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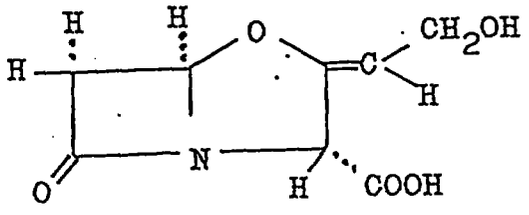
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☑ Description : paged ..1.... to paged ..8.....
☑ Claim(s) : paged ..9.... to paged ..12.....
☐ Drawing(s) : paged ..... to paged .....
☑ Abstract : paged ..13... to paged ..-.....
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NOVEL ALKYLENEDIAMMONIUM DICLAVULANATE DERIVATIVES, A PROCESS FOR THE PREPARATION THEREOF AS WELL AS THE USE THEREOF

IPC C 07 D 498/04, A 61 K 31/42

The present invention belongs to the field of pharmaceutical industry and relates to novel alkylenediammonium diclavulanate derivatives, to a process for the preparation thereof as well as to the use thereof as intermediate compounds for the preparation of clavulanic acid and of pharmaceutically acceptable alkali salts thereof, such as potassium clavulanate.

Clavulanic acid, (2R,5R,Z)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo-[3,2,0]-heptane-2-carboxylic acid, is a known compound of the following structure:



This compound as well as the salts and esters thereof are active as inhibitors of beta-lactamase, i.e. they inhibit beta-lactamases produced by gram-positive and gram-negative microorganisms. Hence clavulanic acid and its salts are used in galenic preparations in order to inhibit the inactivation of beta-lactam antibiotics. Commercial preparations contain a stable potassium salt of clavulanic acid (clavulanic acid itself being rather unstable) in combination with amoxicillin trihydrate.

Clavulanic acid is obtained by a fermentation method from various microorganisms belonging to various *Streptomycetes* strains such as *S. clavuligerus* NRRL 3585, *S. jumoninensis* NRRL 5741, *S. katsurahamanus* IFO 13716 and *Streptomyces sp.* P 6621 FERM P2804.

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Clavulanic acid and its salts were first disclosed in GB 1,508,977. However, the process for the preparation of clavulanic acid described therein is time-consuming and is based on exacting purifications by means of various chromatographic methods. The salts of clavulanic acid are obtained by binding the clavulanate anion present in the filtrate of the fermentation broth on an anionic exchange resin, subsequent elution of the clavulanate anion therefrom by means of an electrolyte, desalting the obtained eluate, passing the latter through another anionic exchange resin and a subsequent chromatographic elution therefrom by means of an electrolyte, repeatedly desalting the obtained eluate and removing the solvent.

The process requires the use of preparative chromatographic columns, which represents a considerable investment expenditure; in addition, the applicability on a large scale is limited.

A further drawback of this process is the fact that the most steps thereof are carried out in an aqueous medium where clavulanic acid is very unstable.

GB 1,543,563 discloses a modified fermentative process, wherein the pH value of the medium is maintained within the range from 6.3 to 6.7, which results in an increased yield of the desired compound. The salts of clavulanic acid such as potassium clavulanate are obtained from lithium clavulanate by double exchange.

According to the process for the preparation of clavulanic acid and of pharmaceutically acceptable salts thereof as disclosed in EP-0-026044, the exacting methods of purification by means of exchange resins are largely avoided. The process is based on the preparation of the tert. butylamine salt of clavulanic acid, preferably in the form of its acetone solvate. The tert. butylamine salt of clavulanic acid is prepared by treating the extract, preferably the ethyl acetate extract, containing crude clavulanic acid, which was prepared according to the process as described in GB 1,508,977, with tert. butylamine in an organic solvent such as acetone, followed by the conversion of the isolated tert. butylamine salt of clavulanic acid to clavulanic acid or a pharmaceutically acceptable salt thereof.

The aim of the invention is to prepare clavulanic acid and pharmaceutically acceptable salts thereof, such as potassium salt, in a new and simple manner, thus obtaining the desired substance in a high yield and high purity by isolation from the concentrate of the extract, obtained in a known manner in an organic solvent after the fermentation with a

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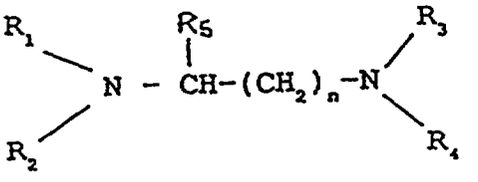
clavulanic-acid-producing microorganism, wherein clavulanic acid is present in crude form.

This aim is achieved by conventionally treating the fermentation broth obtained after the fermentation with a clavulanic acid producing microorganism of the genus *Streptomyces sp.* P 6621 FERM P2804 as described in JP Kokai 80-162993 prior to the solvent extraction, which yields a solution of crude clavulanic acid in an organic solvent.

Solvents suitable for the extraction are ethyl acetate, methyl isobutyl ketone or butanol, the preferred one being ethyl acetate.

The organic phase is then washed with water and concentrated by evaporation to a crude clavulanic acid concentration of at least 20 g/l and to a residual water content of under 6 g/l. The obtained extract in the organic solvent, such as the ethyl acetate extract, is further subjected to a treatment with activated charcoal to eliminate any coloured matter.

The extract obtained in the above-described manner is reacted with substituted alkylenediamines of the formula II



wherein the substituents R_1, R_2, R_3 and R_4 denote

- a hydrogen atom,
- a straight chain or a branched chain alkyl group having 1 to 8 carbon atoms,
- an arylalkyl group wherein the alkyl group is a methyl or ethyl group and the aryl group is a phenyl group, which is optionally para-substituted by a methyl, methoxy, nitro or halo (chloro or bromo) group, the aryl group on the alkyl chain being in α- or in β-position,
- a hydroxyalkyl group having 2 to 4 carbon atoms,

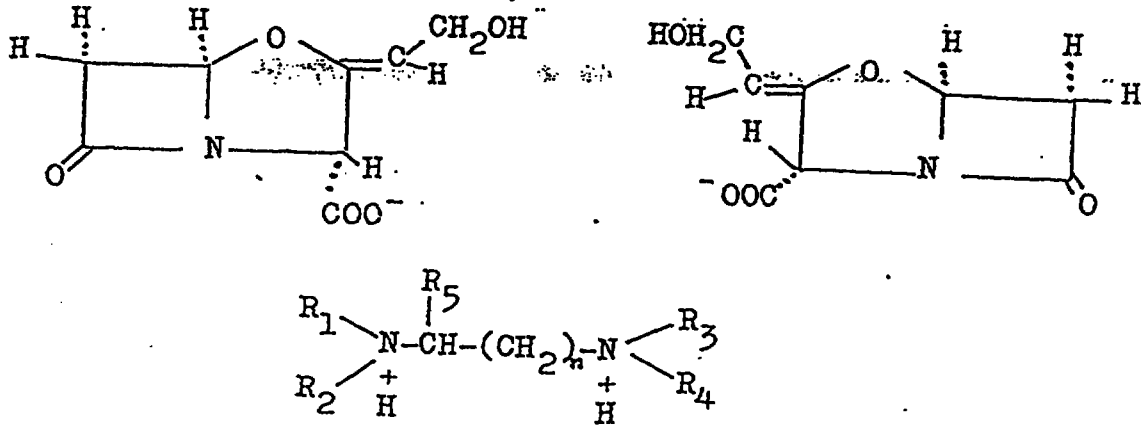
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- an aminoalkyl group having 2 to 4 carbon atoms, which is optionally substituted by an N-alkyl or N,N-dialkyl group having 1 to 4 carbon atoms, or
- R_1 and R_2 or R_3 and R_4 jointly independently denote a cyclic alkylene ring having 3 to 6 methylene groups, one of these groups being optionally substituted by an oxygen or a sulphur atom or by an imino group and

R_5 denotes a hydrogen atom, or a methyl group and

n denotes an integer from 1 to 3,

to yield alkylenediammonium diclavulanates of the formula I



wherein the substituents have the above meanings. These salts are novel and have not yet been described in literature.

The reactions are carried out most advantageously by using N,N'-monosubstituted symmetric ethylenediamines having an alkyl chain of medium length, wherein R_1 and R_3 denote an ethyl or an isopropyl group, R_2 and R_4 denote a hydrogen atom and *n* denotes 1.

As the most preferred alkylenediamine the N,N'-diisopropylethylenediamine is used, which yields the N,N'-diisopropyl-ethylenediammonium diclavulanate.

The alkylenediamine bases can be used either as such or, in the case that they are present in a solid form, in the form of solutions in organic solvents, such as acetone or ethyl acetate.

For the preparation of the salt of the clavulanic acid at least one equivalent of the selected alkylenediamine is used.

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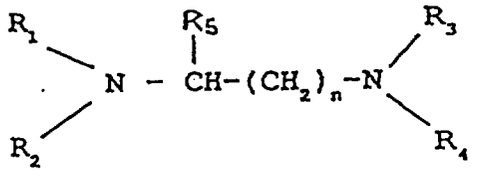
The desired salts of calvulanic acid (1:2) of the formula I, such as N,N'-diisopropylethylenediammonium diclavulanate, can then be isolated whereby the clavulanic acid is obtained free of most or even free of all impurities.

The desired salts of the clavulanic acid of the formula I are stable crystalline salts which excel by high purity, thus rendering a further purification by recrystallization unnecessary.

They are used as intermediate compounds for the preparation of pure clavulanic acid and pharmaceutically acceptable salts thereof, e.g. for the preparation of potassium clavulanate, whereat they are subjected to a double exchange by reaction with a carbonate, hydrogen carbonate or hydroxide of a pharmaceutically acceptable alkali metal or with a salt of an appropriate alkanoic acid, such as potassium 2-ethylhexanoate, in an organic solvent, such as isopropanol. In this reaction an ion exchange between the alkylenediammonium cation and the alkali metal cation takes place.

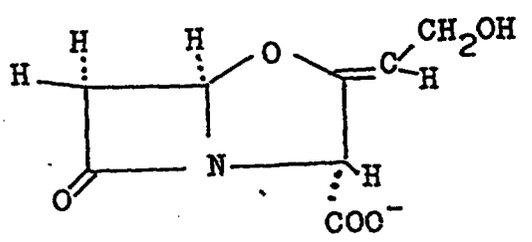
Another object of the present invention is a process for the purification of the clavulanic acid and of the pharmaceutically acceptable salts thereof, which is characterized in that

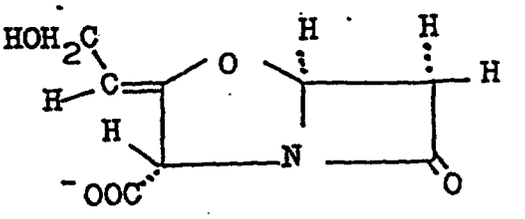
- crude clavulanic acid is reacted in an organic solvent with an appropriate substituted alkylenediamine of the formula II

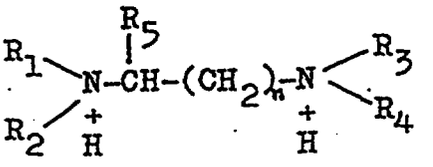


wherein the substituents R_1, R_2, R_3, R_4 and R_5 and n have the meanings as defined herein above,

- the obtained alkylenediammonium diclavulanate of the formula I







wherein the substituents R_1, R_2, R_3, R_4 and R_5 and n have the meanings as defined herein above, is optionally isolated and then coverted to the clavulanic acid or to the pharmaceutically acceptable salts thereof.

All steps of the process of the invention are carried out at temperatures about room temperature.

In comparison with the known method for the preparation of the clavulanic acid and pharmaceutically acceptable salts thereof as described in EP-0-026044, the process of this invention excels by the preparation of the intermediate product, i.e. of the salt of formula I in high yield and purity without any need of additional purification by recrystallization, and this intermediate product can be obtained directly from the ethyl acetate extract of the clavulanic acid without the use of additional organic solvents, such as acetone. According to the process as described in EP-0-026044 the clavulanate is precipitated with tert. butylamine, the obtained precipitate is treated with acetone, purified by reprecipitation with acetone from the isopropanol solution and then transformed in an isopropanolic solution to the potassium salt of the clavulanic acid.

The invention will be illustrated in more detail by the following, in no way limiting Examples.

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Preparation of Starting Materials

Example 1

N,N'-diisopropylethylenediammonium diclavulanate

To the ethyl acetate extract obtained in accordance with known methods (1 l, crude clavulanic acid content 20 g/l, water content 6 g/l), which was previously partly discoloured by treatment with activated charcoal, N,N'-diisopropylethylenediamine (9 ml) was added under vigorous stirring within 10 min. The solution was stirred for another 30 min., whereat a precipitate separated. The obtained precipitate was dissolved in water (20 ml) diluted with acetone (400 ml), whereat N,N'-diisopropylethylenediammonium diclavulanate (13.0 g) separated in form of fine crystals, m.p. 130-132°C.

^{1}H-NMR (D_2O, DSS, 300 Hz): δ = 1.33 (6H, d, J=6.5 Hz, $CH(C\underline{H}_3)_2$), 3.12 (1H, d, J=17.0 Hz, 6-βCH), 3.38 (2H, s, NCH_2), 3.45 (1H, hept, J=6.5 Hz, $C\underline{H}Me_2$), 3.55 (1H, dd, J=17.0 and 2.8 Hz, 6-αCH), 4.18 (1H, d, J=7.4 Hz, $C\underline{H}$', H"OH), 4.19 (1H, d, J=7.8 Hz, CH'$\underline{H}$"OH), 4.88-4.96 (2H, m, 3-CH, =CH-), 5.72 (1H, d, J=2.8 Hz).

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Example 2

N,N'-diethylethylenediammonium diclavulanate

To the ethyl acetate extract obtained in accordance with known methods (1 l, crude clavulanic acid content 20 g/l, water content 6 g/l), which was previously partly discoloured by treatment with activated charcoal, were added acetone (2 l) and then under vigorous stirring within 15 min. N,N'-diethylethylenediamine (6.4 ml). The obtained mixture was stirred for another 30 min., the separated precipitate was filtered off and washed with acetone. The obtained amorphous precipitate was suspended in acetone (1 l), broken up by vigorous stirring and the obtained suspension was filtered once again. The obtained product was dried, dissolved in water (20 ml) and to the solution acetone (200 ml) was added. The clear fraction of the mixture was decanted from the soft mass adhering to the walls of the vessel and filtered. To the filtrate additional acetone (400 ml) was added whereat of a precipitate formed, which was filtered off and washed with

acetone. Thus there was obtained the desired compound (10 g), m.p. 104-108°C.

^{1}H-NMR (D_2O, DSS, 300 Hz): δ = 1.29 (3H, t, J=7.3 Hz, CH_2CH_3), 3.11 (1H, d, J=17.0 Hz, 6-βCH), 3.16 (2H, q, J=7.3 Hz, CH_2CH_3), 3.41 (2H, s, NCH_2), 3.55 (1H, dd, J=17.0 and 2.7 Hz, 6-αCH), 4.18 (1H, d, J=8.2 Hz, CH', H"OH), 4.18 (1H, d, J=8.0 Hz, CH'H"OH), 5.85-5,96 (2H, m, 3-CH, =CH-), 5.72 (1H, d, J=2.7 Hz).

Process according to the Invention

Example 3

Potassium clavulanate

N,N'-diisopropylethylenediammonium diclavulanate (10 g, clavulanic acid content 69%) was dissolved in water (10 ml), the solution was diluted with isopropanol (190 ml) and a solution (15 ml, 2M) of potassium 2-ethylhexanoate in isopropanol was added within 15 min. with stirring. The obtained suspension was stirred for another 30 min. whereat a precipitate separated, which was filtered off, washed with isopropanol and then dried. Thus there was obtained potassium clavulanate (4.8 g; 70%), (USP Grade, clavulanic acid content 81% as determined by the HPLC method).

Example 4

Potassium clavulanate

N,N'-diethylethylenediammonium diclavulanate (10 g, clavulanic acid content 70%) was dissolved in water (10 ml) and to the resulting solution isopropanol (190 ml) was added. The mixture was filtered off and a solution of potassium 2-ethylhexanoate in isopropanol (15 ml, 2M) was added to the filtrate within 15 min. with stirring. The obtained suspension was stirred for another 30 min. whereat a precipitate formed, which was filtered off, washed with isopropanol and then dried. Thus there was obtained potassium clavulanate (4.0 g; 47%), (USP Grade, clavulanic acid content 81% as determined by the HPLC method).

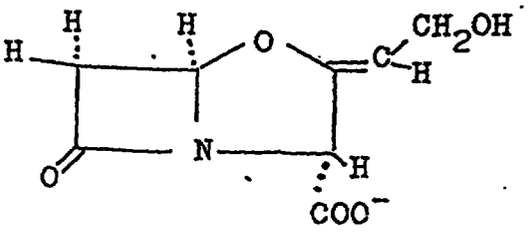
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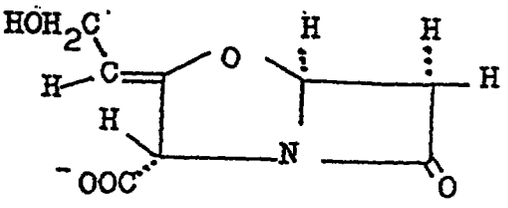


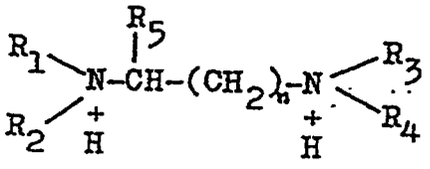
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CLAIMS

1. Alkylenediammonium diclavulanates of the formula I







wherein the substituents R_1, R_2, R_3 and R_4 denote

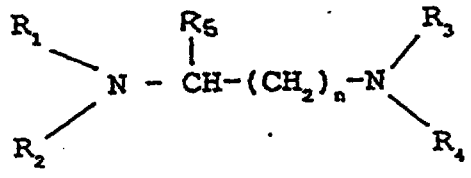
- a hydrogen atom,
- a straight chain or a branched chain alkyl group having 1 to 8 carbon atoms,
- an arylalkyl group wherein the alkyl group is a methyl or ethyl group and the aryl group is a phenyl group, which is optionally para-substituted by a methyl, methoxy, nitro or halo (chloro or bromo) group, the aryl group on the alkyl chain being in α- or in β-position,
- a hydroxyalkyl group having 2 to 4 carbon atoms,
- an aminoalkyl group having 2 to 4 carbon atoms, which is optionally substituted by an N-alkyl or N,N-dialkyl group having 1 to 4 carbon atoms, or
- R_1 and R_2 or R_3 and R_4 jointly independently denote a cyclic alkylene ring having 3 to 6 methylene groups, one of these groups being optionally substituted by an oxygen or a sulphur atom or by an imino group and
  R_5 denotes a hydrogen atom, or a methyl group and
  n denotes an integer from 1 to 3.

2. N,N'-diisopropylethylenediammonium diclavulanate.

3. N,N'-diethylethylenediammonium diclavulanate .

4. A process for the preparation of compounds of the formula I, according to claim 1, characterized in that a solution of clavulanic acid in an organic solvent is reacted with substituted alkylenediamines of the formula II

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wherein the substituents R_1, R_2, R_3, R_4 and R_5 and *n* have the meanings as defined at formula I in claim 1, optionally with solutions thereof in an organic solvent, such as acetone or ethyl acetate.

5. A process according to claim 4, characterized in that ethyl acetate or optionally a mixture of ethyl acetate and acetone, ethyl methyl ketone or methyl isobutyl ketone in a 1:0.5 to 1:5 ratio is used as the organic solvent.

6. Use of the alkylenediammonium diclavulanates of the formula I according to one of the claims 1 to 3 as intermediate compounds in the preparation of clavulanic acid and of pharmaceutically acceptable alkali salts thereof.

7. A process for the preparation of clavulanic acid and of pharmaceutically acceptable alkali salts thereof, characterized in that alkylenediammonium diclavulanates of the formula I according to one of the claims 1 to 3 are converted to clavulanic acid or pharmaceutically acceptable alkali salts thereof.

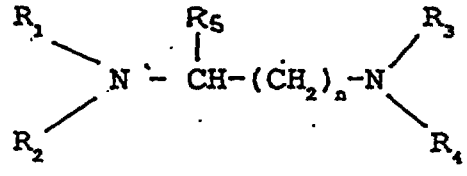
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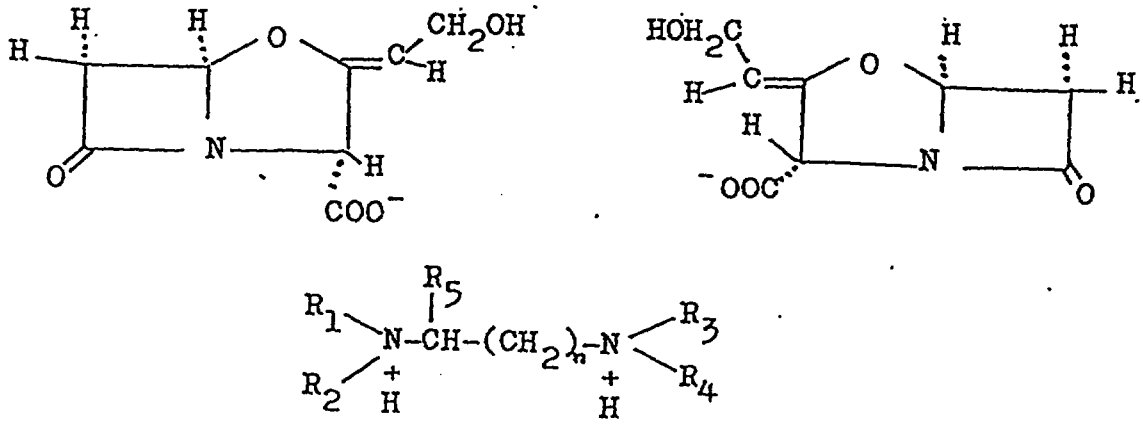
8. A process for the preparation of clavulanic acid and of pharmaceutically acceptable alkali salts thereof, characterized in that

a) crude clavulanic acid is reacted in an organic solvent with a substituted alkylenediamine of the formula II



wherein the substituents R_1, R_2, R_3, R_4 and R_5 and n have the meanings as defined at formula I in claim 1,

b) the thus obtained alkylenediammonium diclavulanate of the formula I



wherein the substituents R_1, R_2, R_3, R_4 and R_5 and n have the meanings as defined at formula I in claim 1, is isolated and

c) then converted to clavulanic acid or pharmaceutically acceptable alkali salts thereof.

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9. A process according to one of the claims 7 or 8, characterized in that clavulanic acid in the form of its stable potassium salt is prepared by reacting alkylenediammonium diclavulanate of the formula I with potassium 2-ethylhexanoate in isopropanol.

10. A process according to one of the claims 7 to 9, characterized in that the organic solvent is selected from the group comprising ethyl acetate or a mixture of ethyl acetate and acetone, ethyl methyl ketone, or methyl isobutyl ketone.

11. A process according to claim 10, characterized in that ethyl acetate is used as the organic solvent.

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