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## PATENT SPECIFICATION

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**L101561**

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### COMPLETE SPECIFICATION

#### Cephalosporin Betaines

WE, GLAXO LABORATORIES LIMITED, a British Company of Greenford, Middlesex, 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 is concerned with improvements in or relating to compounds having antibacterial activity.

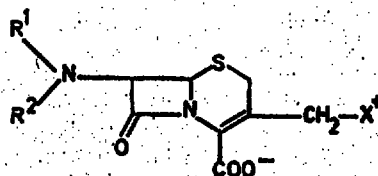
The antibiotic cephalosporin C has recently come into prominence. A number of analogues of cephalosporin C have now been developed, wherein the substitution at the 7-amino group and/or the 3-position side-chain is varied, and several of such analogues have shown conspicuous advantages over the parent antibiotic.

It has previously been found that the acetoxy group of the 3-position acetoxy-methyl group can advantageously be replaced by a quaternary amino group, for example an N-pyridyl group, and some compounds of this type have shown particularly marked activity. The presence of the quaternary ammonium group together with the carboxylate anion at the 4-position results in a betaine or zwitterion structure for the molecule. Such compounds have hitherto been prepared by reacting a tertiary amine with a 7-acyl-aminocephalosporanic acid whereby the betaine is formed directly without the quaternary ammonium group being associated with any anion other than that of the 4-position carboxylate anion.

Such betaines react with acids, particularly acids having a pKa at room temperature of 4.0 or less, to form quaternary ammonium salts wherein the 4-position carboxylate anion is protonated and the positive charge on the quaternary nitrogen atom is satisfied by the anion of the strong acid. Such salts may be made in crystalline, stable forms that are in general less likely than the parent betaines to suffer decomposition on storage.

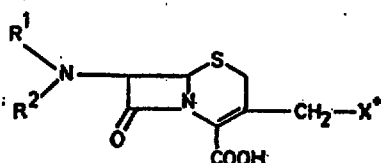
This process may be used with advantage to purify crude solutions or suspensions of the betaine. The betaine may thus be recovered from a crude solution or suspension thereof in the form of a salt as defined above, from which salt the betaine may be regenerated by reaction with a base, for example in the form of an ion-exchange resin.

According to the invention, therefore, there is provided process for the preparation of a betaine of the general formula:—



(II)

where  $R^1$  is a hydrogen atom and  $R^2$  is an acyl group  
 or  $R^1$  and  $R^2$  are both acyl groups,  
 and X is a quaternary ammonium group,  
 which comprises reacting a salt containing cations of the general formula:—



(I)

where  $R^1$ ,  $R^2$  and X have the above defined meanings, with a base.

Purification of the betaine in this way obviates difficulties in handling compounds that are amphoteric and soluble in water and require otherwise to be purified and isolated by tedious methods, such as ion-exchange chromatography and by evaporation of aqueous solutions at low temperatures. The purified betaine may then be converted into another salt as desired.

Whilst  $R^2$  (and  $R^1$  where appropriate) may represent an acyl group in general terms, specific acyl groups which may be used include those having the general formulae:—

(i)  $R^1(CH_2)_nCO$ — where  $R^1$  is aryl, cycloalkyl, substituted aryl, substituted cycloalkyl or heterocyclic group and  $n$  is an integer from 1—8, preferably 1—4. The heterocyclic ring is preferably a 5- or 6-membered ring containing one or more of O, S and N or such a ring fused to benzene. Examples of these groups include phenylacetyl, nitrophenylacetyl, phenylpropionyl, cyclohexylacetyl and thienylacetyl.

(ii)  $C_nH_{2n+1}CO$ — where  $n$  is 0 or an integer from 1—7. The alkyl group may be straight or branched and, if desired, may be interrupted by an oxygen or a sulphur atom. Examples of such groups include formyl, acetyl, hexanoyl, heptanoyl, octanoyl and butylthioacetyl.

(iii)  $C_nH_{2n-1}CO$ — where  $n$  is an integer from 2 to 7. The alkenyl group may be straight or branched and, if desired, may be interrupted by an oxygen or a sulphur atom. Examples of such groups include acrylyl and allylthioacetyl.

(iv)  $R'O CR''R'''CO$ — where  $R'$  has the meaning defined under (i) or is an alkyl group and  $R''$  and  $R'''$  are the same or are different and each is a hydrogen atom or an alkyl group. An example of such a group is phenoxyacetyl.

(v)  $R'SCR''R'''CO$ — where  $R'$ ,  $R''$  and  $R'''$  are as defined under (iv). Examples of such thio groups include S-phenylthioacetyl, S-chlorophenylthioacetyl and S-bromophenylthioacetyl.

(vi)  $R'(CH_2)_mS(CH_2)_nCR''R'''CO$ — where  $R'$ ,  $R''$  and  $R'''$  are as defined under (iv),  $m$  is an integer from 1—4 and  $n$  is 0 or an integer from 1—4. Examples of such a group include S-benzylthioacetyl, benzylthiopropionyl and  $\beta$ -phenethylthioacetyl.

(vii)  $R'CO$ — where  $R'$  has the meaning defined under (i). Examples of such groups include benzoyl, substituted benzoyl and cyclopentanoyl. Where the benzoyl group is substituted the substituents may be alkyl or alkoxy and the substituents may be in the 2- or 2- and 6- positions.

The group X preferably represents an N-pyridyl group or an N-pyridyl group substituted, e.g. with one or more alkyl, carboxy or carbamoyl groups such as an N-picolinyl group, N-isonicotinyl group or N-nicotinamido group.

It is to be noted that in the preparation of the salts the groups  $R^1$  and  $R^2$  will not in general enter into the reaction and from this point of view are relatively unimportant. If  $R^1$  and/or  $R^2$  contain a basic group this may also form a salt group and it will be appreciated that the amount of acid used may have to be adjusted accordingly. The group X although entering into the reaction is also relatively unimportant from this point of view.

The salts may be prepared from an aqueous solution or suspension of the parent betaine by reaction with the desired acid. Formation of salts can be demonstrated by electrophoresis: until they are protonated the betaines behave as uncharged molecules, so that they migrate little in neutral buffers; in acid buffers, for instance an aqueous mixture of acetic and formic acids at pH 2, the betaines are protonated and move, as positively charged ions, to the cathode. The stability of such salts depends on their

dissociation (that is, reversal of the salt-forming reaction) by dilution with water or by evaporation, and on their solubility. Thus, strong organic acids such as trichloroacetic, preferably, mineral acids such as sulphuric, perchloric, nitric, hydrochloric, hydrobromic, and hydriodic, give salts that do not suffer dissociation by evaporation of their aqueous solutions, and some yield insoluble salts; salts with formic acid, which is weaker than those just named, may be soluble and dissociate on evaporation of their aqueous solutions, so that the betaine is recovered and the acid is evaporated with the water.

We have found it convenient with strong acids to add these at a concentration of 0.8N or greater to a solution of the betaine but it should be understood that the concentration of the acid as such is relatively unimportant so long as the desired salt is formed. With strong acids this will normally occur at a pH of 2 or less in the reaction mixture as stated above.

A number of the salts are water soluble and these may be isolated by, for example, evaporation and crystallisation or freeze drying etc. Thus, for example, the compound in which X forms an N-pyridyl group, R<sup>1</sup> is hydrogen and R<sup>2</sup> is a 2-thienyl-acetyl group forms a hydrobromide which has been crystallised on evaporation while its hydrogen phosphate has been isolated by freeze-drying. The hydrochloride may also be isolated by evaporation and crystallisation.

Other salts are water-insoluble and precipitate from the aqueous medium when the pH is reduced below 2. In the case of the above pyridyl-thienylacetyl compound, the perchlorate, tetrafluoroborate, iodide and nitrate all precipitate readily and such substantially insoluble salts afford a useful means of isolating and of purifying the antibiotic. The precipitation may be facilitated by the common ion technique.

It is possible to exchange the anions associated with the salt by, for example, treatment with an anion-exchanger.

The betaine form of the antibiotic can if desired be readily regenerated by slurring the salt in an aqueous medium with an anion exchanger in the form of a salt with a weak acid e.g. the acetate form. The resulting weak acid salt is unstable and loses the weak acid ion on processing e.g. freeze drying to yield the betaine. The betaine may be recovered from the aqueous medium by freeze drying or by direct crystallisation. Alternatively the betaine may be regenerated by contacting an aqueous medium containing the salt with a water-immiscible phase containing a liquid ion exchanger e.g. Amberlite L.A.1 or Amberlite L.A.2 ("Amberlite" is a registered Trade Mark), the betaine being regenerated in the aqueous phase and the residual ions of the salt combining with the ion exchanger. The betaine may also be regenerated by reacting the salt in an aqueous medium with an organic base e.g. a tri (C<sub>1</sub>—C<sub>6</sub> alkyl) amine and recovering the betaine e.g. by freeze drying or direct crystallisation.

When N - (7 - 2' - thienylacetamidoceph - 3 - em - 3 - ylmethyl) - pyridinium-4 - carboxylate is regenerated in an aqueous medium from a salt and is then recovered by direct crystallisation the resulting crystalline form is a non-hygroscopic hydrate. However on subsequent drying the material loses its water of crystalline and becomes hygroscopic although it is relatively stable to heat.

However, the recognition of the fact that said acid addition salts have in general different solubility characteristics in organic media to said betaines has led to an improved process for the recovery of said betaine from acid addition salts. By virtue of this we can dissolve the salt in an organic solvent, convert the salt to the betaine *in situ*, and recover the betaine from the reaction mixture. Although the yield of the betaine may not be any higher than from other methods, the colour and hence the purity of the betaine is in general improved if it is obtained by this process. Moreover the process is relatively simple and inexpensive.

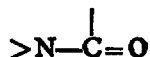
According to a feature of the invention, therefore, there is provided a process for the recovery of betaines of general formula II from acid addition salts containing cations of general formula I which comprises effecting reaction in solution in an organic solvent between said acid addition salt and a base, preferably a strong organic base, i.e. a base having a pK<sub>b</sub> of less than 6, to form a soluble acid addition salt of said base and recovering said betaine from the reaction mixture. The salts preferably derived from an acid having a pK<sub>a</sub> of 4 or less. In general we prefer to use the hydronitrate salt but this is not essential.

This process may be effected at a temperature in the range of 5—60°C.

After the reaction between the base and the acid addition salt has taken place, recovery of the betaine may be facilitated by addition of an anti-solvent e.g. substan-

tially anhydrous alkanols, e.g. methanol or ethanol, ethers, ketones, e.g. acetone and esters e.g. ethyl acetate, and then collecting the resultant precipitate. Alternatively, the betaine may be recovered by direct crystallization from the reaction mixture.

The organic solvent used in this process according to the invention is preferably selected from those containing the following group:



Examples of such solvents are substituted amides of the general formula



where  $\text{R}^3$  is a hydrogen atom or an alkyl group containing 1 to 5 carbon atoms and  $\text{R}^4$  and  $\text{R}^5$ , which may be the same or different, are each an alkyl group containing 1 to 5 carbon atoms, or, alternatively,  $\text{R}^4$  and  $\text{R}^5$  together form a divalent aliphatic group which, together with the adjacent nitrogen atom, forms a heterocyclic ring. Examples of amides of this type are N,N-dimethylformamide, N,N-diethylformamide, N,N-dipropylformamide, N,N-dibutylformamide, N,N-dimethylacetamide, N,N-diethylacetamide, N,N-dimethylvaleramide, N,N-dimethylpropionamide, N-formylpiperidine and N-formylmorpholine. Of this group of compounds, we prefer to use N,N-dimethylacetamide or N,N-dimethylformamide. These two solvents are characterized by having good solubility power for the acid addition salts and low solubility power for the betaines. Thus when a solvent-soluble strong organic base is added to such a solution of the acid-addition salt, the betaine can be crystallized directly from the solution or on the addition of an anti-solvent e.g. methanol.

The N,N-disubstituted amide solvents are particularly to be preferred as solvents in the reaction according to the invention because they yield the betaine in crystalline forms which are non-hygroscopic and hence possess important advantages in handling and formulation over the hygroscopic forms previously prepared.

The non-hygroscopic forms of the betaines of formula II are novel substances and constitute a further feature of the invention. They may be characterised by their X-ray crystallographic constants and by other details given below. Slight differences between forms crystallised from, for example, N,N-dimethylformamide (DMF) and N,N-dimethylacetamide (DMA) have been observed but the invention extends to all the non-hygroscopic crystalline forms of the betaines of formula II.

In the particular case of N-(7-2'-thienylacetamido-ceph-3-em-3-ylmethyl)-pyridinium-4-carboxylate different forms have been isolated from DMA and DMF and these will be termed herein, for convenience, the  $\alpha$ - and  $\beta$ -forms respectively. X-ray crystallographic data in respect of these two forms are given in the following tables showing the value of  $2\theta^\circ$  (twice the Bragg angle) and the corresponding interplanar spacings in Angstroms measured from film taken on a Guinier powder X-ray camera using copper  $K\alpha$ -radiation. The relative intensities of the lines are also given according to the following arbitrary basis:

s = strong  
m = medium  
w = weak  
f = faint  
v = very  
b = broad

TABLE I

 $\alpha$ -form

| 20°   | d(Å) | I       | 20°   | d(Å) | I      | 20°   | d(Å) | I        |
|-------|------|---------|-------|------|--------|-------|------|----------|
| 6.05  | 14.6 | vs (b)  | 24.07 | 3.69 | ms     | 33.50 | 2.67 | m (vb)   |
| 11.97 | 7.38 | ms      | 24.27 | 3.66 | w      | 33.77 | 2.65 |          |
| 12.97 | 6.82 | f       | 25.00 | 3.55 | vw     | 34.70 | 2.58 | f        |
| 13.85 | 6.39 | w       | 25.62 | 3.47 | w (b)  | 35.12 | 2.55 | w-m      |
| 15.57 | 5.68 | vvs     | 26.30 | 3.38 | s      | 35.25 | 2.54 | w        |
| 15.90 | 5.57 | vvs     | 26.57 | 3.35 | ms     | 35.72 | 2.51 | w (b)    |
| 16.75 | 5.29 | ms (vb) | 27.05 | 3.29 | w-m    | 36.35 | 2.47 | vw       |
| 18.02 | 4.92 | w       | 27.82 | 3.20 | f      | 36.90 | 2.43 | w-m      |
| 18.47 | 4.80 | vw      | 28.40 | 3.14 | m (b)  | 37.27 | 2.41 | f        |
| 19.77 | 4.49 | ms (b)  | 28.97 | 3.08 | f      | 37.90 | 2.37 | f        |
| 21.05 | 4.22 | vs (b)  | 29.75 | 3.00 | w (b)  | 38.30 | 2.35 | w (b)    |
| 21.77 | 4.08 | s (b)   | 30.20 | 2.96 | f      | 38.80 | 2.32 | vw (b)   |
| 22.85 | 3.89 | m (b)   | 31.67 | 2.82 | ms (b) | 40.15 | 2.24 | w-m (vb) |
| 23.27 | 3.82 | w       | 32.30 | 2.77 | vw     | 41.35 | 2.18 | m        |
| 23.77 | 3.74 | ms      | 32.80 | 2.73 | w-m    | 42.25 | 2.14 | w (vb)   |

TABLE II

 $\beta$ -form

| 2 $\theta$ ° | d(Å)  | I       | 2 $\theta$ ° | d(Å) | I       | 2 $\theta$ ° | d(Å)  | I      |
|--------------|-------|---------|--------------|------|---------|--------------|-------|--------|
| 6.10         | 14.48 | vs      | 23.42        | 3.79 | w-m     | 33.10        | 2.70  | w (vb) |
| 10.62        | 8.32  | f       | 24.02        | 3.70 | w-m     | 33.40        | 2.68  |        |
| 11.90        | 7.43  | ms      | 24.40        | 3.65 | f       | 34.20        | 2.62  | f      |
| 13.02        | 6.79  | m       | 24.52        | 3.63 | f       | 34.65        | 2.59  | w-m    |
| 13.70        | 6.46  | vw      | 24.85        | 3.58 | vw      | 35.00        | 2.562 | vw     |
| 14.72        | 6.01  | w       | 25.20        | 3.53 | w       | 35.05        | 2.558 | w      |
| 15.35        | 5.77  | vvs     | 25.42        | 3.50 | w       | 36.12        | 2.48  | w (b)  |
| 15.67        | 5.65  | vvs     | 26.00        | 3.42 | w       | 36.45        | 2.46  | w (b)  |
| 15.92        | 5.56  | w       | 26.15        | 3.41 | w       | 36.97        | 2.43  | w      |
| 16.55        | 5.35  | w-m     | 26.62        | 3.35 | w       | 37.62        | 2.39  | w      |
| 16.75        | 5.29  | f       | 26.90        | 3.31 | w       | 37.97        | 2.37  | w      |
| 17.92        | 4.94  | vs (b)  | 27.55        | 3.24 | vw      | 38.30        | 2.35  | vw     |
| 18.40        | 4.82  | w-m     | 28.10        | 3.17 | w       | 38.82        | 2.32  | vw (b) |
| 19.20        | 4.62  | f       | 29.10        | 3.07 | f       | 39.27        | 2.30  | f      |
| 19.67        | 4.51  | w       | 29.67        | 3.01 | ms (b)  | 39.92        | 2.26  | m      |
| 20.47        | 4.33  | vs (vb) | 29.95        | 2.98 | vw      | 40.30        | 2.24  | w (b)  |
| 20.62        | 4.30  |         | 31.10        | 2.87 | w-m (b) | 40.42        | 2.23  |        |
| 21.02        | 4.22  | ms      | 31.67        | 2.82 | m       | 40.77        | 2.21  | vw     |
| 21.70        | 4.09  | ms (b)  | 32.10        | 2.79 | ms      | 41.47        | 2.18  | f      |
| 22.37        | 3.97  | vvs (b) | 32.32        | 2.77 | vw (vb) | 42.00        | 2.15  | w (vb) |
| 23.05        | 3.86  | w-m     |              |      |         | 42.67        | 2.12  | m      |

Differences also exist between the infrared spectra of the aforementioned  $\alpha$ - and  $\beta$ -forms, and Figures 1 and 2 of the drawings accompanying the Provisional Specification of Application No. 28857/64 show infrared spectra of Nujol (Registered Trade Mark) mulls of these two forms respectively. For the sake of comparison, figures 3 and 4 show the infrared spectra of the  $\gamma$ -form crystallised from methanol and the  $\delta$ -form isolated from water by freeze drying respectively. It should be noted that the spectra of the  $\gamma$ - and  $\delta$ -forms refer to material dried to the substantially solvent-free condition. The following Table shows the principal absorption bands in both  $\mu$  and  $\text{cm}^{-1}$  for the  $\alpha$ - and  $\beta$ - forms as compared with those of the  $\gamma$ -form (crystallised from methanol) and the  $\delta$ -form (isolated from water). Intensities are also shown using the same arbitrary basis given above for the X-ray measurements.

TABLE III

Principle absorption bands ( $\mu$  and  $\text{cm}^{-1}$ ) in the infrared spectra of  
Nujol mulls of  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -forms of N-(7-2'-thienylacet-  
amidoceph-3-em-3-ylmethyl)-pyridinium-4-carboxylate

| "α-form"<br>( <i>ex</i> DMA) |                  | "β-form"<br>( <i>ex</i> DMF) |                  | "γ-form"<br>( <i>ex</i> methanol) |                  | "δ-form"<br>( <i>ex</i> water) |                  |
|------------------------------|------------------|------------------------------|------------------|-----------------------------------|------------------|--------------------------------|------------------|
| $\mu$                        | $\text{cm}^{-1}$ | $\mu$                        | $\text{cm}^{-1}$ | $\mu$                             | $\text{cm}^{-1}$ | $\mu$                          | $\text{cm}^{-1}$ |
| 3.21m                        | 3120m            | 3.21m                        | 3120m            | 3.11m                             | 3220m            | 3.08m                          | 3250m            |
| 3.32—                        | 3010—            | 3.32—                        | 3010—            | 3.40x                             | 2940—            | 3.39—                          | 2950—            |
| 3.51s                        | 2850s            | 3.51s                        | 2850s            | 3.52s                             | 2840s            | 3.52s                          | 2840s            |
| 5.64s                        | 1774s            | 5.63s                        | 1776s            | 5.68s                             | 1762s            | 5.68s                          | 1762s            |
| 6.00s                        | 1666s            | 5.99s                        | 1670s            | 5.91m                             | 1692m            | 5.89m                          | 1698m            |
| 6.24s                        | 1602s            | 6.24s                        | 1604s            | 6.19s                             | 1616s            | 6.17s                          | 1622s            |
| 6.34s                        | 1578s            | 6.34s                        | 1578s            | 6.34w                             | 1578w            | 6.30m                          | 1586m            |
| 6.46s                        | 1548s            | 6.45m                        | 1550m            | 6.54s                             | 1530s            | 6.52s                          | 1535s            |
| 6.69s                        | 1495s            | 6.69s                        | 1494s            | 6.64m                             | 1506m            | 6.67s                          | 1498s            |
| 6.72s                        | 1488s            | 6.84m                        | 1462m            | 6.71s                             | 1490s            | 6.83s                          | 1465s            |
| 6.84m                        | 1462m            | 7.15s                        | 1398s            | 6.83s                             | 1465s            | 7.09m                          | 1410m            |
| 6.93m                        | 1442m            | 7.35s                        | 1360s            | 7.25s                             | 1380s            | 7.24s                          | 1382s            |
| 7.15s                        | 1398s            | 7.44m                        | 1344m            | 7.36s                             | 1358s            | 7.39s                          | 1354s            |
| 7.27s                        | 1376s            | 7.63w                        | 1310w            | 7.46s                             | 1340s            | 7.48m                          | 1338m            |
| 7.34s                        | 1362s            | 7.78m                        | 1286m            | 7.55w                             | 1325w            | 7.57m                          | 1322m            |
| 7.42s                        | 1348s            | 7.86w                        | 1272w            | 7.76m                             | 1288m            | 7.75m                          | 1290m            |
| 7.51m                        | 1332m            | 8.03w                        | 1246w            | 8.13m                             | 1230m            | 7.82w                          | 1278w            |
| 7.66w                        | 1306w            | 8.23m                        | 1216m            | 8.39m                             | 1192m            | 8.15s                          | 1228s            |
| 7.78m                        | 1285m            | 8.37w                        | 1194w            | 8.62m                             | 1160m            | 8.39m                          | 1192m            |
| 7.87m                        | 1270m            | 8.61m                        | 1162m            | 8.70m                             | 1150m            | 8.61s                          | 1162s            |
| 8.08w                        | 1238w            | 8.71m                        | 1148m            | 9.07m                             | 1103m            | 8.89w                          | 1125w            |
| 8.20m                        | 1220m            | 8.93w                        | 1120w            | 9.36w                             | 1068w            | 9.01w                          | 1110w            |
| 8.36w                        | 1196w            | 9.03w                        | 1108w            | 9.64w                             | 1038w            | 9.23w                          | 1083w            |
| 8.48m                        | 1180m            | 9.36w                        | 1068w            | 9.88m                             | 1012m            | 9.39w                          | 1065w            |
| 8.62m                        | 1160m            | 9.80m                        | 1020m            | 10.18w                            | 982w             | 9.64w                          | 1038w            |
| 8.77m                        | 1148m            | 10.08w                       | 992w             | 10.50w                            | 952w             | 9.88m                          | 1012m            |
| 9.87w                        | 1128w            | 10.55w                       | 948w             | 11.76w                            | 850w             | 10.05w                         | 995w             |
| 8.93w                        | 1120w            | 11.17w                       | 895w             | 12.22w                            | 818w             | 10.53w                         | 950w             |
| 9.26w                        | 1080w            | 12.12w                       | 825w             | 12.58w                            | 795w             | 11.17w                         | 895w             |
| 9.38w                        | 1066w            | 12.38w                       | 808w             | 12.99m                            | 770m             | 11.74w                         | 852w             |
| 9.80m                        | 1020m            | 12.58w                       | 795w             | 13.66m                            | 732m             | 12.42w                         | 805w             |
| 10.04w                       | 996w             | 12.85m                       | 778m             | 14.71m                            | 680m             | 12.69w                         | 788w             |
| 10.62w                       | 942w             | 13.23w                       | 756w             |                                   |                  | 12.90m                         | 775m             |
| 11.16w                       | 896w             | 13.51w                       | 740w             |                                   |                  | 13.37w                         | 748w             |
| 11.66w                       | 858w             | 14.33m                       | 698m             |                                   |                  | 13.66m                         | 732m             |
| 11.96m                       | 836m             |                              |                  |                                   |                  | 14.12w                         | 708w             |
| 12.50w                       | 800w             |                              |                  |                                   |                  | 14.71m                         | 680m             |
| 12.63w                       | 792w             |                              |                  |                                   |                  |                                |                  |
| 12.85m                       | 778m             |                              |                  |                                   |                  |                                |                  |
| 13.21w                       | 757w             |                              |                  |                                   |                  |                                |                  |
| 13.40w                       | 746w             |                              |                  |                                   |                  |                                |                  |
| 13.55w                       | 738w             |                              |                  |                                   |                  |                                |                  |
| 13.93s                       | 718s             |                              |                  |                                   |                  |                                |                  |
| 14.45m                       | 692m             |                              |                  |                                   |                  |                                |                  |
| 15.38w                       | 650w             |                              |                  |                                   |                  |                                |                  |

The  $\gamma$  and  $\delta$ -forms isolated from methanol and water respectively are hygroscopic. It should be noted that if an alcohol, such as methanol, is used as the antisolvent to increase crystallisation of the betaine from the substituted amide solvent, the product is hygroscopic. It is preferred to obtain the non-hygroscopic crystals by effecting partial crystallisation of the betaine from the substituted amide solvent and then effecting further crystallisation by the addition of a quantity of a lower ketone, e.g. acetone, before harvesting the desired non-hygroscopic crystals. The amount of ketone employed and the rate at which it is added must be carefully regulated if non-hygroscopic crystals of the betaine are to be obtained. The optimum amount may be ascertained by preliminary trial. It has been observed that when acetone is used with dimethylacetamide, the betaine is sometimes obtained as a mixture of non-hygroscopic forms. This does not, however, significantly affect the handling and formulating characteristics of the product.

Another convenient group of solvents are the N,N-dialkylcyanamides, e.g. N,N-dimethylcyanamide.

Yet another group of solvents are tetraalkylureas of the general formula



where  $R^4$  and  $R^5$  have the meanings defined above. Examples of such substituted ureas include, N,N,N',N'-tetramethylurea and N,N,N',N'-tetraethylurea.

Solvents of a different type chemically which may be employed are those of the formula  $R^4.R^5SO$ , where  $R^4$  and  $R^5$  have the meanings defined above. An example of such a solvent is dimethyl sulphoxide.

Where the base used is itself a liquid and the acid addition salt is soluble therein, the base may function as the solvent for the reaction. Pyridine, for example, may be used in this way.

Where organic bases are used these are preferably liquid tertiary amines e.g. tri(lower alkyl) amines or liquid secondary amines e.g. the products sold under the Trade Marks "Amberlite LA<sub>2</sub>" and "Amberlite LA<sub>1</sub>".

Organic bases may be used as such or as a solution in an inert organic solvent.

As an alternative to using organic bases one may use inorganic bases provided that these are otherwise inert to the cephalosporin derivative. Inorganic bases which may be used include alkali metal and alkaline earth metal carbonates and bicarbonates. These may be used in aqueous solution or in solid form.

In order that the invention may be well understood the following Examples are given by way of illustration only. The word "Dowex" used is a registered Trade Mark.

#### EXAMPLE 1.

##### Regeneration of N-(7-2'-thienylacetamidoceph-3-em-3-ylmethyl)pyridinium-4-carboxylate.

To a suspension of the hydrochloride of the above betaine (see Example 1 of Application No. 39172/66 (Serial No. 1,101,552) (3 g.) in water (20 ml.), was added 1.5N-ammonium hydroxide dropwise until the salt dissolved (pH 4.5). The resulting solution was passed down a Dowex-1 ion-exchange column (2.5 cm. x 20 cm.) in the acetate-form, which was eluted with water. The eluant was freeze-dried and the pale yellow product was triturated with methanol to give N-(7-2'-thienylacetamidoceph-3-em-3-ylmethyl)pyridinium-4-carboxylate as a fine white powder (2.55 g; 91%),  $\lambda$  max. 240 m $\mu$  (E<sub>1</sub><sup>1%</sup> 367),  $\lambda$  max. 255 m $\mu$ , (E<sub>1</sub><sup>1%</sup> 335),  $\nu$  max. 1758 ( $\beta$ -lactam), 1628 (COO<sup>-</sup>), and 1675 and 1540 cm.<sup>-1</sup> (—CONH—),  $[\alpha]_D^{20}$  + 47° cc, 0.098; water).

The betaine was produced likewise, in 100% yield, from the hydronitrate (see Example 5 of Application No. 39172/66 (Serial No. 1,101,552).

#### EXAMPLE 2.

N - (7 - 2' - Thienylacetamido - 3 - em - 3 - ylmethyl) - pyridinium - 4 - carboxylate hydronitrate (100 g.) was suspended in distilled water (650 ml.) and shaken with a solution of Amberlite liquid anion exchanger No. 2 (LA 2, 100 ml.) in petrol (b.p. 40—60°, 500 ml.) until all the solid had dissolved. The aqueous layer was separated and washed with LA 2 (20 ml.) in petrol (200 ml.), LA 2 (20 ml.) in petrol (400 ml.) and twice with petrol (250 ml. each). The organic layers were backwashed successively with water (150 ml.). The aqueous layer and backwash were stirred under reduced pressure to remove solvent, filtered and cooled to 0, for 16 hrs. The N-(7-2'-

thienylacetamidoceph-3-em-3-ylmethyl)-pyridinium-4-carboxylate was collected by filtration, washed with ice-cold water (60 ml.), followed by acetone (300 ml.) and dried *in vacuo* at room temperature for 64 hrs. Yield 50.0 g., 57.8% theory,  $[\alpha]_D^{20} + 48.4^\circ$  (c, 1 in water), pH 5.0 (c, 1 in water),  $\lambda_{\text{max}}^{\text{H}_2\text{O}}$  240 m $\mu$ , E  $\frac{1\%}{1 \text{ cm.}}$  394,  $\lambda$  infl.

5 255 m $\mu$ , E  $\frac{1\%}{1 \text{ cm.}}$  361, Ratio  $\frac{\text{E at 240}}{\text{E at 255}} = 1.09$ .

In Examples 3—9 the hydronitrate salt employed is used only for the purpose of illustration and the invention as illustrated therein is not limited to the use of hydronitrate salts. In place of the hydronitrate there could equally have been used any of the following: hydrochloride, hydrogen sulphate, hydrogen orthophosphate, hydrobromide, hydrogen perchlorate, hydrogen trichloroacetate, hydrogen tetrafluoroborate or hydroiodide.

#### EXAMPLE 3.

Triethylamine (20.4 g.; 0.202 moles) was added to a stirred solution of N-(7-2'-thienylacetamidoceph-3-em-3-ylmethyl)pyridinium-4-carboxylate hydronitrate (78 g., 0.163 moles) in N,N-dimethylacetamide (195 ml.). Two minutes after the addition of triethylamine, methanol (780 ml.) was added to the reaction mixture and the stirring was continued for a further ninety minutes. The precipitate was collected by filtration, washed with methanol (350 ml.), diethyl ether (350 ml.) by displacement and dried *in vacuo* at 40° to constant weight to give N-(7-2'-thienylacetamidoceph-3-em-3-ylmethyl)pyridinium-4-carboxylate (60.7 g., 89.7% of theory) with  $[\alpha]_D^{20} + 47.7^\circ$  (c, 1 in water), pH 4.8 (c, 1 in water),  $\lambda_{\text{max}}^{\text{H}_2\text{O}}$  at

240 m $\mu$ , E  $\frac{1\%}{1 \text{ cm.}}$  377, inflection at 255 m $\mu$ , E  $\frac{1\%}{1 \text{ cm.}}$  344, Ratio  $\frac{\text{E at 240}}{\text{E at 255}} = 1.095$ , and giving clear solution in water from 1% to 20% concentrations.

#### EXAMPLE 4

A solution of triethylamine (0.63 g., 6.25 millimoles) in methanol (24 ml.) was added to a solution of N-(7-2'-thienylacetamidoceph-3-em-3-ylmethyl)pyridinium-4-carboxylate hydronitrate (2.4 g.; 4.98 millimoles) in N,N-dimethylformamide (6 ml.) and the reaction mixture was allowed to stand at room temperature for 1 hour with occasional swirling. The crystalline precipitate was collected by filtration, washed with methanol (10 ml.), diethyl ether (10 ml.) by displacement and dried *in vacuo* at 40° to constant weight to give N-(7-2'-thienylacetamidoceph-3-em-3-ylmethyl)pyridinium-4-carboxylate (1.818 g., 87.5% of theory) with  $[\alpha]_D^{20} + 48.4^\circ$  (c, 1 in water), pH 4.8 (c, 1 in water),  $\lambda_{\text{max}}^{\text{H}_2\text{O}}$  at 240 m $\mu$ , E  $\frac{1\%}{1 \text{ cm.}}$  372, inflection at

255 m $\mu$ , E  $\frac{1\%}{1 \text{ cm.}}$  341, Ratio  $\frac{\text{E at 240}}{\text{E at 255}} = 1.09$  and giving clear solutions in water from 1% to 20% concentrations.

#### EXAMPLE 5.

Triethylamine (1.01 g.; 10 millimoles) was added to a solution of N-(7-2'-thienylacetamidoceph-3-em-3-ylmethyl)pyridinium-4-carboxylate hydronitrate (2.4 g.; 4.98 millimoles) in dimethylsulphoxide (6 ml.). Methanol (3 ml.) was added to obtain a homogeneous reaction mixture. After 10 minutes, further methanol (17 ml.) was added portionwise to the stirred reaction solution to give a clear solution from which the product precipitated. The reaction mixture was stirred occasionally during one hour. The precipitate was then collected by filtration washed with methanol (20 ml.) by displacement and dried *in vacuo* to give N-(7-2'-thienylacetamidoceph-3-em-3-ylmethyl)pyridinium-4-carboxylate 1.823 g.; 88.6% of theory) with  $[\alpha]_D^{20} + 46.4^\circ$ , pH 4.9 (c, 1. in water), giving clear solutions for concentrations of 1% to 20% in water.

#### EXAMPLE 6.

Triethylamine (2.1 ml.; 1.52 g.; 15 millimoles) was added to a stirred solution of N-(7-2'-thienylacetamidoceph-3-em-3-ylmethyl)pyridinium-4-carboxylate hydronitrate (4.8 g.; 9.96 millimoles) in N,N-dimethylacetamide (48 ml.) and seeded with N-(7-2'-thienylacetamidoceph-3-em-3-ylmethyl)pyridinium-4-carboxylate. The reaction solution was stirred at room temperature for two hours

during which time the product crystallised out. The precipitate was collected by filtration and washed with N,N-dimethylacetamide (6 ml.) and then with acetone (20 ml.) by displacement and dried *in vacuo* at 40° for 2 hours and then for 16 hours at 30° to give N - (7 - 2' - thienylacetamidoceph - 3 - em - 3 - ylmethyl) - pyridinium 4-carboxylate (3.16 g.; 75.6% of theory) with  $[\alpha]_D^{20} + 47.4$ ; pH 4.5 (c, 1 in water) giving clear solutions at 1% to 20% concentrations in water.

## EXAMPLE 7.

N - (7 - 2' - thienylacetamidoceph - 3 - em - 3 - ylmethyl) - pyridinium - 4-carboxylate hydronitrate (1 g.; 2.09 millimoles) was dissolved with stirring in a mixture of pyridine (5 ml.; 4.9 g.; 62 millimoles) and methanol (5 ml.) at 40°C. After 15 minutes the precipitate was collected by filtration, washed with a 1:1 mixture of pyridine and methanol (10 ml.), methanol (20 ml.) and dried *in vacuo* at 40° to constant weight to give N - (7 - 2' - thienylacetamidoceph - 3 - em - 3 - ylmethyl) - pyridinium-4-carboxylate (0.45 g.; 52% of theory) with  $[\alpha]_D^{20} + 45^\circ$ , pH 4.6 (c, 1 in water) and containing less than 2% of the hydronitrate by potentiometric titration.

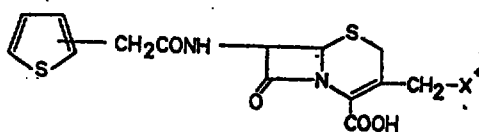
## EXAMPLE 8.

Anhydrous sodium carbonate (0.53 g.; 5.025 millimoles) was added to a solution of the hydronitrate (4.8 g.; 10.05 millimoles) of N - (7 - 2' - thienylacetamido - ceph - 3 - em - 3 - ylmethyl) - pyridinium - 4 - carboxylate in N,N - dimethylacetamide (25 mls) and the suspension was stirred at 40° for 45 minutes. The reaction mixture was clarified by filtration. The filtrate was "seeded" with a small quantity of N-(7-2'-thienylacetamidoceph - 3 - em - 3 - ylmethyl) - pyridinium - 4 - carboxylate and stirred at room temperature for 30 minutes but no precipitate formed. Methanol (70 ml) was then added to the stirred solution and a precipitate slowly formed. After 1 hour the precipitate was collected by filtration, washed with methanol (40 ml.), and dried *in vacuo* at 30° for 24 hours to give N-(7-2'-thienylacetamido-ceph-3-em-3-ylmethyl)-pyridinium-4-carboxylate (2.88 g, 69% of theory) with  $[\alpha]_D^{20} + 47.8^\circ$  pH 3.6 (c 1 in water))  $\lambda_{\text{max}}^{\text{H}_2\text{O}}$  at 240 m $\mu$  E $_{1\text{cm}}^{1\%}$  366,  $\lambda_{\text{infl}}$  at 255 m $\mu$  E $_{1\text{cm}}^{1\%}$  336. Potentiometric titration showed the possible presence of approximately 3.5% of N-(7-2'-thienylacetamido-ceph-3-em-3-ylmethyl)pyridinium-4-carboxylate hydronitrate.

## EXAMPLE 9.

N - (7 - 2' - Thienylacetamidoceph - 3 - em - 3 - ylmethyl) - pyridinium - 4-carboxylate hydronitrate (9.6 g.) was dissolved in N,N'-dimethylacetamide (50 ml.). Triethylamine (3.1 ml.) was added with continued stirring. The N-(7-2'-thienylacetamidoceph - 3 - em - 3 - ylmethyl) - pyridinium - 4 - carboxylate was collected by filtration, washed with 1:1 acetone/dimethylacetamide mixture (25 ml.), followed by acetone (100 ml.) and dried at 30° *in vacuo* for 16 hours. Yield 7.54 g., 90.6% theory,  $[\alpha]_D + 46.7^\circ$  [c, 1.0 in water]; pH 4.75. [c, 1.0 in water].

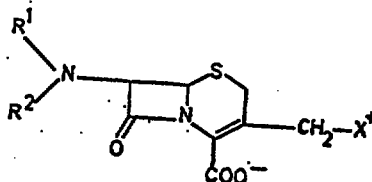
In Application No. 39172/66 (Serial No. 1,101,552), we have described and claimed salts containing cations of the general formula:—



where X is an N-pyridyl or substituted N-pyridyl group. A specific salt of this type which is claimed therein is N - (7 - 2' - thienylacetamidoceph - 3 - em - 3 - ylmethyl) - pyridinium-4-carboxylate hydronitrate.

## WHAT WE CLAIM IS:—

1. A process for the preparation of a betaine of the general formula:—



during which time the product crystallised out. The precipitate was collected by filtration and washed with N,N-dimethylacetamide (6 ml.) and then with acetone (20 ml.) by displacement and dried *in vacuo* at 40° for 2 hours and then for 16 hours at 30° to give N - (7 - 2' - thienylacetamidoceph - 3 - em - 3 - ylmethyl) - pyridinium 4-carboxylate (3.16 g. 75.6% of theory) with  $[\alpha]_D^{20} + 47.4$ ; pH 4.5 (c, 1 in water) giving clear solutions at 1% to 20% concentrations in water.

## EXAMPLE 7.

N - (7 - 2' - thienylacetamidoceph - 3 - em - 3 - ylmethyl) - pyridium - 4-carboxylate hydronitrate (1 g.; 2.09 millimoles) was dissolved with stirring in a mixture of pyridine (5 ml.; 4.9 g.; 62 millimoles) and methanol (5 ml.) at 40°C. After 15 minutes the precipitate was collected by filtration, washed with a 1:1 mixture of pyridine and methanol (10 ml.), methanol (20 ml.) and dried *in vacuo* at 40° to constant weight to give N - (7 - 2' - thienylacetamidoceph - 3 - em - 3 - ylmethyl) - pyridinium-4-carboxylate (0.45 g.; 52% of theory) with  $[\alpha]_D^{20} + 45$ , pH 4.6 (c, 1 in water) and containing less than 2% of the hydronitrate by potentiometric titration.

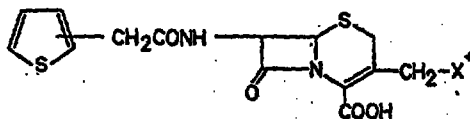
## EXAMPLE 8.

Anhydrous sodium carbonate (0.53 g.; 5.025 millimoles) was added to a solution of the hydronitrate (4.8 g.; 10.05 millimoles) of N - (7 - 2' - thienylacetamido - ceph - 3 - em - 3 - ylmethyl) - pyridium - 4 - carboxylate in N,N - dimethylacetamide (25 mls) and the suspension was stirred at 40° for 45 minutes. The reaction mixture was clarified by filtration. The filtrate was "seeded" with a small quantity of N-(7-2'-thienylacetamidoceph - 3 - em - 3 - ylmethyl) - pyridium - 4 - carboxylate and stirred at room temperature for 30 minutes but no precipitate formed. Methanol (70 ml) was then added to the stirred solution and a precipitate slowly formed. After 1 hour the precipitate was collected by filtration, washed with methanol (40 ml.), and dried *in vacuo* at 30° for 24 hours to give N-(7-2'-thienylacetamido-ceph-3-em-3-ylmethyl)-pyridinium-4-carboxylate (2.88 g, 69% of theory) with  $[\alpha]_D^{20} + 47.8$ ° pH 3.6 (c 1 in water))  $\lambda_{\text{max}}^{\text{H}_2\text{O}}$  at 240 m $\mu$  E 1% 1 cm 366,  $\lambda$  infl. at 255 m $\mu$  E 1% 1 cm 336. Potentiometric titration showed the possible presence of approximately 3.5% of N-(7-2'-thienylacetamido-ceph-3-em-3-ylmethyl)pyridinium-4-carboxylate hydronitrate.

## EXAMPLE 9.

N - (7 - 2' - Thienylacetamidoceph - 3 - em - 3 - ylmethyl) - pyridinium - 4-carboxylate hydronitrate (9.6 g.) was dissolved in N,N'-dimethylacetamide (50 ml.). Triethylamine (3.1 ml.) was added with continued stirring. The N-(7-2'-thienylacetamidoceph - 3 - em - 3 - ylmethyl) - pyridinium - 4 - carboxylate was collected by filtration, washed with 1:1 acetone/dimethylacetamide mixture (25 ml.), followed by acetone (100 ml.) and dried at 30° *in vacuo* for 16 hours. Yield 7.54 g., 90.6% theory,  $[\alpha]_D + 46.7$ ° [c, 1.0 in water]; pH 4.75. [c, 1.0 in water].

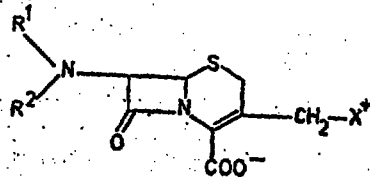
In Application No. 39172/66 (Serial No. 1,101,552), we have described and claimed salts containing cations of the general formula:—



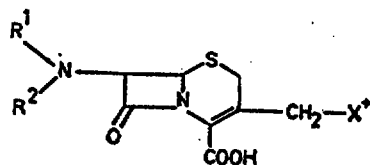
where X is an N-pyridyl or substituted N-pyridyl group. A specific salt of this type which is claimed therein is N - (7 - 2' - thienylacetamidoceph - 3 - em - 3 - ylmethyl) - pyridinium-4-carboxylate hydronitrate.

## WHAT WE CLAIM IS:—

1. A process for the preparation of a betaine of the general formula:—



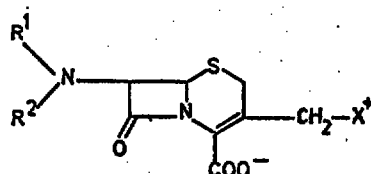
where  $R^1$  is a hydrogen atom and  $R^2$  is an acyl group  
or  $R^1$  and  $R^2$  are both acyl groups,  
and X is a quaternary ammonium group,  
which comprises reacting a salt containing cations of the general formula:—



(I)

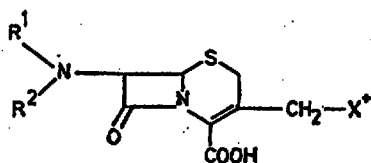
where  $R^1$ ,  $R^2$  and X have the above defined meanings, with a base.

2. A process for the preparation of a betaine having the general formula:—



(II)

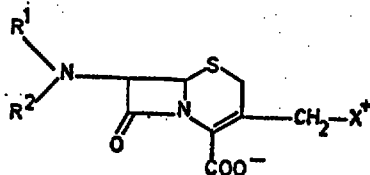
where  $R^1$  is a hydrogen atom,  $R^2$  is an acyl group and X is a quaternary ammonium group, which comprises reacting a salt containing cations of the general formula:—



(I)

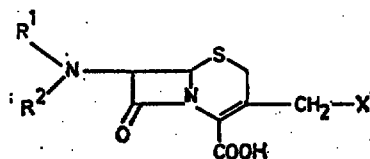
where  $R^1$ ,  $R^2$  and X have the above defined meanings, in an aqueous medium with an anion exchanger in the base or weak acid salt form to yield said betaine or a weak acid salt thereof.

3. A process for the preparation of a betaine having the general formula:—



(II)

where  $R^1$  is a hydrogen atom,  $R^2$  is an acyl group and X is a quaternary ammonium group, which comprises reacting a salt containing cations of the general formula:—



(I)

where  $R^1$ ,  $R^2$  and X have the above defined meanings, in an aqueous medium with an organic base.



4. A process as claimed in claims 2 or 3 in which the betaine is recovered from the aqueous medium by crystallisation or freeze drying.

5. A process for the purification of a crude aqueous solution of a betaine as defined in claim 1 which comprises reacting said solution with an acid having a pKa of 4 or less, recovering the resultant salt and regenerating the betaine from said salt as defined in claim 2 or claim 3.

6. A process for the regeneration of a betaine as defined in claim 1 which comprises reacting a solution of a salt as defined in claim 1 in an organic solvent with a base to form a soluble acid addition salt of said base and recovering said betaine from the reaction mixture.

7. A process as claimed in claim 6 in which said base is an organic base having a pKb of less than 6.

8. A process as claimed in claim 6 or claim 7 in which said base is a liquid tertiary amine or liquid secondary amine.

9. A process as claimed in claim 8 in which the liquid tertiary amine is a tri-(loweralkyl)amine.

10. A process as claimed in claim 6 in which the base is an inert inorganic base.

11. A process as claimed in any of claims 6—10 in which recovery of the betaine is facilitated by addition of an anti-solvent to the reaction mixture.

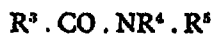
12. A process as claimed in claim 11 in which the anti-solvent is acetone.

13. A process as claimed in any of claims 6—10 in which the betaine is recovered by direct crystallisation from the reaction mixture.

14. A process as claimed in any of claims 6—13 in which the organic solvent has the group:



15. A process as claimed in claim 14 in which the organic solvent has the general formula:



where R<sup>3</sup> is a hydrogen atom or an alkyl group containing 1 to 5 carbon atoms and R<sup>4</sup> and R<sup>5</sup> which may be the same or different are each an alkyl group or, alternatively, R<sup>4</sup> and R<sup>5</sup> together form a divalent aliphatic group which, together with the adjacent nitrogen atom, forms a heterocyclic ring.

16. A process as claimed in claim 15 in which the solvent is N,N-dimethylformamide, N,N-diethylformamide, N,N-dipropylformamide, N,N-dibutylformamide, N,N-dimethylacetamide, N,N-diethylacetamide, N,N-dimethylvaleramide, N,N-dimethylpropionamide, N-formylpiperidine or N-formylmorpholine.

17. A process as claimed in claim 14 in which the solvent is an N,N-dialkylcyanamide.

18. A process as claimed in claim 14 in which the solvent has the general formula



in which R<sup>4</sup> and R<sup>5</sup> have the meanings defined in claim 15.

19. A process as claimed in any of claims 6—13 in which the organic solvent has the general formula:—



in which R<sup>4</sup> and R<sup>5</sup> have the meanings defined in claim 15.

20. A process as claimed in any of the preceding claims in which X is a quaternary amino group derived from a mononuclear heterocycle.

21. A process as claimed in any of the preceding claims in which the anion of the salt is derived from an acid having a pKa of 4 or less.

22. A process as claimed in any of the preceding claims in which the group R<sup>2</sup> and, where appropriate, R<sup>1</sup> is/are the groups R<sup>1</sup>(CH<sub>2</sub>)<sub>n</sub>CO— where R<sup>1</sup> is aryl, cycloalkyl, substituted aryl, substituted cycloalkyl or heterocyclic group and n is an integer from 1 to 8.

23. A process as claimed in claim 22 in which n is an integer from 1 to 4.

24. A process as claimed in claim 22 or 23 in which the heterocyclic ring is a 5- or 6-membered ring containing one or more of O, S, and N, or such a ring fused to a benzene ring.

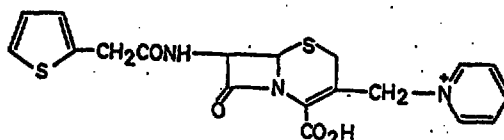


24. A process as claimed in claim 22 in which the group  $R^1(CH_2)_nCO-$  is a phenylacetyl, nitrophenylacetyl, phenylpropionyl or thienylacetyl group.

25. A process as claimed in any of the preceding claims in which X represents an N-pyridyl group or substituted N-pyridyl group.

26. A process as claimed in any of the preceding claims in which the anions of the salt are derived from trichloroacetic, sulphuric, perchloric, nitric, hydrochloric, hydrobromic, hydriodic, acetic or formic acid.

27. A process as claimed in any of the preceding claims utilising salts containing cations of the formula



28. A process as claimed in claim 27 in which the anion of said salt is bromide, hydrogen phosphate, chloride, perchlorate, hydrogen sulphate, tetrafluoroborate, iodide or trichloroacetate.

29. A process as claimed in any of claims 1—27 in which the salt is N-(7-2'-thienylacetamidoceph-3-em-3-ylmethyl)-pyridinium-4-carboxylate hydronitrate.

30. A process for regeneration and recovery of the betaine, N-(7-2'-thienylacetamidoceph-3-em-3-ylmethyl)-pyridinium-4-carboxylate, from an acid addition salt thereof with an acid having a pKa of 4 or less which comprises reacting an organic solution of said salt with a base thereby forming an organic solution of an acid addition salt of said base and recovering said betaine from the organic medium.

31. A process as claimed in claim 30 in which said betaine is recovered from the organic medium by direct crystallisation.

32. A process as claimed in claim 30 in which said betaine is recovered by adding an anti-solvent for the betaine to the organic medium and recovering the precipitated betaine.

33. A process for the regeneration and recovery of the betaine, N-(7-2'-thienylacetamidoceph-3-em-3-ylmethyl)-pyridinium-4-carboxylate, from an acid addition salt thereof with an acid having a pKa of 4 or less which comprises reacting a solution of said salt in a solvent of the formula



where  $R^3$  is selected from the group consisting of hydrogen and alkyl groups containing 1—5 carbon atoms and  $R^4$  and  $R^5$  are each an alkyl group containing 1—5 carbon atoms, with an organic base having a pKb of less than 6 to form a soluble acid addition salt of said base and recovering said betaine from said solvent.

34. A process as claimed in claim 33 in which said acid is nitric acid.

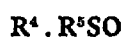
35. A process as claimed in claim 33 or 34 in which said solvent is dimethylformamide.

36. A process as claimed in claim 33 or 34 in which said solvent is dimethylacetamide.

37. A process as claimed in any of claims 33—36 in which the betaine is recovered by direct crystallisation.

38. A process as claimed in any of claims 33—36 in which the betaine is recovered by contacting the solvent with a lower ketone and recovering the precipitated betaine.

39. A process for the regeneration and recovery of the betaine, N-(7-2'-thienylacetamidoceph-3-em-3-ylmethyl)-pyridinium-4-carboxylate, from an acid addition salt thereof with an acid having a pKa of 4 or less which comprises reacting a solution of said salt in a solvent of the formula



where  $R^4$  and  $R^5$  are each an alkyl group containing 1—5 carbon atoms, with an organic base having a pKb of less than 6 to form a soluble acid addition salt of said base and recovering said betaine from said solvent.

40. A process for the regeneration and recovery of the betaine, N-(7-2'-thienylacetamidoceph-3-em-3-ylmethyl)-pyridinium-4-carboxylate, from an acid addition salt



thereof with an acid having a pKa of not greater than 4 which comprises reacting a solution of said salt in a solvent of the formula



where  $R^3$  is selected from the group consisting of hydrogen and alkyl group containing 1—5 carbon atoms and  $R^4$  and  $R^5$  are each alkyl groups containing 1—5 carbon atoms, with an organic tertiary base having a pKb of less than 6 to form an acid addition salt of said base and recovering said betaine from said solvent.

41. A process as defined in claim 40 in which said base is a tri(C1—C6alkyl)-amine.

42. A process as claimed in claim 1 substantially as herein described.

43. A process as claimed in claim 1 substantially as herein described in any of the Examples.

44. Betaines as defined in claim 1 whenever prepared by a process as claimed in any of the preceding claims.

45. The crystalline form of N-(7-2'-thienylacetamidoceph-3-em-3-ylmethyl)-pyridinium-4-carboxylate characterized by the following X-ray diffraction data:—

| Interplanar Spacing Å | 2θ (CuKα), degrees |
|-----------------------|--------------------|
| 14.6                  | 6.05               |
| 7.38                  | 11.97              |
| 6.82                  | 12.97              |
| 6.39                  | 13.85              |
| 5.68                  | 15.57              |
| 5.57                  | 15.90              |
| 5.29                  | 16.75              |
| 4.92                  | 18.02              |
| 4.80                  | 18.47              |
| 4.49                  | 19.77              |
| 4.22                  | 21.05              |
| 4.08                  | 21.77              |
| 3.89                  | 22.85              |
| 3.82                  | 23.27              |
| 3.74                  | 23.77              |
| 3.69                  | 24.07              |
| 3.66                  | 24.27              |
| 3.55                  | 25.00              |
| 3.47                  | 25.62              |
| 3.38                  | 26.30              |
| 3.35                  | 26.57              |



| Interplanar Spacing Å | 2θ (CuKα), degrees |
|-----------------------|--------------------|
| 3.29                  | 27.05              |
| 3.20                  | 27.82              |
| 3.14                  | 28.40              |
| 3.08                  | 28.97              |
| 3.00                  | 29.75              |
| 2.96                  | 30.20              |
| 2.82                  | 31.67              |
| 2.77                  | 32.30              |
| 2.73                  | 32.80              |
| 2.67                  | 33.50              |
| 2.65                  | 33.77              |
| 2.58                  | 34.70              |
| 2.55                  | 35.12              |
| 2.54                  | 35.25              |
| 2.51                  | 35.72              |
| 2.47                  | 36.35              |
| 2.43                  | 36.90              |
| 2.41                  | 37.27              |
| 2.37                  | 37.90              |
| 2.35                  | 38.30              |
| 2.32                  | 38.80              |
| 2.24                  | 40.15              |
| 2.18                  | 41.35              |
| 2.14                  | 42.25              |



46. The crystalline form of N-(7-2'-thienylacetamidoceph-3-em-3-ylmethyl)-pyridinium-4-carboxylate characterized by the following X-ray diffraction data:—

| Interplanar Spacing Å | 2 $\theta$ (CuK $\alpha$ ), degrees |
|-----------------------|-------------------------------------|
| 14.48                 | 6.10                                |
| 8.32                  | 10.62                               |
| 7.43                  | 11.90                               |
| 6.79                  | 13.02                               |
| 6.46                  | 13.70                               |
| 6.01                  | 14.72                               |
| 5.77                  | 15.35                               |
| 5.65                  | 15.67                               |
| 5.56                  | 15.92                               |
| 5.35                  | 16.55                               |
| 5.29                  | 16.75                               |
| 4.94                  | 17.92                               |
| 4.82                  | 18.40                               |
| 4.62                  | 19.20                               |
| 4.51                  | 19.67                               |
| 4.33                  | 20.47                               |
| 4.30                  | 20.62                               |
| 4.22                  | 21.02                               |
| 4.09                  | 21.70                               |
| 3.79                  | 22.37                               |
| 3.86                  | 23.05                               |
| 3.79                  | 23.42                               |
| 3.70                  | 24.02                               |
| 3.65                  | 24.40                               |
| 3.63                  | 24.52                               |
| 3.58                  | 24.85                               |
| 3.53                  | 25.20                               |
| 3.50                  | 25.42                               |
| 3.42                  | 26.00                               |



ylmethyl)  
data: —

| Interplanar Spacing Å | 2 $\theta$ (CuK $\alpha$ ), degrees |
|-----------------------|-------------------------------------|
| 3.41                  | 26.15                               |
| 3.35                  | 26.62                               |
| 3.31                  | 26.90                               |
| 3.24                  | 27.55                               |
| 3.17                  | 28.10                               |
| 3.07                  | 29.10                               |
| 3.01                  | 29.67                               |
| 2.98                  | 29.95                               |
| 2.87                  | 31.10                               |
| 2.82                  | 31.67                               |
| 2.79                  | 32.10                               |
| 2.77                  | 32.32                               |
| 2.70                  | 33.10                               |
| 2.68                  | 33.40                               |
| 2.62                  | 34.20                               |
| 2.59                  | 34.65                               |
| 2.56                  | 35.00                               |
| 2.558                 | 35.05                               |
| 2.48                  | 36.12                               |
| 2.46                  | 36.45                               |
| 2.43                  | 36.97                               |
| 2.39                  | 37.62                               |
| 2.37                  | 37.97                               |
| 2.35                  | 38.30                               |
| 2.32                  | 38.82                               |
| 2.30                  | 39.27                               |
| 2.26                  | 39.92                               |
| 2.24                  | 40.30                               |
| 2.23                  | 40.42                               |



\* C L N S \*

| Interplanar Spacing Å | 2θ (CuKα), degrees |
|-----------------------|--------------------|
| 2.21                  | 40.77              |
| 2.18                  | 41.47              |
| 2.15                  | 42.00              |
| 2.12                  | 42.67              |

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## ERRATA

### SPECIFICATION No. 1,101,561

- Page 3, line 3, after "trichloroacetic" insert  
 "acid"
- Page 6, line 12, for "arbitrary" read "arbi-  
 tary"
- Page 7, Table III, column 5, 2nd entry, for  
 "3.40 x" read "3.40—"
- Page 8, line 49, for "cc, 0.098; water)." read  
 "c, 0.098; water)"
- Page 8, line 60, for "0," read "0°"
- Page 9, line 10, for "trichloroacetate" read  
 "trichloracetate"
- Page 9, line 23, for "solution" read "solu-  
 tions"
- Page 10, line 2, for "dimethacetamide" read  
 "dimethylacetamide"
- Page 10, line 5, for "47.4;" read "47.4°;"
- Page 10, lines 8, 19 and 22, for "pyridium"  
 read "pyridinium"
- Page 10, line 27, after "(c" insert comma
- Page 11, line 1, for "R," read "R<sup>1</sup>"
- Page 12, line 35, for "diethylaceamide" read  
 "diethylacetamide"
- Page 16, 2nd Column, for "75.35" read  
 "15.35"
- Page 17, 1st Column, for "2.56" read "2.562"
- THE PATENT OFFICE  
 25th March 1968

