5 times greater than before reaction.

	Activity (International Units)
Extracted fermentation liquor (volume 50 cc.)	700
Reaction product (volume 66 cc.)	4,092

EXAMPLE 7.

Example 4 was repeated using α-chlorophenylacetyl chloride in place of phenylacetyl chloride.

Both the extracted fermentation liquor and the aqueous solution of the reaction product were assayed by the method described in Example 1 with the following results:

	Activity (International Units)
Extracted fermentation liquor (volume 50 cc.)	700
Reaction product (volume 62 cc.)	5,332

The results show that the activity was more than 7 times as great after reaction with the α-chloro-phenylacetyl chloride as before the reaction.

The penicillins described in Examples 5, 6 and 7 are new substances.

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

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Published by The Patent Office, 25, Southampton Buildings, London, W.C.2, from which copies may be obtained.

ERRATA

SPECIFICATION No. 870,395

Page 3, line 96, *for* "acetates" *read* "acetate"
Page 4, line 64, *for* "Examples" *read* "Example"
Page 5, line 43, *for* "penicillin" *read* "Penicillin"
Page 7, line 1, *after* "hours" *insert* "pH2"
Page 8, line 30, *for* "sulphenobenzoyl" *read* "sulphobenzoyl"
Page 11, line 24, *for* "certan" *read* "certain"
Page 12, line 76, *for* "or air or" *read* "of air or"
Page 13, line 68, *for* "$CaCO_3$" *read* "C_nCO_3"

THE PATENT OFFICE
5th June 1964