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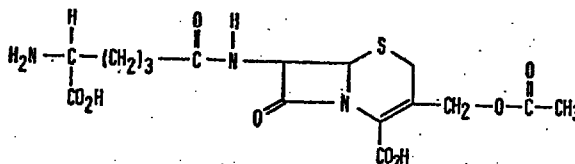
(54) A PROCESS FOR THE PREPARATION OF 7-AMINOCEPHALOSPORANIC ACID AND DERIVATIVES THEREOF

(71) We, BRISTOL-MYERS COMPANY, a corporation organized under the laws of the State of Delaware, United States of America, of 345 Park Avenue, New York, 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 the production of derivatives of cephalosporin C. In particular there are provided methods for the preparation of an acylated cephalosporin C and for the preparation of 7-aminocephalosporanic acid (7-ACA).

Recovery methods for cephalosporin C from fermentation broth are described in United States Patent Numbers 3,093,638 and 3,094,527, wherein the processes described involve adsorption of the cephalosporin C onto an adsorbant as compared to solvent extraction. While the use of (lower)alkyloxycarbonyl- or aryloxycarbonyl- cephalosporin C derivatives are not described as being formed "in situ" in cephalosporin C fermentation broth, or as being useful to aid in the extraction of cephalosporin C from its broth, or as being useful as a starting material in the production of 7-ACA, some of these compounds are described in the Patent literature (British Patent No. 1,014,883) as antibiotics. Several processes for the chemical cleavage of cephalosporin C or certain of its derivatives are described in the Patent literature (United States Patents Numbers 3,188,311; 3,234,223 and 3,124,576 and British Patent No. 1,041,985). None of these processes employ the use of N-(lower)alkyloxycarbonyl- cephalosporin C or a silyl ester thereof as a starting material.

7-Aminocephalosporanic acid (7-ACA) is a most valuable intermediate in the preparation of a multitude of semi-synthetic cephalosporanic acid antibacterial agents. In view of the impracticability of the total synthesis of 7-ACA on a large scale, commercial supplies of the compound are prepared by the chemical degradation of naturally occurring cephalosporanic acids, i.e., cephalosporin C, which is produced by fermentation. Most 7-ACA is derived from cephalosporin C (United States Patent 3,093,638) which has the structure



I.

The production of 7-ACA by currently available methods is fraught with difficulties from the fermentation to the chemical cleavage of cephalosporin C. Low yields of 7-ACA have made it difficult for cephalosporanic acids to take their rightful place in antibiotic therapy. For this reason the processes of the present invention are a significant improvement over the methods of the prior art.

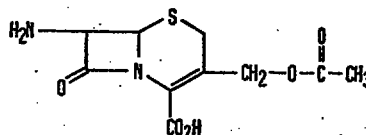
Cephalosporin C is characterized by an amino-acid function in its side chain. The amino-acid exists in the form of a zwitterion in aqueous solution and as such is very water soluble. Because of its highly ionic nature, it is extremely difficult to

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harvest by solvent extraction of the fermentation broth. The harvesting procedure currently used involves the adsorption of the crude cephalosporin C from the fermentation broth onto a suitable adsorbant, e.g., charcoal, or an ion exchange resin, followed by elution, concentration and precipitation at the isoelectric point or by salt formation (United States Patent No. 3,094,527). The low degree of efficiency coupled with the complexity of each step in this process combine to make it very difficult to manufacture 7-ACA.

The building block of the semi-synthetic cephalosporins is 7-ACA, whose structure is shown below:



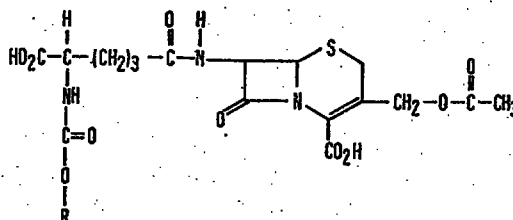
II.

Most 7-ACA is prepared by partial degradation of cephalosporin C or its derivatives by chemical means (United States Patents Numbers 3,124,576; 3,188,311 and 3,234,223).

Again, most of these methods provide commercial yields that are undesirably low. Furthermore, these methods invariably employ as the starting material a purified form of cephalosporin C which is difficult to obtain.

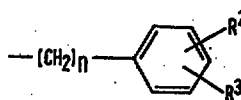
There is therefor a need for a process for improving on the harvestable yields of cephalosporin C from its fermentation broth in such a chemical form as to be directly useable in subsequent cleavage reactions to produce 7-ACA without substantial purification procedures.

This has now been achieved by the provision according to the present invention, of the process for the "in situ" preparation and harvesting of a derivative of cephalosporin C having the formula



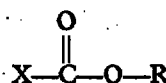
III

wherein R is (lower)alkyl but preferably ethyl, n-propyl, isopropyl, n-butyl or isobutyl; or a group of the formula



wherein n is an integer of 1 to 6 (R is phenethyl for example) and R² and R³ are alike or different and each is H, Cl, Br, F, NO₂, (lower)alkyl or (lower)alkoxy, but preferably hydrogen; which comprises the consecutive steps of:

(A) adding an acylating agent which is a haloformate having the formula



in which R is as defined above, and X is chloro, bromo or iodo to an acid-incubated fermentation broth preferably previously filtered, containing cephalosporin C, in a ratio of at least 2 moles of acylating agent per mole of cephalosporin C, but preferably in a ratio of from 2 to 10 moles of acylating agent per mole of cephalosporin C, and

most preferably 5 to 8 moles of acylating agent per mole of cephalosporin C (e.g. about 6 moles), at a pH of above 7, but preferably about 8, at a temperature no greater than 60°C. to form said derivative of cephalosporin C; and

(B) recovering said derivative of cephalosporin C, preferably by extraction using a water-immiscible organic solvent such as methyl isobutyl ketone, butanol, ethyl acetate, chloroform, methylene chloride or dichloroethane but preferably methyl isobutyl ketone at a pH of from 1 to 3, but preferably about pH 2.

The term "(lower)alkyl" for the purpose of the present invention is defined as an alkyl group comprised of 1 to 10 carbon atoms, including for example, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, t-butyl, and n-pentyl, but especially methyl, ethyl, n-propyl, isopropyl, n-butyl and isobutyl. The terms "(lower)alkoxy" and "halo(lower)alkyl" are also defined as moieties containing 1 to 10 carbon atoms.

The fermentation of the mold known as *Cephalosporium*, or an appropriate mutant thereof, produces a mixture of compounds, predominately cephalosporin C and lesser amounts of cephalosporin N (now known to be a penicillin). Cephalosporin C is acid stable while cephalosporin N is not. Accordingly, prior to the reaction of the fermentation broth with acylating agent (haloformate), it is desirable to destroy *in situ* any co-produced cephalosporin N. This is accomplished by acidification to about pH 2 with a mineral acid such as hydrochloric acid, sulfuric acid or phosphoric acid, followed by an incubation period of from 2 to 20 hours. The fermentation broth may be filtered before or after acidification and incubation.

Following this step, the filtrate of the fermentation broth containing the cephalosporin C is adjusted to from pH 7 to 9, but preferably about 8, by the addition of an alkali metal base such as sodium or potassium hydroxide.

The volume of the prepared broth is increased about 25% by the addition of acetone. The acylating agent is added to this solution with stirring in a ratio of at least 2, preferably from 2 to 10 moles of acylating agent per mole of cephalosporin C (broth concentration having been predetermined) but more preferably in a ratio of from 5 to 8 moles. The pH is held constant during the slow addition and for at least 30 minutes following the addition. The temperature of the broth is preferably maintained below 40°C. e.g. from -5°C to 20°C, but more preferably at 0°C. to 25°C. e.g. from 0°C to 5°C during this time. The reaction is usually complete one hour after the addition is completed.

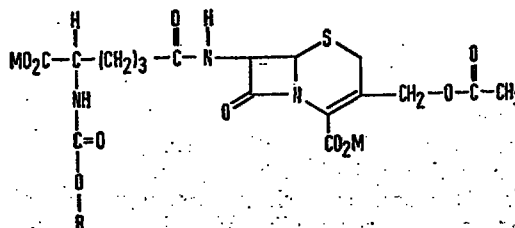
One-half volume of an immiscible organic solvent is added preferably methyl isobutyl ketone (MIBK), and the pH is adjusted to from 1 to 3, but preferably 2. The mixture is stirred and the organic solvent phase containing the cephalosporin C derivative III is collected.

The organic solution is concentrated *in vacuo* to about one-third its original volume at a temperature not to exceed 40°C. The concentrate is cooled to about 20°C. to 30°C. and the sodium salt of the cephalosporin derivative III is precipitated by the addition of a slight excess of sodium 2-ethylhexanoate dissolved in a solvent such as MIBK. The mixture is cooled to 0°C. to 5°C. for 3 to 4 hours and the solid sodium salt of derivative III is collected by filtration. The filter cake is washed with cold MIBK followed by an n-hexane washing. The product is dried *in vacuo* at about 25°C.

Another aspect of the invention is a process for the preparation of 7-ACA which is capable of using the compounds having formula III for instance when prepared by the above process without the necessity of preliminary purification or chemical conversion to cephalosporin C.

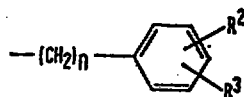
This has been achieved by the provision according to the present invention, of the process for the preparation of 7-aminocephalosporanic acid which comprises the consecutive steps of:

(A) mixing a compound having the formula

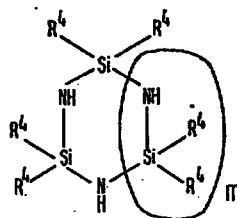


III

wherein each M is hydrogen, metal or an amine cation, and R is (lower)alkyl, but preferably ethyl, n-propyl, isopropyl, n-butyl and isobutyl; or an aralkyl group of the formula

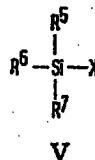


wherein n is an integer of 1 to 6 (R is phenethyl for example) and R^2 and R^3 are alike or different and each is H, Cl, Br, F, NO_2 , (lower)alkyl or (lower)alkoxy, but preferably hydrogen; with at least two equivalents of a silyl compound of the formula



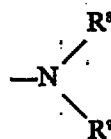
IV

or

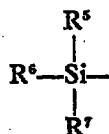


V

wherein R^5 , R^6 and R^7 are the same or different and are each hydrogen, halogen, (lower)alkyl, halo(lower)alkyl or phenyl, at least one of the said R^5 , R^6 and R^7 groups being other than halogen or hydrogen; R^4 is (lower)alkyl, m is an integer of 1 to 2 and X is halogen or



wherein R^8 is hydrogen or (lower)alkyl and R^9 is hydrogen, (lower)alkyl or



but preferably dimethyldichlorosilane or trimethylchlorosilane; under anhydrous conditions in the presence of an acid deactivating tertiary amine such as triethylamine, trimethylamine, dimethylaniline, quinoline, lutidine, or pyridine, in an inert solvent such as methylene chloride, dichloroethane, chloroform, tetrachloroethane, nitromethane, or diethylether, to produce the corresponding silyl di-ester of compound III.

(B) mixing said silyl di-ester with a halogenating agent such as phosphorus pentachloride, phosphorus pentabromide, phosphorus trichloride, phosphorus tribromide,

oxalyl chloride, p-toluenesulfonyl halide, phosphorus oxychloride, or phosgene, in a molar ratio of 2 or more moles of halogenating agent per mole of silyl ester (e.g. 2 to 4 moles), preferably 2 to 3 moles, but more preferably about 2 moles, under anhydrous conditions in an inert solvent such as methylene chloride, dichloroethane, chloroform, tetrachloroethane, nitromethane, or diethyl ether in the presence of an acid deactivating tertiary amine such as triethylamine, dimethylaniline, quinoline, lutidine, or pyridine at a temperature below 0°C., but preferably from -10°C. to -60°C. and more preferably in the range of -40°C. to -60°C., to produce in solution the corresponding imino-halide;

(C) mixing with said solution of imino-halide an alcohol which may be an aliphatic alcohol having 1 to 12 carbon atoms or a phenylalkyl alcohol having 1 to 7 alkyl carbon atoms, at a temperature below 0°C., but preferably -40°C. to -70°C. to produce in the solution the corresponding imino-ether; and

(D) mixing said solution of imino-ether under acidic conditions with water or an alcohol particularly an aliphatic alcohol, for example methanol, n-propanol, isopropanol or a mixture thereof or a mixture of both, to produce 7-aminocephalosporanic acid.

The process of the invention unexpectedly produces high yields under both laboratory and commercial scale conditions. The yields, which may be in the order of 50% to 70% are attributed to the use of silyl esters on the carboxyl groups of the compounds of formula III. The silyl esters may be prepared and hydrolyzed to the acid again without the loss of product, especially if the reaction proceeds at a temperature below -20°C., preferably -40°C. to -70°C., during formation of the imino-ether. Moreover, the use of silyl esters rather than the esters employed in the previously cited Patents, simplifies the process since the silyl ester hydrolyzes simultaneously with the splitting of the double bond of the imino-group and avoids the separate step of splitting the 4-carboxylic esters of the prior art processes.

The formation of the silyl ester is accomplished by reacting a silyl compound of formula IV or V, under anhydrous conditions, in an inert organic solvent, with the compound of formula III, or a salt thereof, in the presence of an acid deactivating tertiary amine.

Inert solvents which can be used include amongst others methylene chloride, dichloromethane, dichloroethane, chloroform, tetrachloroethane, nitromethane, benzene and diethyl ether.

Salts of compound III which can be used include amongst others alkali metal and alkaline earth metal salts such as potassium, sodium and calcium, and also aluminum salts. Also acceptable are ammonium and amine salts, preferably tertiary amines such as triethylamine, dibenzylamine, trimethylamine, N-methylmorpholine, pyridine, N-benzyl-β-phenethylamine, 1-phenamine, N,N'-dibenzylethylene-diamine, dehydroabiethylamine, and N-(lower)alkylpiperidines such as N-ethylpiperidine. Tertiary-amine salts are preferable.

Acid deactivating tertiary amines which can be used include amongst others triethylamine, trimethylamine, dimethylaniline, quinoline, lutidine, and pyridine. The quantity of acid deactivating amine used is preferably an amount equivalent to about 75% of the total acid generated in the process by the halogenating agent and the halosilane compound reacting with compound III.

Silyl compounds of formulas IV and V which can be used are: trimethyl chlorosilane, hexamethyl disilazane, triethyl chlorosilane, methyl trichlorosilane, dimethyl dichlorosilane, triethyl bromosilane, tri-n-propyl chlorosilane, bromomethyl dimethyl chlorosilane, tri-n-butyl chlorosilane, methyl diethyl chlorosilane, dimethyl ethyl chlorosilane, phenyl dimethyl bromosilane, benzyl methyl ethyl chlorosilane, phenyl ethyl methyl chlorosilane, triphenyl chlorosilane, triphenyl fluorosilane, tri-o-tolyl chlorosilane, tri-p-dimethylaminophenyl chlorosilane, N-ethyl triethyl silylamine, hexaethyl disilazane, triphenyl silylamine, tri-n-propyl silylamine, tetraethyl dimethyl disilazane, tetramethyl diethyl disilazane, tetramethyl diphenyl disilazane, hexaphenyl disilazane, and hexa-p-tolyl disilazane. The same effect is produced by hexa-alkylcyclotrisilazanes or octa-alkylcyclotetrasilazanes. Other suitable silylating agents are silylamides and silylureides such as trialkylsilylacetamide and a bis-trialkylsilylacetamide.

The imino compound is preferably an imino chloride or bromide which can be prepared by reacting the silyl ester of compound III with a halogenating agent such as phosphorus pentachloride, phosphorus pentabromide, phosphorus trichloride, phosphorus tribromide, oxalyl chloride, p-toluene sulfonic acid halide, phosphorus oxychloride, or phosgene, under anhydrous conditions in the presence of acid binding agents at temperatures below 0°C. such as -40° to -60°C.

A very important step for high yields of the process of the present invention is

the formation of the imino ether by reacting the imino halide under anhydrous conditions with a primary or secondary alcohol at temperatures below 0°C, preferably between -20° and -70°C., and more preferably about -40°C. to -70°C. Temperatures higher than -40°C. result in a substantial reduction in yield.

Alcohols which can be used for producing the imino ethers from the imino-halides are primary and secondary alcohols having the general formula $R^{10}OH$ in which R^{10} is (A) alkyl having 1 to 12 carbon atoms, preferably having 1 to 3 carbon atoms, such as methanol, ethanol, propanol, isopropanol, n-butanol, amyl alcohol and decanol; (B) phenylalkyl having 1 to 7 alkyl carbon atoms, such as benzyl alcohol, and 2-phenylethanol-1; (C) cycloalkyl, such as cyclohexylalcohol; (D) hydroxyalkyl having 2 to 12 carbon atoms, preferably at least 3 carbon atoms, such as 1,6-hexanediol; (E) alkoxyalkyl having 3 to 12 carbon atoms, such as 2-methoxyethanol, 2-isopropoxyethanol, 2-butoxyethanol; (F) aryloxyalkyl, having 3 to 7 carbon atoms in the aliphatic chain, such as 2-p-chlorophenoxyethanol; (G) aralkoxyalkyl, having 3 to 7 carbon atoms in the aliphatic chain, such as 2-(p-methoxybenzyloxy)-ethanol; (H) hydroxy-alkoxyalkyl, having 4 to 7 carbon atoms, such as diglycol. Also mixtures of these alcohols are suitable for forming the imino ethers.

After formation of the imino-ether from the imino-halide, the imino bond must be split to produce 7-ACA. The process is accomplished by mild acidic hydrolysis or alcoholysis. If the quantity of acid deactivating tertiary amine present in the process is a quantity less than the acid produced by the silylation and halogenation, the cleavage will probably proceed simultaneously upon completion of the formation of the imino-ether. If however the quantity of acid deactivating amine was more than the acid produced, the cleavage step will require the careful addition of a quantity of H^+ to effect the cleavage. This step is carried out at a temperature of from +10°C. to -10°C. and preferably at about 0°C.

The 7-ACA is harvested from the reaction mixture by adjusting the pH of the mixtures to or near the isoelectric point of the 7-ACA, following which the 7-ACA crystallizes and is collected by filtration.

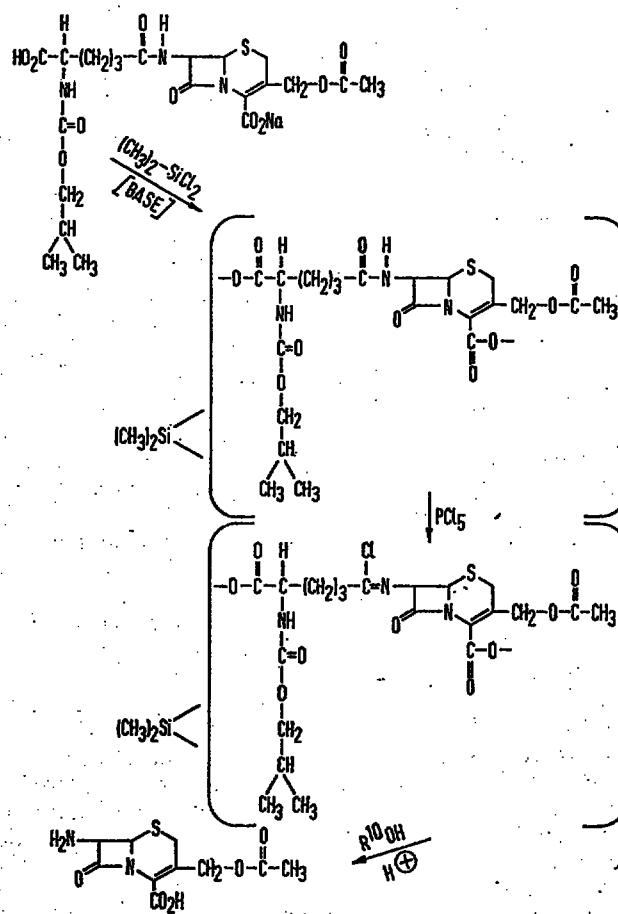
For optimum yields of 7-ACA, it is preferred to use high concentrations of the reactants. For example, in the formation of the silyl esters, a 20% to 30%, preferably 25% by weight of compound III is suspended in an inert organic solvent and a base for the results. The silane is employed preferably in excess, i.e., 10% to 60% above theoretical amounts.

One molecule of cephalosporin C derivative III possesses two reactive carboxyl groups capable of forming silyl esters. Therefore, in terms of the silylation reaction, one mole of III is equal to two equivalent weights.

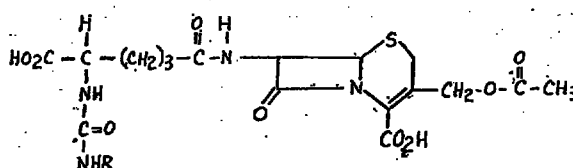
Accordingly, when compound III is treated with dichlorodimethylsilane, one molecule of compound III (two equivalent weights) is treated with at least one molecule (two equivalent weights) of dichlorodimethylsilane. Similarly, when compound III is treated with chlorotrimethylsilane, one molecule of compound III (two equivalent weights) is treated with at least two molecules (two equivalent weights) of chlorotrimethylsilane.

This enables the use of solvents which are not absolutely dry because trace amounts of water are removed therefrom by reacting with the excess silylating agent. Obviously the quantity of the silane compound required is dependent upon whether one or both carboxyl groups of the compound III are available for silyl ester formation. In a preferred embodiment the ratio of equivalents of silylating agent to compound III is from 1.2:1 to 2:1.

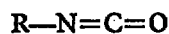
Thus if, as is the case in the reaction sequence set out below by way of illustration, the compound of formula III is a monosodium salt with the sodium on the carboxyl group of the nucleus (the side chain carboxyl group being in a free acid form) the silylating agent will react first with the carboxyl salt group on the nucleus. If there is insufficient of the silane compound, due for example to the presence of water, the free carboxyl group will remain unreacted and the reaction will proceed in this manner.



Our copending Application No. 8682/72 (Serial No. 1283223) describes and claims a process for the preparation of a derivative of cephalosporin C of the formula



wherein R is as defined for the compounds prepared by the process of this invention except that n is an integer of from 0 to 6, which process is analogous to the present process except that an isocyanate of formula



wherein R is as defined for the cephalosporin C derivatives resulting from the process, is employed as an acylating agent.

Also described and claimed in our copending Application No. 8682/72 (Serial No. 1283223) is a process for the preparation of 7-ACA from the aforementioned cephalosporin C derivatives in a process analogous to that of the present invention.

Embodiments of the invention will now be illustrated in the following Examples.

In these Examples no incubation period is specified since as is described above the fermentation broth is incubated when cephalosporin N is present. The fermentation broth used in these Examples does not necessarily contain cephalosporin N.

EXAMPLE 3

"In Situ" Preparation of Sodium N-Carbisobutoxycephalosporin C

The procedure of Example 1 was repeated substituting for the 5.9:1 molar ratio of isobutylchloroformate:cephalosporin C used therein a 4.5:1 molar ratio 10.4 kg. of product was produced. Assay of the spent broth after extraction indicated the presence of less than 14% cephalosporin C activity.

EXAMPLE 4

Preparation of 7-Aminocephalosporanic Acid

Sodium N-carbisobutoxycephalosporin C [13.5 g.] obtained from Example 3, 50 ml. of methylene chloride, 3 ml. of dimethylaniline and 3.67 ml. of triethylamine were mixed together. Dichlorodimethylsilane (6.2 ml.) was added with stirring at a temperature of 25°–29°C. The solution was stirred for 30 minutes, then cooled to –60°C. and 12.0 grams of PCl₅ in 100 ml. of methylene chloride was added. The temperature was maintained at –40°C. for 2 hours. The solution was cooled to –73°C. and a solution of 60 ml. methanol and 2.5 ml. dimethylaniline cooled to –78°C. was added. The temperature rose to –46°C. The temperature was held in the range of –45°C. to –50°C. for 2 hours. Fifty-five ml. of water warmed to 95°C. was added with stirring. The temperature rose to +5°C. After 4 minutes of stirring (pH 0.2), 22 ml. of NH₄OH was added over a 7 minute interval to pH 3.8. Stirring was continued for several days with cooling. The precipitated 7-ACA was collected by filtration, washed with 50 ml. methylene chloride, 40 ml. water, 50 ml. methanol, then 50 ml. acetone. The dried solid weighed 2.3 grams and was 95.4% 7-ACA.

EXAMPLE 5

"In Situ" Preparation of Sodium N-Carbethoxycephalosporin C

The procedure of Example 1 was repeated with substitution for the 5.9:1 molar ratio of isobutylchloroformate:cephalosporin C used therein of a 7.0:1 molar ratio of ethylchloroformate per mole of cephalosporin C. Sodium N-carbethoxycephalosporin C was produced.

EXAMPLE 6

7-ACA From Sodium N-Carbethoxycephalosporin C

Sodium N-carbethoxycephalosporin C (12.0 grams) 45 ml. methylene chloride, 3.47 ml. of triethylamine and 7.0 ml. of N,N-dimethylaniline was added to a 500 ml. flask. The slurry was stirred and 4.75 ml. of dichlorodimethylsilane was added dropwise at 25°–28°C. The reaction was held at ~25°C. for 45 minutes. The reaction was chilled to –60°C. and 10.8 grams of PCl₅ (0.052 moles) in 100 ml. of methylene chloride was added at such a rate as to maintain the temperature at below –40°C. This was followed by 11.7 ml. of N,N-dimethylaniline while maintaining the temperature at –40°C. The reaction was maintained at –40°C. for two hours. The reaction was cooled to –73°C. and 60 ml. of methanol containing 2.36 ml. of N,N-dimethylaniline at –78°C. was added in a quick pour to the reaction. The temperature rose to –43°C. The reaction was cooled to –50°C. and maintained at –50°C. for 2 hours. 45 ml. of water at +65°C. was added to the reaction and the reaction was warmed to +5°C. and stirred for 4 minutes. The pH was ≈0. Ten mls. of concentrated ammonium hydroxide was added dropwise over ~6 minutes (pH=1.6). The temperature was maintained at 0 to +5°C. with an ice bath. Five grams of ammonium carbonate in 10 ml. of water was added to the mixture (pH=3.0). The pH was then adjusted to 3.8 with 3.0 ml. of ammonium hydroxide. The crystallization mixture was stirred for two hours in an ice-bath and held in ice overnight for 16 hours. The crystals were filtered, washed with methylene chloride, 20 ml. of water, 50 ml. of methanol and acetone. The crystals were dried. 3.72 Grams yield=58.2%.

EXAMPLE 7

"In Situ" Preparation of Sodium N-Carbobenzycephalosporin C

In the procedure of Example 1 there was substituted for the 5.9:1 molar ratio of isobutylchloroformate:cephalosporin C used therein an 8.0:1 molar ratio of carbobenzychloride per mole of cephalosporin C. Sodium N-carbobenzycephalosporin C was produced.

EXAMPLE 8

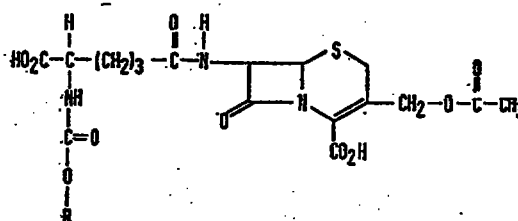
7-ACA From Sodium N-Carbobenzycephalosporin C

The procedure of Example 6 is repeated, except that the sodium N-carbethoxy-

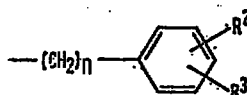
cephalosporin C used therein is replaced by an equivalent amount of sodium N-carboxybenzoylcephalosporin C, to produce 7-ACA.

WHAT WE CLAIM IS:—

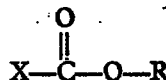
1. A process for the preparation of a derivative of cephalosporin C of the formula:



wherein R is (lower)alkyl or a group of the formula



wherein n is an integer of 1 to 6 and R² and R³ are the same or different and each is H, Cl, Br, F, NO₂, (lower)alkyl or (lower)alkoxy; which process comprises adding an acylating agent which is a haloformate of formula



wherein R is as defined above and X is chloro, bromo or iodo, to an acid-incubated fermentation broth containing cephalosporin C, in a ratio of at least 2 moles of acylating agent per mole of cephalosporin C, at a pH above 7 and at a temperature no greater than 60°C., and recovering said derivative of cephalosporin C from the reaction mixture.

2. A process as claimed in claim 1, wherein the acylating agent is added to a previously filtered and acid incubated fermentation broth in a ratio of from 2 to 10 moles per mole of cephalosporin C.

3. A process as claimed in claim 1 or claim 2, wherein the mole ratio of acylating agent to cephalosporin C is from 5:1 to 8:1.

4. A process as claimed in any one of the preceding claims, wherein the pH is from 7 to 9.

5. A process as claimed in any one of claims 1 to 3, wherein the pH is about 8.

6. A process as claimed in any one of the preceding claims wherein the temperature is below 40°C.

7. A process as claimed in claim 6, wherein the temperature is from 0°C. to 25°C.

8. A process as claimed in any one of the preceding claims, wherein R₃ is H.

9. A process as claimed in claim 8, wherein R_2 and R_3 are H.

10. A process as claimed in any one of claims 1 to 7, wherein R is ethyl, n-propyl, isopropyl, n-butyl or isobutyl.

11. A process as claimed in any one of the preceding claims, wherein the derivative is recovered by extraction with a water-immiscible organic solvent at a pH of from 1 to 3.

12. A process as claimed in claim 11, wherein the solvent is methyl isobutyl ketone, ethylacetate, butanol, chloroform, methylene chloride or dichloroethane.

13. A process as claimed in claim 11, wherein the recovery is at a pH of about 2 using methyl isobutyl ketone.

14. A process as claimed in any one of the preceding claims, wherein the haloformate is a chloroformate.

15. A process as claimed in any one of claims 1 to 7 or 10 to 14, wherein R is isobutyl and the acylating agent is isobutyl chloroformate.



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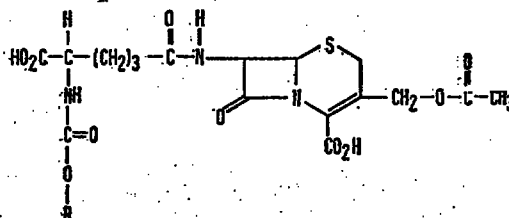
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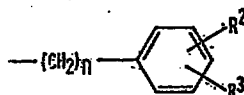
cephalosporin C used therein is replaced by an equivalent amount of sodium N-carbo-benzoxyccephalosporin C, to produce 7-ACA.

WHAT WE CLAIM IS:—

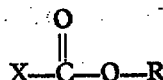
1. A process for the preparation of a derivative of cephalosporin C of the formula:



wherein R is (lower)alkyl or a group of the formula



wherein n is an integer of 1 to 6 and R² and R³ are the same or different and each is H, Cl, Br, F, NO₂, (lower)alkyl or (lower)alkoxy; which process comprises adding an acylating agent which is a haloformate of formula



wherein R is as defined above and X is chloro, bromo or iodo, to an acid-incubated fermentation broth containing cephalosporin C, in a ratio of at least 2 moles of acylating agent per mole of cephalosporin C, at a pH above 7 and at a temperature no greater than 60°C., and recovering said derivative of cephalosporin C from the reaction mixture.

2. A process as claimed in claim 1, wherein the acylating agent is added to a previously filtered and acid incubated fermentation broth in a ratio of from 2 to 10 moles per mole of cephalosporin C.

3. A process as claimed in claim 1 or claim 2, wherein the mole ratio of acylating agent to cephalosporin C is from 5:1 to 8:1.

4. A process as claimed in any one of the preceding claims, wherein the pH is from 7 to 9.

5. A process as claimed in any one of claims 1 to 3, wherein the pH is about 8.

6. A process as claimed in any one of the preceding claims wherein the temperature is below 40°C.

7. A process as claimed in claim 6, wherein the temperature is from 0°C. to 25°C.

8. A process as claimed in any one of the preceding claims, wherein R₃ is H.

9. A process as claimed in claim 8, wherein R₂ and R₃ are H.

10. A process as claimed in any one of claims 1 to 7, wherein R is ethyl, n-propyl, isopropyl, n-butyl or isobutyl.

11. A process as claimed in any one of the preceding claims, wherein the derivative is recovered by extraction with a water-immiscible organic solvent at a pH of from 1 to 3.

12. A process as claimed in claim 11, wherein the solvent is methyl isobutyl ketone, ethylacetate, butanol, chloroform, methylene chloride or dichloroethane.

13. A process as claimed in claim 11, wherein the recovery is at a pH of about 2 using methyl isobutyl ketone.

14. A process as claimed in any one of the preceding claims, wherein the haloformate is a chloroformate.

15. A process as claimed in any one of claims 1 to 7 or 10 to 14, wherein R is isobutyl and the acylating agent is isobutyl chloroformate.



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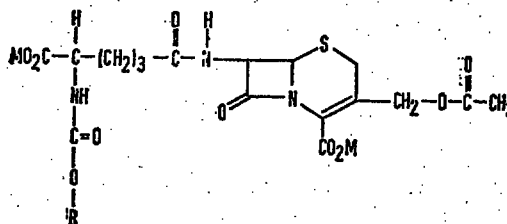
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16. A process as claimed in claim 1 substantially as hereinbefore described in any one of Examples 1, 3, 5, or 7.

17. A derivative of cephalosporin C of the formula I defined in claim 1 when prepared by a process as claimed in any one of the preceding claims.

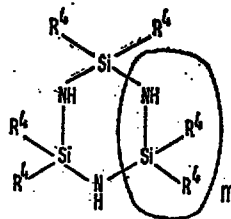
18. A process for the preparation of 7-aminocephalosporanic acid, which process comprises the steps of:

(A) mixing a compound having the formula

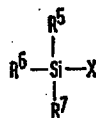


III

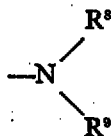
wherein each M is hydrogen or a metal or amine cation, and R is as defined in claim 1 with at least two equivalents of a silyl compound of the formula



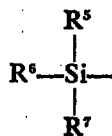
or



wherein R⁵, R⁶ and R⁷ are the same or different and are each hydrogen, halogen, (lower)alkyl, halo(lower)alkyl or phenyl, at least one of R⁵, R⁶ and R⁷ being other than halogen or hydrogen; R⁴ is (lower)alkyl, m is 1 or 2 and X is halogen or



wherein R⁸ is hydrogen or (lower)alkyl and R⁹ is hydrogen, (lower)alkyl or



under anhydrous conditions, in the presence of an acid deactivating tertiary amine in an inert solvent to produce the corresponding silyl diester of compound III;

(B) mixing said silyl diester with a halogenating agent in a molar ratio of 2 or more moles of halogenating agent per mole of silyl ester, under anhydrous conditions in an inert solvent in the presence of an acid deactivating tertiary amine at a temperature below 0°C., to produce in solution the corresponding iminoaldehyde;



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(C) mixing with said solution of imino-halide an alcohol at a temperature below 0°C., to produce in the solution the corresponding imino-ether; and

(D) mixing said solution of imino-ether under acidic conditions with water or an alcohol or a mixture of both to produce 7-aminocephalosporanic acid.

5 19. A process as claimed in claim 18, wherein the ratio of silylating agent to compound III is stoichiometric. 5

20. A process as claimed in claim 18 or claim 19, wherein the ratio of equivalents of silylating agent to compound III is from 1.2:1 to 2:1.

10 21. A process as claimed in any one of claims 18 to 20, wherein the deactivating tertiary amine in each stage is triethylamine, trimethylamine, dimethylaniline, quino- 10 line, lutidine or pyridine.

22. A process as claimed in any one of claims 18 to 21, wherein the inert solvent in each stage is methylene chloride, dichloroethane, chloroform, tetrachloroethane, nitromethane or diethylether.

15 23. A process as claimed in any one of claims 18 to 22, wherein the halogenating agent is phosphorus pentachloride, phosphorus pentabromide, phosphorus trichloride, phosphorus tribromide, oxalyl chloride, p-toluenesulfonyl halide, phosphorus oxy- 15 chloride or phosgene.

24. A process as claimed in any one of claims 18 to 23, wherein there is used a molar ratio of 2 to 4 moles of halogenating agent per mole of silyl ester. 20

25. A process as claimed in any one of claims 18 to 23, wherein the mole ratio of halogenating agent to silyl ester is 2:1 to 3:1.

26. A process as claimed in any one of claims 18 to 23, wherein the mole ratio of halogenating agent to silyl ester is 2:1.

25 27. A process as claimed in any one of claims 18 to 26, wherein the halogenation is carried out at a temperature of from -10°C. to -60°C. 25

28. A process as claimed in claim 27, wherein the halogenation is carried out at a temperature of from -40°C. to -60°C.

30 29. A process as claimed in any one of claims 18 to 28, wherein the alcohol in step (C) is an aliphatic alcohol having from 1 to 12 carbon atoms or a phenyl alkyl 30 alcohol having from 1 to 7 carbon atoms in the alkyl group.

30. A process as claimed in claim 29, wherein the aliphatic alcohol is methanol, ethanol, n-propanol or isopropanol.

35 31. A process as claimed in any one of claims 18 to 30, wherein the temperature in step (C) is from -20°C. to -70°C. 35

32. A process as claimed in claim 31, wherein the temperature in step (C) is from -40°C. to -70°C.

33. A process as claimed in any one of claims 18 to 32, wherein the alcohol in step (D) is an aliphatic alcohol.

40 34. A process as claimed in claim 33, wherein the aliphatic alcohol is methanol, 40 n-propanol or isopropanol or a mixture thereof.

35. A process as claimed in any one of claims 18 to 34, wherein step (D) is carried out at a temperature of from +10°C. to -10°C.

45 36. A process as claimed in any one of claims 18 to 34, wherein step (D) is carried out at a temperature of about 0°C. 45

37. A process as claimed in any one of claims 18 to 36, wherein R is ethyl, n-propyl, isopropyl, n-butyl or isobutyl, or phenethyl.

38. A process as claimed in any one of claims 18 to 36, wherein R is isobutyl.

39. A process as claimed in any one of claims 18 to 36, wherein R₃ is hydrogen.

50 40. A process as claimed in any one of claims 18 to 39, wherein the silyl compound is dimethyldichlorosilane or trimethylchlorosilane. 50

41. A process as claimed in any one of claims 18 to 40, wherein the compound III is produced by a process as claimed in any one of claims 1 to 16.

55 42. A process as claimed in claim 18 substantially as hereinbefore described in any one of Examples 2, 4, 6 or 8. 55

43. 7-Aminocephalosporanic acid when produced by a process as claimed in any one of claims 18 to 42.

44. A cephalosporin when produced from 7-aminocephalosporanic acid as claimed in claim 43 by acylation.

60 45. A process according to claim 1, wherein the acylating agent is added to a previously filtered and acid-incubated fermentation broth in a ratio of from 2 to 10 moles per mole of cephalosporin C, at a pH of from 7 to 9, at a temperature of from 0°C. to 25°C. and the derivative is recovered by extraction with a water-im- 60 miscible organic solvent at a pH of from 1 to 3.

65 46. A process according to claim 1 wherein R₃ is H, the mole ratio of acylating 65



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agent to cephalosporin C is from 5:1 to 8:1, the temperature is from 0°C. to 15°C. and the solvent is methyl isobutyl ketone, ethylacetate, butanol, chloroform, methylene chloride or dichloroethane.				
47. A process according to claim 1, wherein R is ethyl, n-propyl, isopropyl, n-butyl or isobutyl, R ₂ and R ₃ are H and about 6 moles of acylating agent are employed at a pH of about 8, at a temperature of from 0°C. to 5°C. and the recovery is at a pH of about 2 using methyl isobutyl ketone.	5	5		5
48. A process according to claim 18, wherein the ratio of equivalents of silylating agent to compound III is 1:1, the deactivating tertiary amine in each stage is triethylamine, trimethylamine, dimethylaniline, quinoline, lutidine or pyridine, the inert solvent in each stage is methylene chloride, dichloroethane, chloroform, tetrachloroethane, nitromethane or diethylether, the halogenating agent is phosphorus pentachloride, phosphorus pentabromide, phosphorus trichloride, phosphorus tribromide, oxalyl chloride, p-toluenesulfonyl halide, phosphorus oxychloride or phosgene, and is in a molar ratio of 2 to 4 moles of halogenating agent per mole of silyl ester, the temperature of the halogenation is from -10°C. to -60°C., the alcohol in the imino ether production stage is an aliphatic alcohol of from 1 to 12 carbon atoms or a phenyl alkyl alcohol of 1 to 7 alkyl carbon atoms and the temperature in that stage is from -20°C. to -70°C., and the alcohol in the acid production stage is an aliphatic alcohol, the temperature at that stage being about 0°C.	10	10		10
49. A process according to claim 18, wherein R is ethyl, n-propyl, isopropyl, n-butyl or isobutyl and R ^a is H, the ratio of equivalents of silylating agent to compound III is from 1.2:1 to 2:1, the halogenating agent is phosphorus pentachloride or phosphorus oxychloride and the mole ratio of halogenating agent to silyl ester is from 2:1 to 3:1, the temperature of halogenating agent is from -40°C. to -60°C., the alcohol in the imino-ether production stage is methanol, ethanol, n-propanol or isopropanol, the etherification is conducted at from -40°C. to -70°C., and the alcohol when used in the acid production stage is methanol, n-propanol or isopropanol or mixtures thereof, the temperature in the last stage being from -10°C. to +10°C.	15	15		15
50. A process according to claim 18, wherein R is ethyl, n-propyl, isopropyl, n-butyl or isobutyl, or phenethyl, the silyl compound is dimethyldichlorosilane or trimethylchlorosilane, the inert solvent in each stage is methylene chloride or dichloroethane and the halogenating agent is phosphorus pentachloride used in a molar ratio to silyl ester of 2:1.	20	20		20
	25	25		25
	30	30		30
	35	35		35
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