1 565 209

PATENT SPECIFICATION

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A5B 170 180 213 216 21Y H

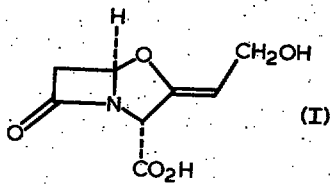
(72) Inventors THOMAS TREFOR HOWARTH and ROGER JOHN PONSFORD

(54) CLAVULANIC ACID DERIVATIVES

(71) We, BEECHAM GROUP LIMITED, a British Company of Beecham House, Great West Road, Brentford, 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:—

The present invention relates to β-lactam containing compounds, to their preparation and to compositions containing them.

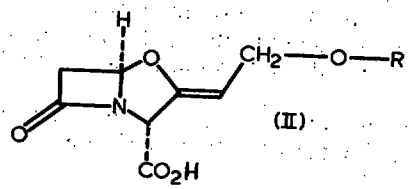
British Patent Specification Nos. 1508977 and 1508978 (see also Belgian Patent Specification No. 827926) disclose *inter alia* clavulanic acid and its salts and esters, which substance clavulanic acid has the formula (I):



(I)

Clavulanic acid and its salts and esters are able to inhibit β-lactamases from a range of bacteria and owing to this useful property are able to enhance the effectiveness of penicillins and cephalosporins against many gram-positive and gram-negative bacteria. It has now been discovered that certain derivatives of clavulanic acid also possess useful anti-bacterial and β-lactamase inhibitory activity.

Accordingly the present invention provides ethers of the formula (II):

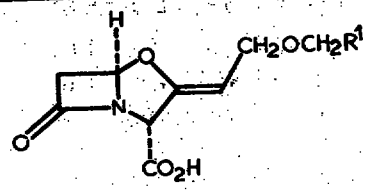


(II)

and salts and esters thereof wherein R is an inert etherifying organic group of up to 18 carbon atoms.

The terms "inert" and "inertly substituted" herein means that the moiety to which the term refers is not unstable. It will be appreciated that a chemically unstable compound is inappropriate for use as a medicinal agent in the treatment of bacterial infections.

Aptly the compound of the formula (II) is of the formula (III):



(III)

wherein CH_2R^1 is an inert etherifying group R as defined in relation to formula (II).

Suitable groups R for inclusion in the compounds of the formula (II) include hydrocarbon groups such as alkyl, alkenyl or alkynyl group or such groups substituted by a phenyl group. Other suitable groups include hydrocarbon groups inertly substituted by a halogen atom or a OR^2 group, a OH group, a $O.CO.R^2$ group, a CO_2R^2 group, a NR^3COR^2 group, a $NR^3CO_2R^2$ group, a SOR^2 group, a SO_2R^2 group, a CO_2R^2 group, a NH_2 group, a NR^2R^3 group, a NO_2, a CN group or a $CONR^2R^3$ group wherein R^2 is a hydrocarbon group of up to 8 carbon atoms and R^3 is a hydrocarbon group of up to 4 carbon atoms. Favoured values for the R^2 groups include the methyl, ethyl, propyl, butyl, phenyl and benzyl groups. A preferred value for the R^2 group is the methyl group. A preferred value for the R^3 group is the methyl group.

An apt value for R is the methyl group. Other favoured values for R include the groups of the formula CH_2R^1 where R^1 is an alkyl group of 1 to 4 carbon atoms, which is to say R^1 is favourably a methyl, ethyl, propyl or butyl group. Further favoured values for R are those of the formula CH_2R^1 where R^1 is a phenyl group or a naphthyl group or a phenyl group substituted by a halgen or a group R^4 or OR^4 wherein R^4 is a methyl, ethyl or propyl group. Such compounds have good anti-staphyloccocal activity.

Other suitable values for R are those of the formula

$$—CH_2—X—CO_2H \quad \text{and} \quad —CH_2—X—CO_2R^5$$

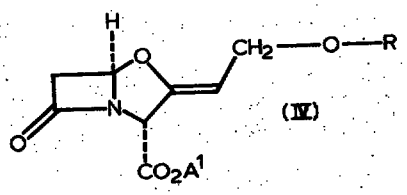
wherein X is a bond or CH_2 and R^5 is a methyl, ethyl, propyl or butyl group or a methyl, ethyl, propyl or butyl group substituted by a phenyl group.

Further suitable values for R are those of the formula $—CH_2—Y—NR^6R^7$ wherein $—CH_2—Y—$ is an alkylene group of 2 to 4 carbon atoms or an alkylene group of 2 to 4 carbon atoms substituted by a phenyl group; R^6 is a hydrogen atom or a methyl, ethyl, propyl or butyl group; and R^7 is a hydrogen atom or a group R^8, COR^8 or CO_2R^8 wherein R^8 is a methyl, ethyl, propyl or butyl group or a methyl, ethyl, propyl or butyl group substituted by a phenyl group. Aptly Y is a CH_2 group. Aptly R_6 is a methyl. Aptly R^7 is a hydrogen atom. Aptly R^7 is a benzyloxycarbonyl group.

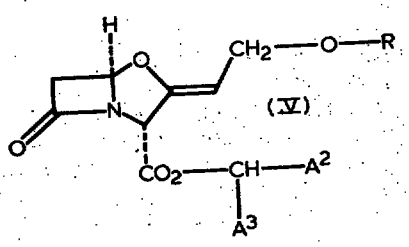
Other values of R worthy of mention include the ethoxycarbonylmethyl benzyloxycarbonylmethyl, carboxymethyl, cyanomethyl, *p*-nitrobenzyl, allyl, 2-benzyloxycarbonylethyl, 2-carboxyethyl, acetonyl, 4-chlorophenacyl, 3-hydroxypropyl and 2-(N-benzyloxycarbonyl-N-methylamino)ethyl groups.

The compounds of this invention are suitably provided as the acid or more suitably as a salt which, since the compounds of this invention are intended for pharmaceutical use, is normally a pharmaceutically acceptable salt. Suitable pharmaceutically acceptable salts include the sodium, potassium, calcium and magnesium salts. Other suitable salts include the lithium, ammonium and conventional substituted ammonium salts such as monoalkylamine, dialkylamine, trialkylamine (for example trimethylamine or triethylamine) or quaternary ammonium salts.

The compounds of this invention may be provided in the form of an ester. Suitable esters include those of the formulae (IV) and (V):



(IV)



(V)

wherein R is as defined in relation to formula (II) and A^1 is an alkyl group of 1 to 8 carbon atoms optionally substituted by halogen or a group of the formula OA^4, $OCOA^4$, SA^4 or SO_2A^4 wherein A^4 is a hydrocarbon group of up to 6 carbon atoms; A^2 is a hydrogen atom, an alkyl group of up to 4 carbon atoms or a phenyl group optionally substituted by halogen or by a group A^5 or OA^5 where A^5 is an alkyl group of up to 6 carbon atoms; and A^3 is a phenyl group optionally substituted by halogen or by a group A^5 or OA^5 where A^5 is an alkyl group of up to 6 carbon atoms.

An apt value for A^1 is the methyl group. An apt value for CHA^2A^3 is the benzyl group.

The esters within this invention are preferably *in vivo* hydrolysable. Suitable esters include those described in Belgian Patent Specification No. 827926 as being *in vivo* hydrolysable when attached to clavulanic acid. Particularly suitable *in vivo* hydrolysable ester forming groups include acetoxymethyl, α-acetoxyethyl, pivaloyloxymethyl, phthalidyl, ethoxycarbonyloxymethyl and α-ethoxycarbonyloxyethyl.

Compounds worthy of mention include 9-O-methylclavulanic acid and its pharmaceutically acceptable salts and esters. Suitable esters of 9-O-methylclavulanic acid include its methyl and benzyl esters. Suitable pharmaceutically acceptable salts of 9-O-methylclavulanic acid include the sodium, potassium, calcium and magnesium salts.

The present invention also provides pharmaceutical compositions which comprise a compound of this invention and a pharmaceutically acceptable carrier therefor.

The compositions of the invention include those in a form adapted for oral, topical or parenteral use and may be used for the treatment of infection in mammals, including humans.

Suitable forms of the compositions of this invention include tablets, capsules, creams, syrups, suspensions, solutions, reconstitutable powders and sterile forms suitable for injection or infusion. Such compositions may contain conventional pharmaceutically acceptable materials such as diluents, binders, colours, flavours, preservatives and disintegrants in accordance with conventional pharmaceutical practice in the manner well understood by those skilled in the art of formulating antibiotics.

Injectable or infusable compositions of salts of a compound of the formula (II) are particularly suitable as high tissue levels of a compound of the formula (II) can occur after administration by injection or infusion. Thus, one preferred composition aspect of this invention comprises a salt of a compound of the formula (II) in sterile form.

Unit dose compositions comprising a compound of the formula (II) or a salt or ester thereof adapted for oral administration form a further preferred composition aspect of this invention.

The compound of the formula (II) or its pharmaceutically acceptable salt or ester may be present in the composition as sole therapeutic agent. However, the compound of the formula (II) or its pharmaceutically acceptable salt or ester may favourably be present with a penicillin or cephalosporin. Suitable penicillins for inclusion in such synergistic compositions include benzylpenicillin, phenoxymethylpenicillin, propicillin, ampicillin, amoxycillin, epicillin, cyclacillin, hetacillin, metampicillin or the acetoxymethyl, pivaloyloxymethyl or phthalidyl esters of benzylpenicillin, ampicillin or amoxycillin or the phenyl, tolyl or indanyl α-esters of carbenicillin or ticarcillin. Other penicillins suitable for inclusion in such compositions include ticarcillin and carbenicillin. Suitable cephalosporins for use in such compositions include cephaloridine, cephalothin, cefazolin, cephalexin, cephacetrile, cephradine, cephapirin and cephaloglycine. Such penicillins and cephalosporins may be used in the form of their pharmaceutically acceptable salt or hydrate.

Naturally if the penicillin or cephalosporin present in the composition is not suitable or oral administration then the composition will be adapted for parenteral administration.

When in a pharmaceutical composition together with a penicllin or cephalosporin the weight ratio of a compound of the formula (II) or its pharmaceutically acceptable salt or ester present to penicillin or cephalosporin present may vary over a wide range of ratios, for example 10:1. Aptly a ratio between 3:1 and 1:3 may be used; advantageously this may be a ratio such as 1:2 or 1:1.

The total quantity of antibacterial agents present in any unit dosage form will normally be between 50 and 1500 mg and will usually be between 100 and 1000 mg.

Compositions of this invention may be used for the treatment of bacterial infections of mammals excluding humans, e.g. of, *inter alia*, the respiratory tract, the urinary tract and soft tissues and mastitis in cattle.

Normally between 50 and 3000 mg of the compounds of the invention will be administered each day of treatment but more usually between 100 and 1000 mg of the compounds of the invention will be administered per day, for example as 1 to 6 doses, more usually 2 to 4 doses.

The penicillin or cephalosporin in synergistic compositions of this invention will normally be present by up to or at approximately the amount at which it is conventionally used.

Particularly favoured compositions of this invention will contain from 150—1000 mg of amoxycillin, ampicillin or a pro-drug therefor and from 50—500 mg of a compound of the formula (II) or a salt or *in vivo* hydrolysable ester thereof and more suitably from 200—500 mg of amoxycillin, ampicillin or a pro-drug therefor and from 50—250 mg of a compound of the formula (II) or a salt or *in vivo* hydrolysable ester thereof.

The materials present in such compositions may be hydrated, if required, for example ampicillin trihydrate or amoxycillin trihydrate may be employed. The weights of the antibiotics in such compositions are expressed on the basis of antibiotic theoretically available from the composition and not on the basis of the weight of pro-drug.

The present invention also provides a process for the preparation of a compound of the formula (II) as hereinbefore defined which process comprises the etherification of an ester of clavulanic acid and thereafter if desired cleaving a thus formed ester of the compound of the formula (II) by hydrogenolysis or hydrolysis to yield the compound of the formula (II) or its salt.

The preferred method of preparing esters of the compound of the formula (II) is by the reaction of the ester of clavulanic acid and a diazo compound.

Normally this diazo process will be adapted to the preparation of an ester of a compound of the formula (III) by the reaction of an ester of clavulanic acid with a diazoalkane of the formula N_2—CHR^1.

Naturally any reactive moiety present in the diazo compound, such as an amino, carboxylic or hydroxyl group, is protected during a condensation reaction in a conventional manner such as protection with a benzyloxycarbonyl group for the amino or hydroxyl group and benzyl group for the carboxyl group. Such protecting groups may be removed by hydrogenation in conventional manner thereafter.

The reaction with the diazo compound will suitably take place in the presence of a Lewis acid catalyst such as boron trifluoride, for example as boron trifluoride etherate. Suitably the reaction takes place in a solvent such as tetrahydrofuran, methylene chloride, methanol, chloroform or dioxane. The reaction normally takes place at a depressed or non-elevated temperature, for example −80° to +30°C, and preferably at a depressed temperature, for example −30° to +10°C. A convenient temperature for carrying out the reaction is about −20°C to 0°C.

Esters of the compound of the formula (II) may also be prepared by the reaction of alcohol of the formula ROH where R is as defined in relation to formula (II) with the corresponding ester of clavulanic acid in the presence of a Lewis acid catalyst such as boron trifluoride or its equivalent, for example as $BF_3 . O(C_2H_5)_2$.

The preceding reaction normally takes place in a solvent inert under the reaction conditions such as chloroform, dichloromethane, tetrahydrofuran or dioxane at a depressed or non-elevated temperature, for example −80°C to +30°C, and preferably at a depressed temperature, for example −50°C to 0°C, and conveniently at about −30°C.

An alternative form of this method of preparation of esters of the compounds of the formula (II) comprises the reaction in the presence of a Lewis acid of an alcohol of the formula ROH wherein R is as defined in relation to formula (II) with the corresponding ester of an acylated derivative of clavulanic acid wherein the 9-position hydroxyl group is acylated so as to be of the sub-formula O . CO . R^9 wherein R^9 is a C_{1-6} alkyl group optionally inertly substituted by one or more halogen atoms.

The conditions for this reaction are similar to those outlined above for the reaction of the alcohol ROH with an ester of clavulanic acid.

The acylated compounds may be prepared as described in Belgian Patent No.

834645, that is by the reaction of a compound of the formula $R^9.CO_2H$ or a reactive acylating derivative thereof with the corresponding ester of clavulanic acid.

Salts of the compound of the formula (II) may be prepared from esters of the compound of the formula (II) by very mild basic hydrolysis, for example by hydrolysis in an aqueous solution maintained at pH 7 to 9 by the slow addition of base.

The compounds of the formula (II) and salts thereof may be prepared from hydrogenolysable esters of compounds of formula (II) by hydrogenation, e.g. using a medium or low pressure of hydrogen in the presence of a transition metal catalyst.

Palladium, for example 10% palladium on charcoal, has proved a particularly useful catalyst. We have found that a suitable ratio for the weight of 10% palladium on charcoal used to the weight of ether used is 1:3.

The hydrogenation reaction preferably occurs in a solution in a solvent which consists of or contains tetrahydrofuran.

Suitable hydrogenolysable esters include those of the formula (V) as hereinbefore defined. A preferred hydrogenolysable ester is the benzyl ester. A preferred hydrolysable ester is the methyl ester.

If a base such as sodium hydrogen carbonate, potassium hydrogen carbonate, lithium carbonate or calcium carbonate is included in the reaction mixture then the resulting compound is in the form of a salt. If no such base is included the product is in the form of the free acid.

Acids of the formula (II) may also be prepared by the careful acidification of the corresponding sodium or like salt.

From the preceding comments it will be appreciated that this invention provides a process for the preparation of the compounds of the formula (II) or a salt thereof which process comprises the de-esterification of an ester of a corresponding compound of the formula (II).

Esters of the compounds of the formula (II) may be prepared by the reaction of a salt of a compound of the formula (II) such as an alkali metal salt with an esterifying agent such as a halide or active ester such as a chloride, bromide, iodide, methanesulphonate or toluenesulphonate or by reaction of an acid of the formula (II) with a diazocompound. Such reactions proceed under conventional conditions.

Thus the present invention provides a process for the preparation of esters of the compounds of the formula (II) which process comprises the esterification of the corresponding compound of the formula (II) or a salt thereof.

Normally and preferably the processes of this invention are adapted to prepare those compounds of the formula (II) as hereinbefore described as being preferred compounds of this invention.

The esters of the compounds of the formula (II) may also be prepared by the etherification of the corresponding ester of clavulanic acid by reaction with a compound of the formula R—Q wherein Q is a group readily displaceable by an electron-rich moiety.

Suitably Q is a chlorine, bromine or iodine atom. Most suitably Q—R is a group of the formula $Q—CH_2R^1$ where R^1 is as hereinbefore defined.

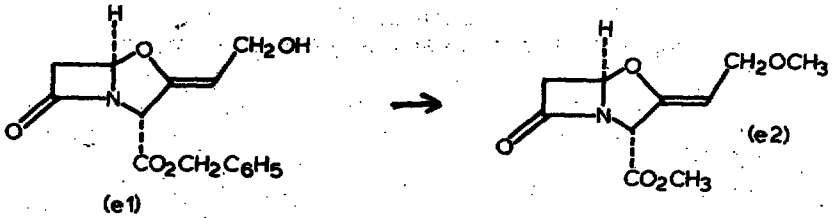
Normally the reaction is carried out in the presence of an acid acceptor such as collidine or dicyclohexylamine.

[The preparation of diazo compounds is described in Houben-Weyl, Methoden der Organischen Chemie, Vol 10/4. 4th Edition.]

The following Examples are illustrative of the invention:

Example 1

Preparation of Methyl 9-O-methylclavulanate



Benzyl clavulanate (e1) (1.5 g) in methanol (10 ml) was hydrogenated over 10%

palladium on charcoal (0.4 g) for ½ hour at ambient temperature and pressure. The solution was filtered through Celite (Registered Trade Mark) and treated with a solution of diazomethane in ether at 0°C. The solution was left at 0°C overnight, the solvent evaporated and the oil chromatographed on silica gel to yield as the second eluted product crude (e2) (35 mg). Re-chromatography provided the title product as a colourless oil (15 mg).

I.r. ($CHCl_3$): 1800, 1750, 1695 cm^{-1};

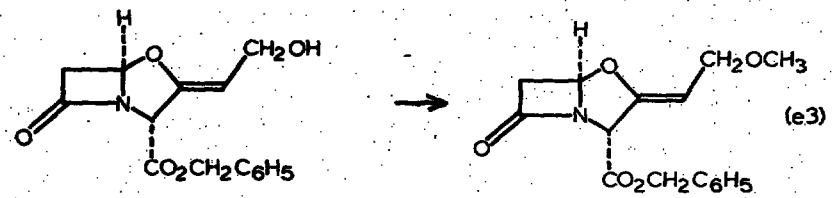
N.m.r. ($CDCl_3$): 3.03 (1H, dd, J 17Hz, J' 1Hz, 6β-*CH*), 3.53 (1H, dd, J 17Hz, J' 2.5Hz, 6α-*CH*), 3.31 (3H, s, OCH_3), 3.79 (3H, s, CO_2CH_3), 4.03 (2H, d, J 7Hz, CH_2OCH_3), 4.88 (1H, brt. J 7Hz, olefinic *CH*), 5.08 (1H, m, 3-*CH*), 5.70 δ (1H, dd, J 2.5Hz, J' 2.5Hz, 5-*CH*).

The approximate β-lactamase inhibition I_{50} values in μg/ml for (e2) were as follows:

Escherichia coli JT4	0.9
Klebsiella aerogenes E70	0.25
Staphylococcus aureus Russell	0.5
Escherichia coli JT410	3.1
P. moig. Cr.	0.68
Pseudomonas aeruginosa	1.9
Citrobacter mantio	0.45

Example 2

Preparation of Benzyl 9-O-methylclavulanate



(e1)

Benzyl clavulanate (e1) (300 mg) was dissolved in dry methylene dichloride (25 ml) and cooled to 0°C. Boron trifluoride etherate (5 drops) was added at 0°C, followed by a solution of diazomethane in ether. The reaction mixture was stirred at 0°C for one hour and washed with 3% sodium bicarbonate solution (2×25 ml). The organic phase was dried over magnesium sulphate and evaporated; chromatographic purification gave the title compound (e3) (66 mg).

I.r. ($CHCl_3$): 1800, 1745, 1695 cm^{-1};

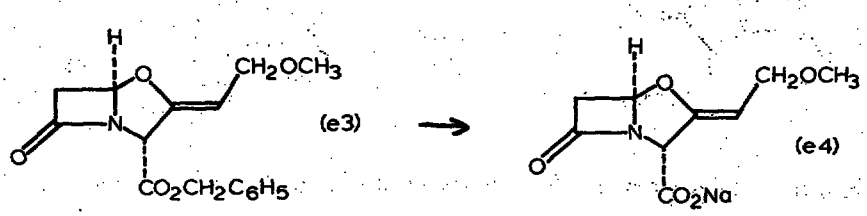
N.m.r. ($CDCl_3$): 3.10 (1H, d, J 17Hz, 6β-*CH*), 3.35 (3H, s, OCH_3), 3.60 (1H, dd, J 17Hz, J' 2.5Hz, 6α-*CH*), 4.12 (2H, d, J 8Hz, CH_2OCH_3), 4.94 (1H, t, J 8Hz, =*CH*—CH_2), 5.24 (1H, br.s, 3-*CH*), 5.32 (2H, s, CO_2CH_2Ph), 5.82 (1H, d, J 2.5Hz, 5-*CH*), 7.51 δ (5H, s, CO_2CH_2Ph).

The approximate β-lactamase inhibition I_{50} values in μg/ml for (e3) were as follows:

Escherichia coli JT4	0.1
Klebsiella aerogenes E70	0.05
Staphylococcus aureus Russell	0.05
Proteus mirabilis C889	<0.07
Pseudomonas aeruginosa	1.4
Pseudomonas dalgleish	<0.07
Citrobacter mantio	1.1

Example 3

Preparation of Sodium 9-O-methylclavulanate



The ether (e3) (30 mg) was dissolved in tetrahydrofuran (3 ml) and 10% palladium on charcoal (10 mg) added. The solution was hydrogenated at ambient temperature and pressure for 15 minutes, filtered and sodium bicarbonate (8.4 mg) in 0.5 ml water was added. The solvent was evaporated to yield the sodium salt (e4) as an amorphous solid after trituration with ether (15 mg).

I.r. (KBr disc): 1790, 1690, 1615 cm^{-1};

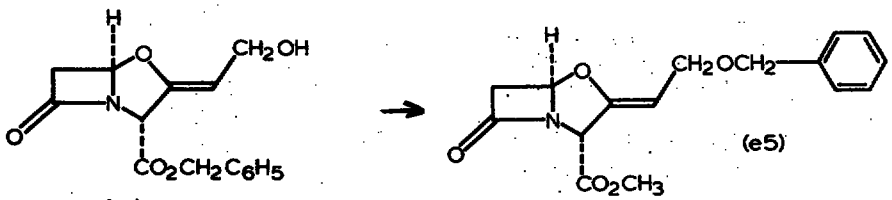
N.m.r. (D_2O): 3.07 (1H, d, J 17Hz, 6β-*CH*), 3.27 (3H, s, OCH_3), 3.54 (1H, dd, J 17Hz, J' 2.5Hz, 6α-*CH*), 4.04 (2H, d, J 8Hz, CH_2OCH_3), 4.87 (1H, t, J 8Hz, =*CH*—CH_2), 4.93 (1H, s, 3-*CH*), 5.69 δ (1H, d, J 2.5Hz, 5-*CH*).

The approximate β-lactamase inhibition I_{50} values in μg/ml for (e4) were as follows:

Escherichia coli JT4	0.18
Klebsiella aerogenes E70	0.07
Staphylococcus aureus Russell	0.05
Proteus mirabilis C889	0.01

Example 4

Preparation of Methyl 9-O-benzylclavulanate



(e1)

Benzyl clavulanate (e1) (300 mg) in methylene chloride (25 ml) was cooled to −30°C and treated with boron trifluoride etherate (5 drops), followed by a solution of diazotoluene in ether. The solution was stirred at −30°C for one hour and washed with 3% sodium bicarbonate solution (3×25 ml). The organic phase was dried over magnesium sulphate and evaporated to yield after chromatography crude benzyl 3 - (2 - benzyloxyethylidene) - 7 - oxo - 4 - oxa - 1 - aza - bicyclo[3,2,0]heptane - 2 - carboxylate (e5) (150 mg) as an oil. The oil was dissolved in tetrahydrofuran (5 ml) and hydrogenated at ambient temperature and pressure over 10% Pd/C (20 mg) for 20 minutes. The solution was filtered and treated with a solution of diazomethane in ether at 0°C. Evaporation of the solvent and chromatography provided the title product (e5) (30 mg) as a colourless oil.

I.R. ($CHCl_3$): 1800, 1750, 1695 cm^{-1};

N.m.r. ($CDCl_3$): 3.12 (1H, d, J 17Hz, 6β-*CH*), 3.62 (1H, dd, J 17Hz, J' 2.5Hz, 6α-*CH*), 3.89 (3H, s, CO_2CH_3), 4.25 (2H, d, J 8Hz, =CH—CH_2O), 4.61 (2H, s, OCH_2Ph), 5.02 (1H, br.t., J 8Hz, =*CH*—CH_2), 5.18 (1H, m, 3-*CH*), 5.82 (1H, d, J 2.5Hz, 5-*CH*), 7.49 δ (5H, s, OCH_2*Ph*);

$[\alpha]_D^{20}$=+15° (c=1.37; MeOH).

Antibacterial Activity In Vitro (μg/ml)

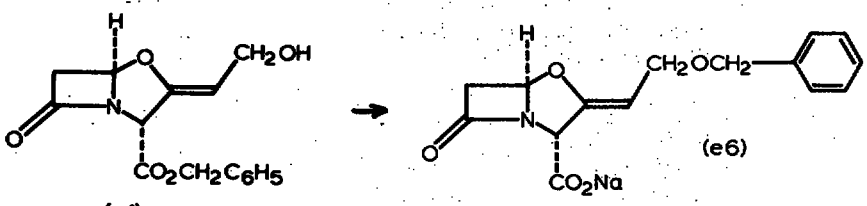
Staphylococcus aureus Oxford	15—31
Staphylococcus aureus Russell	8—15
Klebsiella aerogenes A.	>500

β-Lactamase Inhibition I_{50} (μg/ml)

Escherichia coli JT4	0.8
Klebsiella aerogenes E70	0.8
Staphylococcus aureus Russell	0.005

Example 5

Preparation of Sodium 9-O-benzylclavulanate



(e1)

Crude benzyl clavulanate (e1) (150 mg) was dissolved in tetrahydrofuran (5 ml) and hydrogenated at ambient temperature and pressure over 10% Pd/C (20 mg) for 20 minutes. The solution was filtered and treated with sodium bicarbonate (15 mg) in 1 ml water. The solvent was evaporated and the residue taken up in water (10 ml) and washed with ethyl acetate (3×10 ml). The aqueous phase was evaporated to yield the required sodium salt as an amorphous solid after trituration with ether (e6) (35 mg).

I.r. (KBr): 1790, 1690, 1615 cm^{-1};

N.m.r. (D_2O): 3.09 (1H, d, J 17Hz, 6β-*CH*), 3.59 (1H, dd, J 17Hz, J' 2.5Hz, 6α-*CH*), 4.20 (2H, d, J 8Hz, =CH—CH_2O), 4.48 (2H, s, OCH_2Ph), 4.97 (1H, br.t., J 8Hz, =*CH*—CH_2O), 4.99 (1H, m, 3-*CH*), 5.74 (1H, d, J 2.5Hz, 5-*CH*), 7.42 δ (5H, s, OCH_2*Ph*);

$[\alpha]_D^{20}$=+22.5° (c=1.15; 50% aqueous MeOH).

Antibacterial Activity In Vitro (μg/ml)

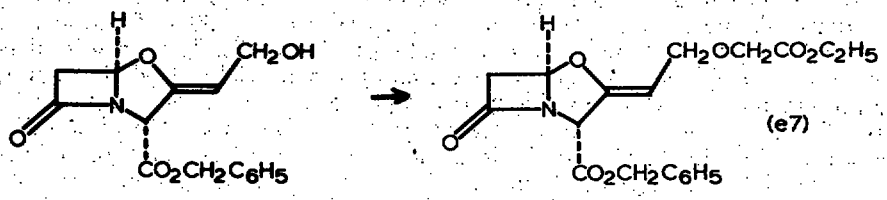
Klebsiella aerogenes A.	125
Staphylococcus aureus Oxford	4.0
Staphylococcus aureus Russell	8.0

β-Lactamase Inhibition I_{50} (μg/ml)

Escherichia coli JT4	0.1
Klebsiella aerogenes E70	0.04
Staphylococcus aureus Russell	0.005
Proteus mirabilis C889	0.02
Pseudomonas dalgleish	0.008

Example 6

Preparation of Benzyl 9-O-(ethoxycarbonylmethyl)clavulanate



Benzyl clavulanate (e1) (1.44 g) was dissolved in dry methylene dichloride (100 ml) and boron trifluoride etherate (25 drops; 0.3 ml) was added at −30°C. Ethyl diazoacetate (2.90 g; 5 equivalents) in methylene dichloride (10 ml) was added dropwise over half an hour and the mixture stirred at −30°C to −10°C for one hour. The solution was washed with 3% sodium bicarbonate solution (3×50 ml) and dried over magnesium sulphate. The solvent was evaporated at room temperature and the residue chromatographed to yield the title compound (e7) as a colourless oil (600 mg).

I.r. ($CHCl_3$): 1800, 1745, 1695 cm^{-1};

N.m.r. ($CDCl_3$): 1.29 (3H, t, J 8Hz, —$CO_2CH_2CH_3$), 3.00 (1H, d, J 17Hz, 6β-*CH*), 3.50 (1H, dd, J 17Hz, J' 2.5Hz, 6α-*CH*), 3.97 (2H, s, CH_2CO_2Et), 3.90 to 4.50 (4H, complex pattern, =CH—CH_2 and $CO_2CH_2CH_3$, 4.79 (1H, t, J 8Hz, =*CH*—CH_2), 5.14 (1H, brs, 3-*CH*), 5.23 (2H, s, CO_2CH_2Ph), 5.72 (1H, d, 2.5Hz, 5-*CH*), 7.37 δ (5H, s, CO_2CH_2*Ph*).

$[\alpha]_D^{21}$=+35.3° (c=1.29; MeOH).

The approximate β-lactamase inhibition I_{50} values in μg/ml for (e7) were as follows:

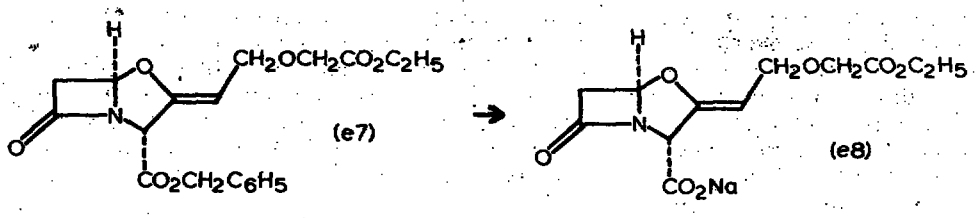
Escherichia coli JT4	0.14
Klebsiella aerogenes E70	0.30
Staphylococcus aureus Russell	0.12
Proteus mirabilis C889	1.0

Antibacterial Activity In Vitro (μg/ml)

Klebsiella aerogenes A.	>500
Staphylococcus aureus Oxford	125
Staphylococcus aureus Russell	125

Example 7

Preparation of Sodium 9-O-(ethoxycarbonylmethyl)clavulanate



The ester (e7) (188 mg) was dissolved in dry tetrahydrofuran (5 ml) and 10% Pd/C (65 mg) was added. The solution was hydrogenated at ambient temperature and pressure for fifteen minutes, the solution filtered through kieselguhr and sodium bicarbonate (42 mg) in water (1 ml) was added. The solvent was evaporated and the residue chromatographed on silica gel, eluting with butanol/ethanol/water, 4/1/1. Trituration of the product with ether gave the sodium salt as an amorphous solid (e8) (95 mg).

I.r. (KBr): 1790, 1740, 1690, 1600 cm^{-1};

N.m.r. (D_2O): 1.27 (3H, t, J 8Hz, $CO_2CH_2CH_3$), 3.09 (1H, d, J 17Hz, 6β-*CH*), 3.57 (1H, dd, J 17Hz, J' 2.5Hz, 6α-*CH*), 4.14 (2H, s, OCH_2CO_2Et), 4.21 (4H, doublet and quartet superimposed, J=J' 8Hz, =CH—CH_2— and $CO_2CH_2CH_3$), 4.86 (1H, t, J 8Hz, =*CH*—CH_2—), 4.96 (1H, brs, 3-*CH*), 4.71 δ (1H, d, J 2.5Hz, 5-*CH*).

$[\alpha]_D^{21}$=36.5° (c=1.15; 50% aqueous MeOH).

The approximate β-lactamase inhibition I_{50} values in μg/ml for (e8) were as follows:

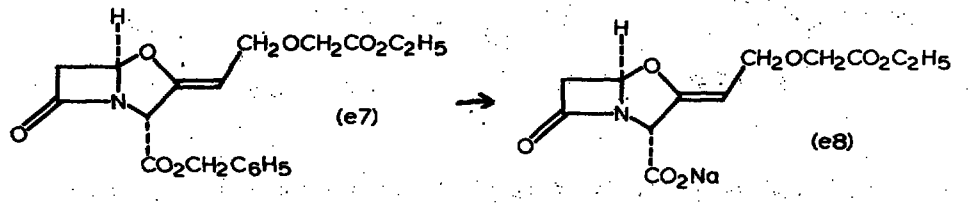
Escherichia coli JT4	0.16
Klebsiella aerogenes E70	0.034
Staphylococcus aureus Russell	0.044
Proteus mirabilis C889	0.025

Antibacterial Activity In Vitro (μg/ml)

Escherichia coli 10418	31.0
Klebsiella aerogenes A.	15.0
Proteus mirabilis C977	62.0
Staphylococcus aureus Oxford	15.0
Staphylococcus aureus Russell	15.0

Example 8

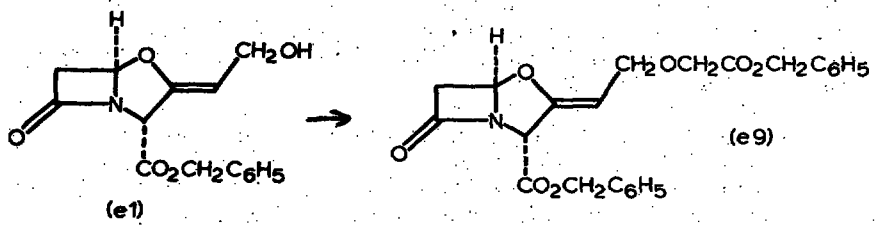
Preparation of Sodium 9-O-(ethoxycarbonylmethyl)clavulanate



The ester (e7) (112 mg) was hydrolysed using N NaOH at constant pH (using a pH stat) until hydrolysis was complete. Chromatography yielded the sodium salt as an amorphous solid after trituration with ether (e8) (15 mg).

Example 9

Preparation of Benzyl 9-O-(benzyloxycarbonylmethyl)clavulanate



Benzyl clavulanate (e1) (1.8 g) was dissolved in dry methylene chloride (100 ml) and boron trifluoride etherate (25 drops; 0.3 ml) was added at −30°C. Benzyl diazoacetate (5 g of 66% pure material) in methylene chloride (20 ml) at −30°C was added dropwise over half an hour and the mixture stirred at −30°C to −10°C for one hour. The solution was washed with 3% sodium bicarbonate solution (2×50 ml) and dried over magnesium sulphate. The solvent was evaporated at room temperature and the residue chromatographed over silica gel, eluting with hexane/ethyl acetate. The resulting somewhat impure oil was rechromatographed to yield pure title material (e9) as a colourless oil (0.32 g).

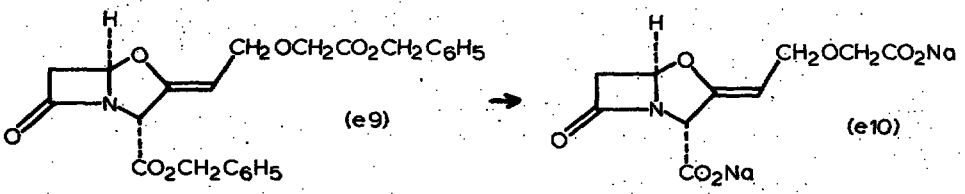
I.r. (film): 1800, 1760, 1750, 1695 cm^{-1};

N.m.r. ($CDCl_3$): 7.29 (10H, aromatic H), 5.58 (1H, d, J 2.5Hz, 5-*CH*), 5.13 (4H, CH_2Ph), 5.03 (1H, 3-*CH*), 4.79 (1H, t, J 7Hz, =C*H*—CH_2), 4.16 (2H, d, J 8Hz, =CH—CH_2—), 4.00 (2H, s, =CH—CH_2—O—CH_2—), 3.40—2.95 δ (2H, dd, J 18Hz, 6-CH_2).

The benzyl diazoacetate was prepared by the reaction of benzyl chloroformate and diazomethane at 4°C for 3 days which yielded a 66% pure product as an oil.

Example 10

Preparation of Di-sodium 9-O-(carboxymethyl)clavulanate

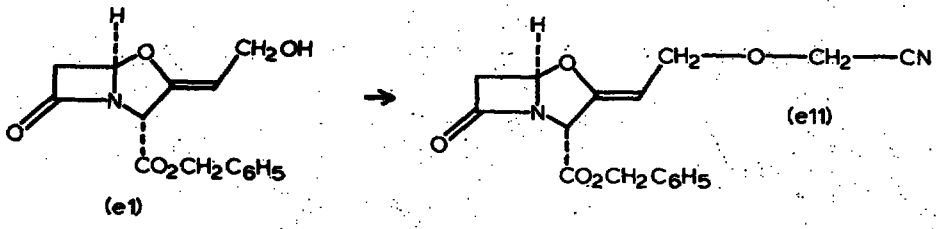


The ester (e9) (260 mg) was dissolved in a mixture of tetrahydrofuran and water (5:1, 12 ml) and sodium bicarbonate (100 mg) and 10% Pd/C (150 mg) were added. The solution was hydrogenated at ambient temperature and pressure for 30 minutes, filtered and evaporated to yield the desired disodium salt (e10).

I.r. (KBr): 1780, 1685, 1600, 1425 cm^{-1}.

Example 11

Preparation of Benzyl 9-O-(cyanomethyl)clavulanate

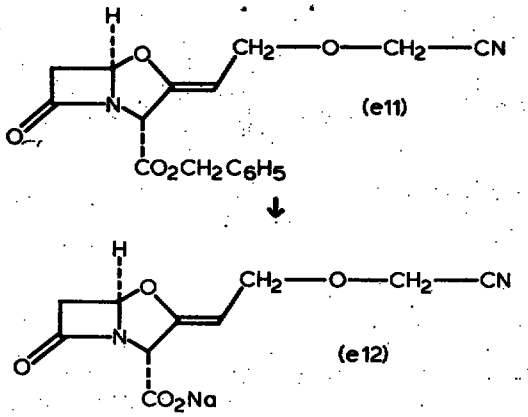


A solution of benzyl clavulanate (e1) (5.18 g) in methylene dichloride (50 ml) was dried over 4A molecular sieves and filtered. The solution was cooled to −40 — −30°C and 1 ml boron trifluoride etherate was added. A solution of diazoacetonitrile (prepared from aminoacetonitrile hydrochloride (9.3 g) as in Houben-Weyl vol 10/4) in methylene chloride (250 ml) was added dropwise over 1 hour, maintaining the temperature at −40 — −30°C. The mixture was then allowed to warm to −10°C. After 1 hour at −10°C the solution was washed with 3 portions of dilute sodium bicarbonate solution (200 ml), dried over calcium chloride and evaporated to give a brown gum. The product was purified by column chromatography (Kieselgel; cyclohexane:ethyl acetate 3:1 followed by 1:1) to give a slightly impure product. Repeat of the chromatography (same system) gave the desired product (e11) (1.56 g).

I.r. (film): 1805, 1750, 1698 cm^{-1}.

N.m.r. ($CDCl_3$): δ 3.02 (1H, doublet, J 17Hz), 3.45 (1H, double doublet, J 17Hz and 3Hz), 4.01 (2H, singlet), 4.14 (2H, doublet, J 7Hz), 4.70 (1H, triplet with fine coupling, Jtrip 7Hz), 5.06 (1H, singlet), 5.14 (2H, singlet), 5.66 (1H, singlet with fine coupling), 3.28 (5H, singlet).

Example 12
Preparation of Sodium 9-O-(cyanomethyl)clavulanate

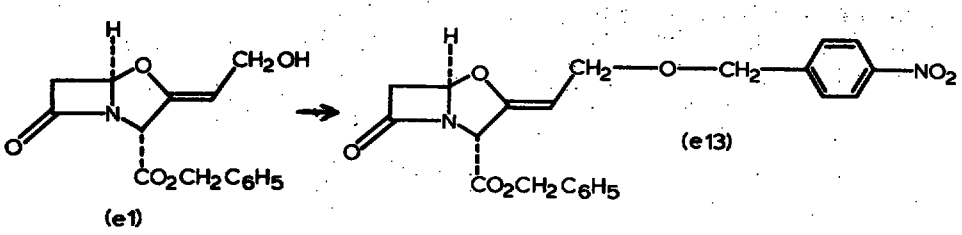


A mixture of benzyl 9-(O-cyanomethyl)clavulanate (e11) (1.56 g) and sodium bicarbonate (0.399 g) in tetrahydrofuran/water (30 ml 5:1 tetrahydrofuran:water) was hydrogenated over 10% palladium charcoal (0.5 g) at room temperature and atmospheric pressure, until the requisite amount of hydrogen had been absorbed. The mixture was then filtered and the catalyst washed with water. The combined filtrates were then evaporated until most of the tetrahydrofuran had been removed. The remaining aqueous solution was washed with ethyl acetate and freeze-dried to yield the desired product (e12) (913 mg) as a brown solid. A sample (535 mg) was passed through a short column of Kieselgel using n-butanol:H_2O:EtOH (4:1:1) solvent to yield (e12) (213 mg) as a pale yellow solid.

N.m.r. (D_2O): δ 3.24 (1H, doublet, J 16Hz), 3.66 (1H, double doublet, J 16Hz and 3Hz), 4.30 (2H, doublet, J 7Hz), 4.40 (2H, singlet), 4.91 (1H, triplet with fine coupling, J 7Hz), 5.00 (1H, singlet), 5.78 (1H, doublet, 3Hz).

I.r. (KBr disc): 1780, 1610 (broad) cm^{-1}.

Example 13
Preparation of Benzyl 9-O-(4-nitrobenzyl)clavulanate

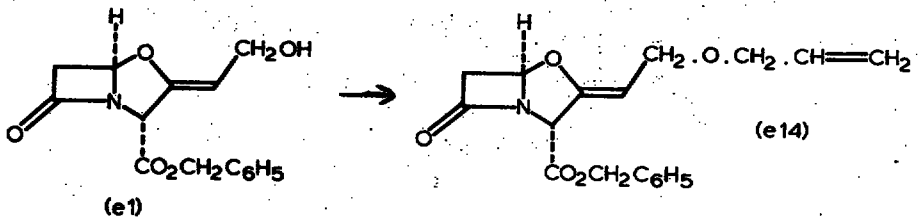


A solution of benzyl clavulanate (e1) (4.33 g) in methylene chloride (50 ml) was dried over 4A molecular sieves for ½ hour. The solution was then filtered and cooled to −30°C. Boron trifluoride etherate (0.8 ml) was then added. A solution of 4-nitrophenyl diazomethane (7.34 g) in methylene chloride (150 ml) was then added to the stirred solution at −30°C, over 1 hour. The mixture was then warmed to −10°C over a period of 1 hour. The solution was then washed with sodium bicarbonate solution (3×150 ml), dried over magnesium sulphate and evaporated. Repeated chromatography (twice with cyclohexane:ethyl acetate 1:1, and once with methylene chloride as eluant) on Kieselgel H gave the pure ether (e13) (230 mg).

I.r. (film): 1800, 1750, 1700, 1520 cm^{-1}.

N.m.r. ($CDCl_3$): 2.98 (1H, doublet, J 17Hz), 3.44 (1H, double doublet, J 17Hz and 3Hz), 4.09 (2H, doublet, J 7Hz), 3.45 (2H, singlet), 4.8 (1H, triplet with fine coupling, J 7Hz), 5.05 (1H, singlet), 5.14 (2H, singlet), 5.63 (1H, doublet with fine coupling), 7.27 (5H, singlet), 7.38 (2H, doublet, J 9Hz), 8.11 (2H, doublet, J 9Hz).

Example 14
Preparation of Benzyl 9-O-allylclavulanate



(i) N-Nitroso-N-allylbenzamide

A solution of nitrogen dioxide (24.1 g) in carbon tetrachloride (150 ml) was again to a stirred mixture of anhydrous sodium acetate (45 g) and carbon tetrachoride (150 ml) at −20°C. N-allylbenzamide (28.4 g) in carbon tetrachloride (150 ml) was added to the stirred mixture at −5°C which was then stirred at 0°C for 0.5 hour. This was washed with aqueous sodium bicarbonate (2×400 ml), dried, and evaporated to give N-nitroso-N-allylbenzamide (28.5 g).

ν_{max} (liquid film): 1710, 1650, 1600, 1570, 1355, 1285, 1155, 1040 and 920 cm^{-1},

δ ($CDCl_3$): 7.83—7.20 (5H, m, Ar*H*), 6.03—4.83 (3H, m, =C*H*), and 4.33 (2H, broad, d, *J* 4Hz).

(ii) 3-Diazoprop-1-ene

Pyrrolidine (4.53 ml) was added to a solution of N-nitroso-N-allylbenzamide (9.5 g) in methylene chloride (100 ml) at −20°C and the mixture was stirred at −20°C for 15 minutes to give a solution of 3-diazoprop-1-ene.

ν_{max} (CH_2Cl_2): 2030 cm^{-1}. This solution was then stored at 0°C until use.

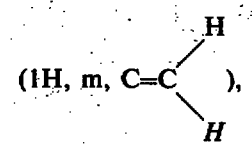
(iii) Benzyl 9-(O)-allylclavulanate

Boron trifluoride etherate (1 ml) was added to a solution of benzyl clavulanate (2.89 g) in methylene chloride (150 ml) at −30°C. The solution of 3-diazoprop-1-ene prepared above was added to the mixture at −30°C and the solution was stirred at −30°C to −10°C for hour. This was washed with aqueous sodium bicarbonate (2×200 ml), dried and evaporated to give the crude product as an oil which was chromatographed over silica gel (50 g). Elution of the column with cyclohexane-ethyl acetate gave benzyl 9-(O)-allylclavulanate (e14) (0.92 g) as a colourless liquid,

$[\alpha]_D$+45.3° ($CHCl_3$; *c*, 1.0),

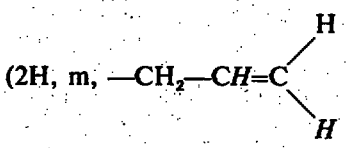
ν_{max} (liquid film): 1800, 1750, 1695, 1300, 1175, 1030, 1010, 995 cm^{-1}.

δ ($CDCl_3$): 7.37 (5H, s, Ar-*H*), 6.05—5.70



(1H, m,),

5.60 (1H, d, *J* 2.5Hz, 5-C*H*), 5.30—5.10



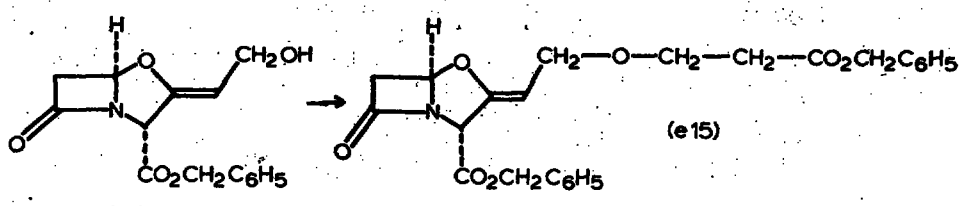
(2H, m,)

5.13 (2H, s, —CH_2Ph), 5.03 (1H, m, 3-C*H*), 4.79 (1H, broad t, *J* 8Hz, 8-C*H*), 4.01 (2H, d, *J* 8Hz, 9-CH_2), 3.86 (2H, broad d, *J* 7Hz, —CH_2—CH=CH_2), 3.41 (1H, dd, *J* 18Hz, *J'* 2.5Hz, 6α-C*H*), 2.98 (1H, d, *J* 18Hz, 6β-C*H*).

(Found: C, 65.85; H, 5.65; N, 4.45%. $C_{18}H_{19}NO_5$ requires C, 65.65; H, 5.8; N, 4.25%).

Example 15

Preparation of Benzyl 9-O-(2-benzyloxycarbonylethyl)clavulanate



(e1) (e15)

(i) Benzyl N-nitroso-N-benzyloxycarbonyl-β-alanate

A solution of nitrogen dioxide (6.9 g) in carbon tetrachloride (40 ml) was added to a stirred solution of anhydrous sodium acetate (12.3 g) and carbon tetrachloride (40 ml) at −20°C. Benzyl N-benzyloxycarbonyl-β-alanate (12.95 g) in carbon tetrachloride (50 ml) was added to the stirred mixture at −5°C which was then stirred at 0°C for 0.5 hour. The reaction mixture was washed with aqueous sodium bicarbonate (2×100 ml) and the carbon tetrachloride solution was dried and evaporated to give benzyl N-nitroso-N-benzyloxycarbonyl-β-alanate (12.5 g) as an oil,

ν_{max} (CCl_4): 1745, 1705, 1520, 1400, 1350, 1175, 1140, 1095, 960 cm^{-1},

δ ($CDCl_3$): 7.30 (10H, s, Ar*H*), 5.46 (2H, s, CH_2Ph), 4.90 (2H, s, CH_2Ph), 3.92 (2H, t, *J* 7Hz), and 2.36 (2H, t, *J* 7Hz).

(The n.m.r. spectrum indicated that the starting material and this oil were only about 73% pure).

(ii) Benzyl 9-O-(2-benzyloxycarbonylethyl)clavulanate

Pyrrolidine (0.58 ml) was added to a solution of benzyl N-nitroso-N-benzyloxycarbonyl-β-alanate (3.24 g) in methylene chloride (25 ml) at −20°C and the mixture was stirred at −20°C for 15 minutes to give a solution of benzyl 3-diazopropionate which was kept at 0°C for the next stage.

Boron trifluoride etherate (0.5 ml) was added to a solution of benzyl clavulanate (1 g) in methylene chloride (150 ml) at −30°C. The solution of benzyl 3-diazopropionate prepared above was added to the mixture at −30°C and the solution was stirred at −30°C to −10°C for 1 hour. This was washed with aqueous sodium bicarbonate (2×50 ml), dried and evaporated to give the crude product as an oil which was chromatographed over silica gel (30 g). Elution of the column with cyclohexane - ethyl acetate gave benzyl 9 - [O - (2 - benzyloxycarbonylethyl)] - clavulanate (e15) (0.21 g) as a colourless liquid, $[\alpha]_D$ 29.3° ($CHCl_3$: c, 1.0),

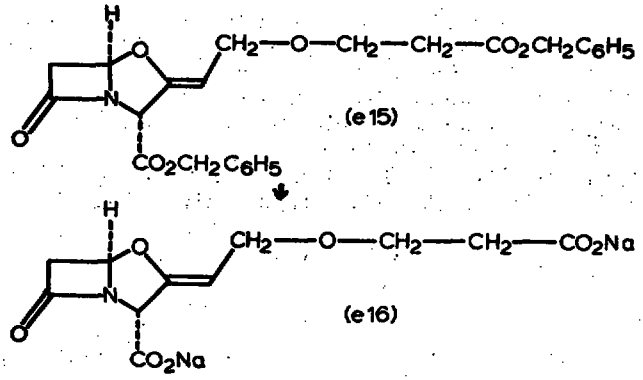
ν_{max} (liquid film): 1805 1740, 1700, 1240, 1170, 1100, 1040 and 1020 cm^{-1},

δ ($CDCl_3$): 7.28 (10H, s, Ar-*H*), 5.60 (1H, d, *J* 2.5Hz, 5-C*H*), 5.14 (2H, s, CH_2Ph), 5.09 (2H, s, CH_2Ph), 5.02 (1H, m, 3-C*H*), 4.74 (1H, broad t, *J* 8Hz, 8-C*H*), 4.01 (2H, broad d, *J* 8Hz, 9-CH_2), 3.60 (2H, t, *J* 7Hz), 3.41 (1H, dd, *J* 18Hz, *J'* 2.5Hz, 6α-C*H*), 2.99 (1H, d, *J* 18Hz, 6β-C*H*) and 2.57 (2H, t, *J* 7Hz).

M^+ 451.1626, $C_{25}H_{25}NO_7$ requires M^+ 451.1631.

Example 16

Preparation of Disodium 9-O-(2-carboxyethyl)clavulanate



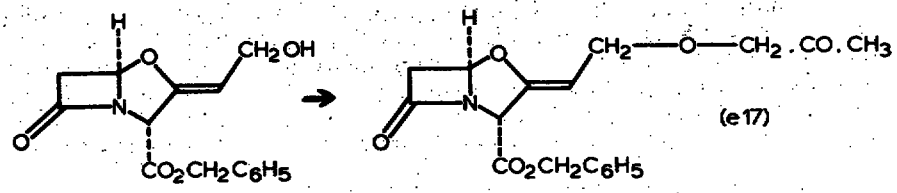
(e15) (e16)

A mixture of benzyl 9-[O-(2-benzyloxycarbonylethyl)]clavulanate (e15) (0.17 g) and sodium bicarbonate (0.063 g) in tetrahydrofuran/water 5:1, 12 ml) was hydrogenated in the presence of 10% palladium charcoal (0.1 g) at room temperature and pressure for 0.5 hour. The mixture was filtered and the filtrate evaporated to give disodium 9-[O-(2-carboxyethyl)]clavulanate (e16) (0.084 g).

ν_{max} (KBr): 1780, 1690, 1620, 1390, 1310, 1195, 1155, 1085, 1040 and 895 cm^{-1}.

δ (D_2O): 5.65 (1H, d, *J* 2.5Hz, 5-C*H*), 4.87 (1H, s, 3-C*H*), 4.81 (1H, broad t, *J* 8Hz, 8-C*H*), 4.06 (2H, d, *J* 8Hz, 9-CH_2), 3.63 (3H, t, *J* 7Hz), 3.51 (1H, dd, *J* 18Hz, *J'* 2.5Hz, 6α-C*H*), 3.05 (1H, d, *J* 18Hz, 6β-C*H*), and 2.49 (3H, t, *J* 7Hz).

Example 17
Preparation of Benzyl 9-O-acetonylclavulanate



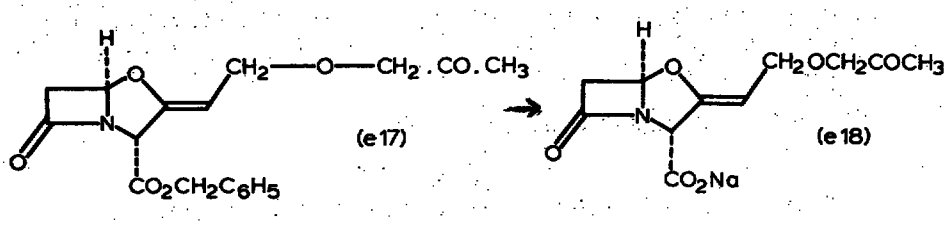
To a solution of benzyl clavulanate (e1) (1.0 g) and diazoacetone (1.4 g) in methylene chloride (30 ml) was added boron trifluoride diethyletherate (20 drops), and the solution stirred at −10°C for 2.0 hours. The reaction mixture was quenched with dilute bicarbonate solution. The organic extract was washed with sodium chloride solution, dried over magnesium sulphate and evaporated. Silica gel chromatography (eluting with ethyl acetate/cyclohexane) yielded the title product (e17) (190 mg) as a colourless oil.

I.r. ($CHCl_3$): 1800, 1730—1760, 1695 cm^{-1};

N.m.r. ($CDCl_3$): 2.10 (3H, s, —*C*H_3), 2.96 (1H, d, J 17Hz, 6β-C*H*), 3.36 (1H, dd, J 17Hz, J' 2.5Hz, 6α-C*H*), 3.99 (2H, d, J 7Hz, =CH—*C*H_2), 4.68 (1H, t, J 7Hz, =C*H*—CH_2), 5.03 (1H, brs, 3-C*H*), 5.20 (2H, s, —*C*H_2Ph), 5.70 (1H, d, J 2.5Hz, 5-C*H*), 7.33 (5H, s, CO_2CH_2*Ph*).

M (mass spectrometry) 345.

Example 18
Preparation of Sodium 9-O-acetonylclavulanate



The acetonyl ether benzyl ester (e17) (80 mg) was hydrogenated in a tetrahydrofuran/water mixture (5:1) in the presence of sodium bicarbonate (5 mg) and 10% palladium/charcoal (30 mg.). After twenty minutes the catalyst was filtered off. The filtrate was diluted with water and extracted with ethyl acetate before freeze-drying. This yielded the title product as an off-white solid.

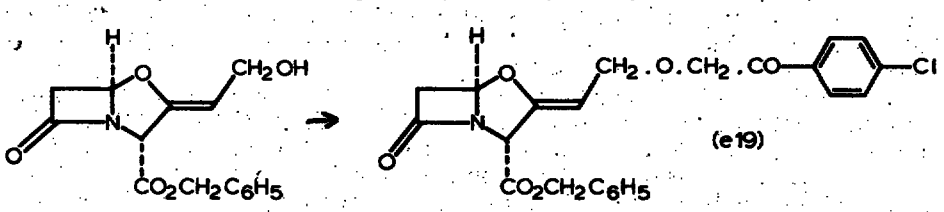
I.r. (KBr): 1780, 1720, 1690, 1610 cm^{-1};

N.m.r. ($CDCl_3$): 2.09 (3H, s, —*C*H_3), 3.00 (1H, d, J 17Hz, 6β-C*H*), 3.45 (1H, dd, J 17Hz, J' 2.5Hz, 6α-C*H*), 4.08 (2H, d, J 8Hz, =CH—*C*H_2), 4.18

(2H, s, *C*H_2C(=O)),

4.80 (1H, t, J 8Hz, =C*H*), 4.90 (1H, s, 3-C*H*), 5.65 (1H, d, J 2.5Hz, 5-C*H*).

Example 19
Preparation of Benzyl 9-O-(4-chlorophenacyl) clavulanate



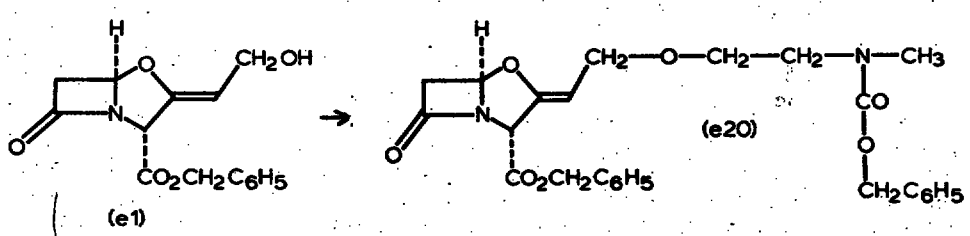
Benzyl clavulanate (e1) (500 mg) was dissolved in methylene chloride (25 ml) and treated with α-diazo-p-chloroacetophenone (0.90 g). To the stirring solution at −20°C was added boron trifluoride etherate (0.1 ml). The solution was stirred at −20°C to −10°C for 2.0 hours, washed with dilute sodium bicarbonate solution (×2), and the organic extract dried over magnesium sulphate. Evaporation of the solvent and silica gel chromatography (eluting with chloroform) yielded the title compound (e19) (130 mg).

I.r. ($CHCl_3$): 1805, 1750, 1695 cm^{-1};

N.m.r. ($CDCl_3$): 3.10 (1H, d, J 17Hz, 6β-*CH*), 3.60 (1H, dd, J 17Hz, J' 2.5Hz, 6α-*CH*), 4.36 (2H, s, J 7Hz, =CH—CH_2O—), 4.74

5.00 (1H, t, J, 7Hz, =*CH*—CH_2), 5.26 (1H, brs, 3-*CH*), 5.37 (2H, s, CO_2CH_2Ph), 5.82 (1H, d, J 2.5Hz, 5-*CH*), 7.48—8.12 (9H, m, Aryl).

Example 20
Preparation of Benzyl 9-O-[2-(N-benzyloxycarbonyl-N-methylamino)ethyl]clavulanate



(i) N-Methy-N'-nitroso-N,N'-dibenzyloxycarbonyl-1,2-diaminoethane

To carbon tetrachloride (90 ml), stirred between −5° and 10°C, was added anhydrous sodium acetate (27.8 gm), followed by a solution of nitrogen dioxide (15.6 gm) in carbon tetrachloride (90 ml) at such a rate that the temperature did not rise above −5°C. A solution of N-methyl-N,N'-dibenzyloxycarbonyl-1,2-diaminoethane (29 gm) in carbon tetrachloride (90 l) was then added to the stirred solution over 40 minutes, maintaining the temperature at −10°C. When the addition was complete the mixture was stirred for 2 hours. The mixture was then washed with excess sodium bicarbonate solution, the aqueous phase was extracted with carbon tetrachloride and the combined organic phases were dried over magnesium sulphate and evaporated to yield 29.8 g of approximately 80% pure material.

(ii) N-Methyl-N,N'-dibenzyloxycarbonyl-1,2-diaminoethane

To a stirred solution of N-methyl-1,2-diaminoethane (7.4 gm) and triethylamine (20.2 gm) in chloroform (50 ml) at 0°C was added dropwise over 30 minutes benzyl chloroformate (34.1 gm). The mixture was then left to warm to room temperature over one hour. The organic solution was then washed with water, citric acid solution, sodium bicarbonate solution and finally water again. The chloroform solution was dried over magnesium sulphate and evaporated to give a yellow oil (29.6 g).

(iii) Benzyl 9-[2-Benzyloxycarbonyl-N-Methylamino)ethyl]clavulanate

A stirred solution of N-methyl-N'-nitroso-N,N'-dibenzyloxycarbonyl-1,2-diaminoethane (3.71 gm) in methylene chloride (10 ml) was cooled to −30°C and treated with pyrrolidine (0.71 gm). The mixture was stirred at −25° to 30°C for half an hour and then used as described below.

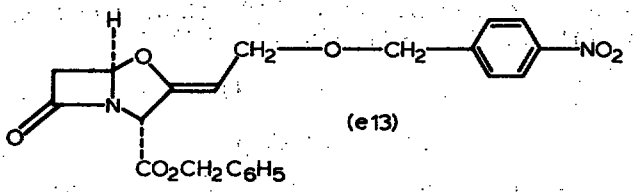
A solution of benzyl clavulanate (1.0 gm) in methylene chloride (10 ml) was cooled to −30°C and treated with boron trifluoride etherate (0.5 ml). The solution of the diazo compound, prepared as described above, was then added to the stirred solution at such a rate as to keep the temperature below −25°C. The mixture was then stirred for one hour, gradually raising the temperature to −10°C. Excess ice-cold sodium bicarbonate solution was then added and the organic phase separated. The aqueous phase was extracted with methylene chloride and the combined organic phases were washed with water, dried over magnesium sulphate and evaporated. Repeated chromatography of the residue gave the desired ether (e20).

I.r. (film), [No OH absortion], 1800, 1745 and 1700 cm^{-1}.

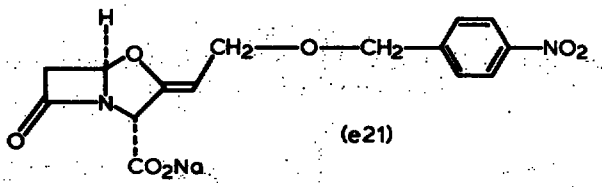
Thin layer chromatographic properties consistent with formula.

Example 21

Preparation of Sodium 9-O-(4-nitrobenzyl)clavulanate



(e13)



(e21)

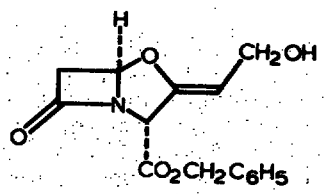
A solution of benzyl 9-[O-(4-nitrobenzyl)]clavulanate (e13) (110 mgs) and sodium bicarbonate (21.7 mgs) in tetrahydrofuran—water (5:1, 8 mls) was hydrogenated over 10% palladium on charcoal (68 mgs) for 15 minutes. The solution was filtered through Celite, and the solid was washed with water. The combined filtrates were evaporated to remove most of the tetrahydrofuran, and the remaining aqueous solution was washed with ethyl acetate. The aqueous solution was then evaporated and the residual gum was purified by column chromatography (Kieselgel H, n-butanol:water:ethanol, 4:1:1 as eluant) to give the product (e21) (39 mgs) as a yellow solid (estimated 70% pure).

I.r. (KBr) 1790, 1605 (broad) cm^{-1}.

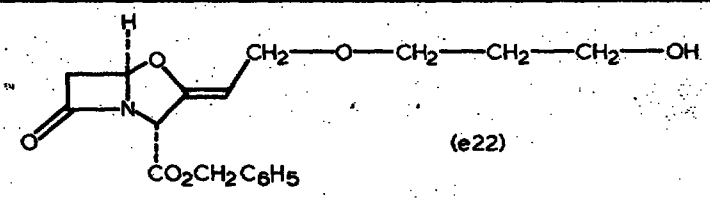
N.m.r. (DMSO) 2.83 (IH, doublet, J 17Hz) 3.41 (IH, double doublet, J 17Hz and 3Hz) 3.88 (2H, doublet, J 7Hz), 4.4 (2H, singlet), 4.52 (IH singlet), 4.67 (IH, multiplet), 5.57 (IH, singlet with fine coupling), 6.46 (2H, doublet J 9Hz), 6.89 (2H, doublet, J 9Hz).

Example 22

Preparation of Benzyl 9-O-(3-hydroxypropyl)clavulanate



(e1)



(e22)

(i) N,O-bis-(benzyloxycarbonyl)-3-aminopropanol

Benzyl chloroformate (47 ml.) was added dropwise to a stirred solution of 3-aminopropanol (11.4 ml) in pyridine (150 ml) at 0°C. The mixture was then stirred at room temperature overnight. This was poured into ice-water (300 ml) and extracted with ethyl acetate (3×200 ml). The organic extract was washed with hydrochloric acid (5m, 3×400 ml), dried with anhydrous sodium carbonate—magnesium sulphate and evaporated to give the desired compound as an oil contaminated with a little dimethyl carbonate (30.23 g).

(ii) Nitroso-N,O-bis-(benzyloxycarbonyl)-3-Aminopropanol

Anhydrous sodium acetate (45 g) was added to carbon tetrachloride (150 ml) at 0°C and the mixture was cooled to −20°C and then added to a solution of nitrogen dioxide (24.1 g) in carbon tetrachloride (150 ml). A solution of N,O-bis-(benzoyloxycarbonyl)-3-aminopropanol (30.23 g) in carbon tetrachloride (150 ml) was added to the stirred mixture at −5°C which was then stirred at 0°C for half and hour. The reaction mixture was washed with aqueous sodium bicarbonate (2×400 ml), dried, and evaporated to give the desired compound as an oil (31.23 g), ν_{max} (liquid film) 1750, 1510, 1400, 1260, 1125 and 1035.

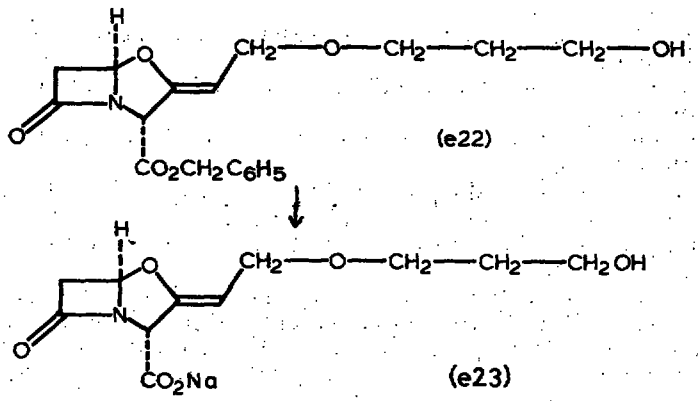
(iii) Benzyl 9-O-(3-benzyloxycarbonyloxypropyl)clavulanate

Pyrrolidine (4.94 ml) was added to a solution of N-nitroso-N,O-bis-(benzyloxycarbonyl)-3-aminopropanol (31.22 g) 0.056 mole in the mixture) in methylene chloride (100 ml) at −20°C for 15 minutes to give a solution of 3-diazo-O-benzyloxycarbonyl-propanol, ν_{max} (CH_2Cl_2) 2030 cm^{-1}. This solution was kept at 0°C for the next stage.

Boron trifluoride etherate (1 ml) was added to a solution of benzyl clavulanate (3 g) in methylene chloride (150 ml) at −30°C. The solution of 3-diazo-O-benzyloxycarbonylpropanol prepared above was added to the mixture at −30°C and the solution was stirred at −30° to −10°C for 1 hour. This was washed with aqueous sodium bicarbonate (2×200 ml), dried, and evaporated to give an oil which was chromatographed using silica gel (80 g). Elution of the column with cyclohexaneethyl acetate afforded the title compound (e22) (1.5 g), δ ($CDCl_3$) 7.36 (10H, s, Ar*H*), 5.70 (IH, d, *J*=2.5Hz, 5-C*H*), 5.18 (4H, s, CH_2Ph), 5.13 (IH, m, 3-C*H*), 4.84 (IH, broad t, *J*=8Hz, 8-C*H*), 4.26 (IH, 5, *J*=6Hz, CH_2 CH_2 OCBz), 4.06 (IH, d, *J*=8Hz, 9-CH_2), 3.60—3.26 (4H, m, O—CH_2CH_2 and 6α-C*H*), 3.02, (IH, d, J=18Hz, 6β-C*H*), and 1.86 (IH, m, $CH_2CH_2CH_2$).

Example 23

Preparation of Sodium 9-O-(3-hydroxypropyl)clavulanate



(e22)

(e23)

A mixture of benzyl 9-O-(3-benzyloxycarbonyloxypropyl)clavulanate (0.5 g) and sodium bicarbonate (0.05 g) in tetrahydrofuran—water (S=1, 12 ml) was

hydrogenated at room temperature and pressure in the presence of 10% palladium-charcoal (0.3 g) for 15 minutes. The mixture was filtered and the filtrate evaporated to give an oil which was partitioned between water and ethyl acetate. The aqueous fraction was freeze-dried to give the title compound (e23) with a consistent n.m.r. spectrum.

Example 24
Biological Activity

a. When administered to mice the compounds of this invention do not exhibit a high level of toxicity, for example on sub-cutaneous administration the compounds of Examples 5 and 12 have LD_{50} values of greater than 250 mg/kg.

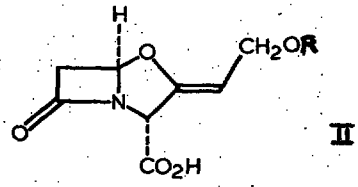
b. When tested in standard MIC tests in combination with ampicillin the compounds of this invention are seen to enhance the effectiveness of the penicillin against various gram-positive and gram-negative organisms such as strains of *Staphylococcus aureus* Russell, *Klebsiella aerogenes*, *Proteus mirabilis* and *Escherichia coli*: for example the following MIC values against *Staphylococcus aureus* Russell were obtained for ampicillin in the presence of 1 μg/ml of certain compounds of the invention:

Compound of Example No:	3	5	9	10	11	14	15	16
MIC (μg/ml):	2	0.3	1.6	3	1.25	1.25	1	1

Ampicillin in the absence of synergist did not inhibit the growth of the test organism at a concentration of 250 μg/ml. None of the synergists inhibited the growth of the test organism at 5 μg/ml.

WHAT WE CLAIM IS:—

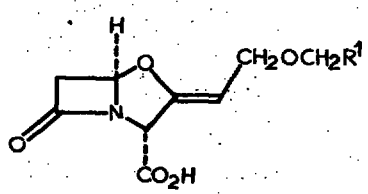
1. A compound of the formula (II):



II

or a salt or ester thereof wherein R is an inert etherifying organic group of up to 18 carbon atoms.

2. A compound as claimed in claim 1 of the formula (III):



(III)

or a salt or ester thereof wherein CH_2R^1 is an inert etherifying organic group of up to 18 carbon atoms.

3. A compound as claimed in claim 1 or 2 wherein the inert etherifying group is a hydrocarbon group.

4. A compound as claimed in any of claims 1, 2 or 3 wherein the etherifying group is an alkyl group.

5. A compound as claimed in any of claims 1, 2 or 3 wherein the etherifying group is an alkyl group substituted by a phenyl group.

6. A compound as claimed in any of claims 1, 2 or 3 wherein the etherifying group is an alkenyl group.

7. A compound as claimed in any of claims 1, 2 or 3 wherein the inert etherifying group is a hydrocarbon group substituted by a halogen atom.

8. A compound as claimed in claims 1 or 2 wherein the inert etherifying group is a hydrocarbon group substituted by a group OR^2 wherein R^2 is a hydrocarbon group of up to 8 carbon atoms.

9. A compound as claimed in claims 1 or 2 wherein the inert etherifying group is a hydrocarbon group substituted by a hydroxyl group.

hydrogenated at room temperature and pressure in the presence of 10% palladium-charcoal (0.3 g) for 15 minutes. The mixture was filtered and the filtrate evaporated to give an oil which was partitioned between water and ethyl acetate. The aqueous fraction was freeze-dried to give the title compound (e23) with a consistent n.m.r. spectrum.

Example 24

Biological Activity

a. When administered to mice the compounds of this invention do not exhibit a high level of toxicity, for example on sub-cutaneous administration the compounds of Examples 5 and 12 have LD_{50} values of greater than 250 mg/kg.

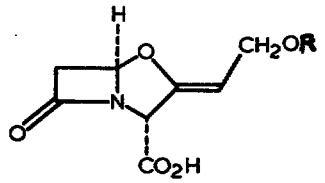
b. When tested in standard MIC tests in combination with ampicillin the compounds of this invention are seen to enhance the effectiveness of the penicillin against various gram-positive and gram-negative organisms such as strains of *Staphylococcus aureus* Russell, *Klebsiella aerogenes*, *Proteus mirabilis* and *Escherichia coli*: for example the following MIC values against *Staphylococcus aureus* Russell were obtained for ampicillin in the presence of 1 μg/ml of certain compounds of the invention:

Compound of Example No:	3	5	9	10	11	14	15	16
MIC (μg/ml):	2	0.3	1.6	3	1.25	1.25	1	1

Ampicillin in the absence of synergist did not inhibit the growth of the test organism at a concentration of 250 μg/ml. None of the synergists inhibited the growth of the test organism at 5 μg/ml.

WHAT WE CLAIM IS:—

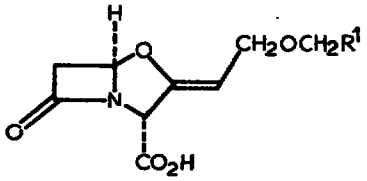
1. A compound of the formula (II):



II

or a salt or ester thereof wherein R is an inert etherifying organic group of up to 18 carbon atoms.

2. A compound as claimed in claim 1 of the formula (III):



(III)

or a salt or ester thereof wherein CH_2R^1 is an inert etherifying organic group of up to 18 carbon atoms.

3. A compound as claimed in claim 1 or 2 wherein the inert etherifying group is a hydrocarbon group.

4. A compound as claimed in any of claims 1, 2 or 3 wherein the etherifying group is an alkyl group.

5. A compound as claimed in any of claims 1, 2 or 3 wherein the etherifying group is an alkyl group substituted by a phenyl group.

6. A compound as claimed in any of claims 1, 2 or 3 wherein the etherifying group is an alkenyl group.

7. A compound as claimed in any of claims 1, 2 or 3 wherein the inert etherifying group is a hydrocarbon group substituted by a halogen atom.

8. A compound as claimed in claims 1 or 2 wherein the inert etherifying group is a hydrocarbon group substituted by a group OR^2 wherein R^2 is a hydrocarbon group of up to 8 carbon atoms.

9. A compound as claimed in claims 1 or 2 wherein the inert etherifying group is a hydrocarbon group substituted by a hydroxyl group.

10. A compound as claimed in claims 1 or 2 wherein the inert etherifying group is a hydrocarbon group substituted by a $O.CO.R^2$ group where R^2 is a hydrocarbon group of up to 8 carbon atoms.

11. A compound as claimed in claims 1 or 2 wherein the inert etherifying group is a hydrocarbon group substituted by a CO_2R^2 group where R^2 is a hydrocarbon group of up to 8 carbon atoms.

12. A compound as claimed in claims 1 or 2 wherein the inert etherifying group is a hydrocarbon group substituted by a NR^3COR^2 group where R^2 is a hydrocarbon group of up to 8 carbon atoms and R^3 is a hydrocarbon group of up to 4 carbon atoms.

13. A compound as claimed in claims 1 or 2 wherein the inert etherifying group is a hydrocarbon group substituted by a $SO.R^2$ group wherein R^2 is a hydrocarbon group of up to 8 carbon atoms.

14. A compound as claimed in claims 1 or 2 wherein the inert etherifying group is a hydrocarbon group substituted by a SO_2R^2 group wherein R^2 is a hydrocarbon group of up to 8 carbon atoms.

15. A compound as claimed in claims 1 or 2 wherein the inert etherifying group is a hydrocarbon group substituted by a NH_2 group.

16. A compound as claimed in claims 1 or 2 wherein the inert etherifying group is a hydrocarbon group substituted by a NR^2R^3 group wherein R^2 is a hydrocarbon group of up to 8 carbon atoms and R^3 is a hydrocarbon group of up to 4 carbon atoms.

17. A compound as claimed in claims 1 or 2 wherein the inert etherifying group is a hydrocarbon group substituted by a NO_2 group.

18. A compound as claimed in claims 1 or 2 wherein the inert etherifying group is a hydrocarbon group substituted by a CN group.

19. A compound as claimed in claims 1 or 2 wherein the inert etherifying group is a hydrocarbon group substituted by a $CO.NR^2R^3$ wherein R^2 is a hydrocarbon group of up to 8 carbon atoms and R^3 is a hydrocarbon group of up to 4 carbon atoms.

20. A compound as claimed in any of claims 9, 10, 11, 12, 13, 14, 16 or 17 wherein R^2 is a methyl, ethyl, propyl, butyl, phenyl or benzyl group.

21. A compound as claimed in any of claims 10, 11, 12, 13, 14, 16, or 17 wherein R^2 is a methyl group.

22. A compound as claimed in any of claims 4, 10, 11, 12, 13, 14, 16, 19 or 21 wherein R^3 is a methyl group.

23. A compound as claimed in claim 1 wherein the inert etherifying organic group is a methyl group.

24. A compound as claimed in claim 2 wherein R^1 is a methyl group.

25. A compound as claimed in claim 2 wherein R^1 is an ethyl group.

26. A compound as claimed in claim 2 wherein R^1 is a propyl group.

27. A compound as claimed in claim 2 wherein R^1 is a butyl group.

28. A compound as claimed in claim 2 wherein R^1 is a phenyl group.

29. A compound as claimed in claim 2 wherein R^1 is a naphthyl group.

30. A compound as claimed in claim 2 wherein R^1 is a phenyl group substituted by a halogen or a group OR^4 or R^4 wherein R^4 is a methyl, ethyl or propyl group.

31. A compound as claimed in claims 1 or 2 wherein the etherifying group is of the sub-formula (a):

$$—CH_2—X—CO_2H \qquad (a)$$

wherein X is a bond or CH_2 group.

32. A compound as claimed in claims 1 or 2 wherein the etherifying group is of the sub-formula (b):

$$—CH_2—X—CO_2R^5 \qquad (b)$$

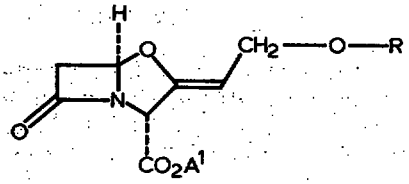
wherein X is a bond or CH_2 group and R_5 is a methyl, ethyl, propyl or butyl group or is a methyl, ethyl, propyl or butyl group substituted by a phenyl group.

33. A compound as claimed in claims 1 or 2 wherein the etherifying group is of the sub-formula (c):

$$—CH_2—Y—NR^6R^7 \qquad (c)$$

wherein $—CH_2—Y$ is an alkylene group of 2 to 4 carbon atoms or an alkylene group of 2 to 4 carbon atoms substituted by a phenyl group; R^6 is a hydrogen atom or a methyl, ethyl, propyl or butyl group; and R^7 is a hydrogen atom or a group R^8, $CO.R^8$ or CO_2R^8 wherein R^8 is a methyl, ethyl, propyl or butyl group substituted by a phenyl group.

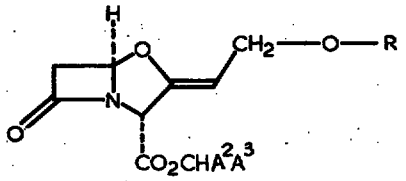
34. A compound as claimed in claim 33 wherein Y is a CH_2 group.
35. A compound as claimed in claims 33 or 34 wherein R^6 is a methyl group.
36. A compound as claimed in any of claims 33, 34 or 35 wherein R^7 is a benzyloxycarbonyl group.
37. A compound as claimed in any of claims 33, 34 or 35 wherein R^7 is a hydrogen atom.
38. A compound as claimed in claim 1 wherein the etherifying group is the ethoxycarbonylmethyl group.
39. A compound as claimed in claim 1 wherein the etherifying group is the benzoyloxycarbonylmethyl group.
40. A compound as claimed in claim 1 wherein the etherifying group is the carboxymethyl group.
41. A compound as claimed in claim 1 wherein the etherifying group is the cyanomethyl group.
42. A compound as claimed in claim 1 wherein the etherifying group is the *p*-nitrobenzyl group.
43. A compound as claimed in claim 1 wherein the etherifying group is the allyl group.
44. A compound as claimed in claim 1 wherein the etherifying group is the 2-benzyloxycarbonylethyl group.
45. A compound as claimed in claim 1 wherein the etherifying group is the 2-carboxyethyl group.
46. A compound as claimed in claim 1 wherein the etherifying group is the acetonyl group.
47. A compound as claimed in claim 1 wherein the etherifying group is the 4-chlorophenacyl group.
48. A compound as claimed in claim 1 wherein the etherifying group is the 3-hydroxypropyl group.
49. A compound as claimed in claim 1 wherein the etherifying group is the 2-(N-benzyloxycarbonyl-N-methylamino)ethyl group.
50. A compound as claimed in claim 1 in the form of the acid or a pharmaceutically acceptable salt thereof.
51. A compound as claimed in claim 50 which is a sodium salt.
52. A compound as claimed in claim 50 which is a potassium salt.
53. A compound as claimed in claim 50 which is a calcium salt.
54. A compound as claimed in claim 50 which is a magnesium salt.
55. A compound as claimed in any of claims 2, 3 or 4 in the form of the acid or a pharmaceutically acceptable salt thereof.
56. A compound as claimed in claim 55 which is a sodium salt.
57. A compound as claimed in claim 55 which is a potassium salt.
58. A compound as claimed in claim 55 which is a calcium salt.
59. A compound as claimed in claim 55 which is a magnesium salt.
60. A compound as claimed in any of claims 24 to 28 in the form of the acid or a pharmaceutically acceptable salt thereof.
61. A compound as claimed in claim 60 which is a sodium salt.
62. A compound as claimed in claim 60 which is a potassium salt.
63. A compound as claimed in claim 60 which is a calcium salt.
64. A compound as claimed in any of claims 8 to 22 or 29 to 49 in the form of the acid or a pharmaceutically acceptable salt thereof.
65. A compound as claimed in claim 64 which is a sodium salt.
66. A compound as claimed in claim 64 which is a potassium salt.
67. A compound as claimed in claim 64 which is a calcium salt.
68. A compound as claimed in claim 64 which is a magnesium salt.
69. A compound as claimed in claim 1 which is an ester of the compound of the formula (II).
70. A compound as claimed in claim 69 of the formula (IV):



wherein R is as defined in claim 1 and A^1 is an alkyl group of 1 to 8 carbon atoms optionally substituted by a halogen or a group of the formula OA^4, $OCOA^4$, SA^4 or SO_2A^4 wherein A^4 is a hydrocarbon group of up to 6 carbon atoms.

71. A compound as claimed in claim 70 wherein A^1 is methyl.

72. A compound as claimed in claim 69 of the formula (V):



wherein R is as defined in claim 1 and A^2 is a hydrogen atom or an alkyl group of up to 4 carbon atoms or a phenyl group optionally substituted by a halogen or a group A^5 or OA^5 where A^5 is an alkyl group of up to 6 carbon atoms, and A^3 is a phenyl group optionally substituted by halogen or by a group A^5 or OA^5 where A^5 is an alkyl group of up to 6 carbon atoms.

73. A compound as claimed in claim 72 wherein A^2 is a hydrogen atom.

74. A compound as claimed in claim 72 wherein CHA^2A^3 is a benzyl group.

75. A compound as claimed in claim 69 which is hydrolysable *in vivo*.

76. A compound as claimed in claim 69 which is a phthalidyl ester.

77. A compound as claimed in claim 69 which is an α-acetoxyethyl ester.

78. A compound as claimed in claim 69 which is an ethoxycarbonyloxymethyl ester.

79. A compound as claimed in claim 69 which is an α-ethoxycarbonyloxyethyl.

80. 9-O-Methylclavulanic acid or a pharmaceutically acceptable salt or ester thereof.

81. 9-O-Methylclavulanic acid or a pharmaceutically acceptable salt thereof.

82. Methyl 9-O-Methylclavulanate.

83. Benzyl 9-O-methylclavulanate.

84. Sodium 9-O-methylclavulanate.

85. Potassium 9-O-methylclavulanate.

86. Calcium 9-O-methylclavulanate.

87. Magnesium 9-O-methylclavulanate.

88. A compound as claimed in claim 1 in the form of a triethylamine salt.

89. A compound as claimed in claim 1 in the form of a trimethylamine salt.

90. A pharmaceutical composition which comprises a compound of the formula (II) as claimed in claim 1 or a pharmaceutically acceptable salt or ester thereof and a pharmaceutically acceptable carrier therefor.

91. A composition as claimed in claim 90 wherein the compound as claimed in claim 1 is a compound as claimed in any of claims 50 to 54.

92. A composition as claimed in claim 90 wherein the compound as claimed in claim 1 is a compound as claimed in any of claims 55 to 59.

93. A composition as claimed in claim 90 wherein the compound as claimed in claim 1 is a compound as claimed in any of claims 60 to 63.

94. A composition as claimed in claim 90 wherein the compound as claimed in claim 1 is a compound as claimed in any of claims 64 to 68.

95. A composition as claimed in claim 90 wherein the compound as claimed in claim 1 is a compound as claimed in any of claims 75 to 79.

96. A pharmaceutical composition which comprises a compound as claimed in claim 80 and a pharmaceutically acceptable carrier.

97. A pharmaceutical composition which comprises a compound as claimed in claim 81 and a pharmaceutically acceptable carrier.

98. A pharmaceutical composition which comprises the compound claimed in claim 82 and a pharmaceutically acceptable carrier.

99. A pharmaceutical composition which comprises the compound claimed in claim 83 and a pharmaceutically acceptable carrier.

100. A pharmaceutical composition which comprises the compound claimed in claim 84 and a pharmaceutically acceptable carrier.

101. A pharmaceutical composition which comprises the compound claimed in claim 85 and a pharmaceutically acceptable carrier.

102. A pharmaceutical composition which comprises the compound claimed in claim 86 and a pharmaceutically acceptable carrier.

103. A pharmaceutical composition which comprises the compound claimed in claim 87 and a pharmaceutically acceptable carrier.

104. A composition as claimed in claim 90 which also comprises a penicillin or cephalosporin.

105. A composition as claimed in claim 91 which also comprises a penicillin or cephalosporin.

106. A composition as claimed in claim 92 which also comprises a penicillin or cephalosporin.

107. A composition as claimed in claim 93 which also comprises a penicillin or cephalosporin.

108. A composition as claimed in claim 94 which also comprises a penicillin or cephalosporin.

109. A composition as claimed in claim 95 which also comprises a penicillin or cephalosporin.

110. A composition as claimed in claim 96 which also comprises a penicillin or cephalosporin.

111. A composition as claimed in claim 97 which also comprises a penicillin or cephalosporin.

112. A composition as claimed in claim 98 which also comprises a penicillin or cephalosporin.

113. A composition as claimed in claim 99 which also comprises a penicillin or cephalosporin.

114. A composition as claimed in claim 100 which also comprises a penicillin or cephalosporin.

115. A composition as claimed in claim 101 which also comprises a penicillin or cephalosporin.

116. A composition as claimed in claim 102 which also comprises a penicillin or cephalosporin.

117. A composition as claimed in claim 103 which also comprises a penicillin or cephalosporin.

118. A composition as claimed in claim 104 which comprises benzylpenicillin, phenoxymethylpenicillin, propicillin, ampicillin, amoxycillin, epicillin, cyclacillin, hetacillin, metampicillin or the acetoxymethyl, pivaloyloxymethyl or phthalidyl esters of benzylpenicillin, ampicillin or amoxycillin or the phenyl, tolyl or indanyl α-ester of carbenicillin or ticarcillin.

119. A composition as claimed in claim 104 which comprises carbenicillin or ticarcillin.

120. A composition as claimed in claim 104 which comprises cephaloridine, cephalothin, cefazolin, cephalexin, cephacetrile, cephradine, cephapirin or cephaloglycine.

121. A composition as claimed in any of claims 105 to 107 which comprises a penicillin named in claim 118.

122. A composition as claimed in any of claims 105 to 107 which comprises a penicillin as named in claim 119.

123. A composition as claimed in any of claims 105 to 107 which comprises a cephalosporin as named in claim 120.

124. A composition as claimed in any of claims 108 to 110 which comprises a penicillin named in claim 118.

125. A composition as claimed in any of claims 108 to 110 which comprises a penicillin named in claim 119.

126. A composition as claimed in any of claims 108 to 110 which comprises a cephalosporin named in claim 120.

127. A composition as claimed in any of claims 111 to 117 which comprises a penicillin named in claim 118.

128. A composition as claimed in any of claims 111 to 117 which comprises a penicillin named in claim 119.

129. A composition as claimed in any of claims 111 to 117 which comprises a cephalosporin as claimed in claim 120.

130. A composition as claimed in any of claims 104, 118, 119 or 120 of this invention wherein the weight ratio of the compound of the formula (II) or its pharmaceutically acceptable salt or ester to penicillin or cephalosporin is 1:3 to 3:1.

131. A composition as claimed in any of claims 105 to 107 and 121 to 123 wherein the weight ratio of the compound of the formula (II) or its pharmaceutically acceptable salt to penicillin or cephalosporin is 1:3 to 3:1.

132. A composition as claimed in any of claims 108 and 109 and 124 to 126

wherein the weight ratio of compound of the formula (II) or its pharmaceutically acceptable salt or ester to penicillin or cephalosporin is 1:3 to 3:1.

133. A composition as claimed in any of claims 110 to 117 and 127 to 129 wherein the weight ratio of compound as claimed in claims 80 to 87 to penicillin or cephalosporin is 1:3 to 3:1.

134. A composition as claimed in claim 90 adapted for oral administration.

135. A composition as claimed in claim 80 adapted for administration by injection.

136. A composition as claimed in any of claims 118, 124, or 127 adapted for oral administration.

137. A composition as claimed in any of claims 119, 122 or 128 adapted for administration by injection.

138. A composition as claimed in any of claims 120, 123 or 129 adapted for administration by injection.

139. A composition as claimed in claim 135 which comprises a sterile salt of compound as claimed in claims 1 or 23.

140. A composition as claimed in any of claims 90, 96 to 104, 110 to 118 and 121 which is in the form of a tablet.

141. A composition as claimed in any of claims 90, 96 to 104, 110 to 117, 119 or 120 in the form of an injectable composition.

142. A pharmaceutical composition which comprises from 150 to 1000 mg of amoxycillin or ampicillin or a pro-drug for amoxycillin or ampicillin, from 50 to 500 mg of a compound of the formula (II) as claimed in claim 1 or a pharmaceutically acceptable salt or *in vivo* hydrolysable ester thereof, and a pharmaceutically acceptable carrier.

143. A composition as claimed in claim 142 which comprises from 200 to 500 mg of amoxycillin or ampicillin or a pro-drug for amoxycillin or ampicillin.

144. A composition as claimed in claims 142 or 143 which comprises from 50 to 250 mg of a compound of the formula (II) as claimed in claim 1 or a pharmaceutically acceptable salt or *in-vivo* hydrolysable ester thereof.

145. A composition as claimed in any of claims 142 to 144 which comprises amoxycillin.

146. A composition as claimed in any of claims 142 to 144 which comprises ampicillin.

147. A composition as claimed in any of claims 142 to 146 which comprises a compound as claimed in claims 80 or 81.

148. A composition as claimed in any of claims 142 to 146 which comprises the compound claimed in claim 82.

149. A composition as claimed in any of claims 142 to 146 which comprises a compound as claimed in any of claims 84 to 87.

150. A composition as claimed in any of claims 142 to 149 which comprises amoxycillin trihydrate.

151. A composition as claimed in any of claims 142 to 150 which comprises ampicillin trihydrate.

152. A composition as claimed in any of claims 142 to 148 adapted for oral administration.

153. A composition as claimed in any of claims 149 to 151 adapted for oral administration.

154. A composition as claimed in any of claims 142 to 150 adapted for administration by injection.

155. A composition as claimed in any of claims 142 to 149 in the form of a tablet or capsule.

156. A composition as claimed in claim 150 or 151 in the form of a tablet or capsule.

157. A process for the preparation of a composition as claimed in any of claims 104—156 which comprises bringing the components thereof together in known manner.

158. A composition prepared by the process of claim 157.

159. A process for the preparation of a compound of the formula (II) as claimed in claim 1 or a salt or ester thereof which process comprises the etherification of an ester of clavulanic acid and thereafter if desired cleaving a thus produced ester of the compound of the formula (II) by hydrogenolysis or hydrolysis to yield the compound of the formula (II) or its salt.

160. A process as claimed in claim 159 adapted for the preparation of a compound as claimed in claim 2 wherein the etherification is effected by reaction

of an ester of clavulanic acid with a diazoalkane of the formula N_2—CHR^1 where R^1 is as defined in claim 2.

161. A process as claimed in claim 159 adapted to the preparation of a compound as claimed in claim 23.

162. A process as claimed in any of claims 159—161 wherein the ester of clavulanic acid is the methyl or benzyl ester.

163. A process for the preparation of a salt of a compound as claimed in claim 1 which comprises the very mild basic hydrolysis of an ester of the compound as claimed in claim 1.

164. A process as claimed in claim 163 adapted to the preparation of a salt as claimed in any of claims 51 to 54 and 84 to 87.

165. A process for the preparation of a compound as claimed in claim 1 or a salt thereof which comprises the hydrogenation of a hydrogenolysable ester of the compound as claimed in claim 1 optionally in the presence of a base.

166. A process as claimed in claim 165 wherein the hydrogenolysable ester is the benzyl ester.

167. A process as claimed in claim 165 or 166 wherein the hydrogenation is effected in the presence of a 10% palladium on charcoal catalyst.

168. A process as claimed in claim 159 which process comprises the reaction of the corresponding ester of clavulanic acid with a compound of the formula RQ where R is as defined in claim 1 and Q is a group readily displaceable by an electron-rich moiety.

169. A process as claimed in claim 168 wherein Q is a chlorine, bromine or iodine atom.

170. A process as claimed in claim 169 adapted to the preparation of a compound as claimed in claim 2.

171. A compound as claimed in claim 1 when prepared by a process as claimed in claim 159.

172. A compound as claimed in claim 1 when prepared by a process as claimed in claim 161.

173. A compound as claimed in claim 1 when prepared by a process as claimed in claim 162.

174. A salt of a compound of the formula (II) as claimed in claim 1 when prepared by the process as claimed in claim 163.

175. A salt of a compound of the formula (II) as claimed in any of claims 51 to 54 and 84 to 87 when prepared by a process as claimed in claim 164.

176. A compound of the formula (II) or a salt thereof when prepared by a process as claimed in any of claims 165 to 167.

177. The compound of claim 82 when prepared by the methylation of methylclavulanate.

178. A compound as claimed in any of claims 84 to 87 when prepared by a process as claimed in claim 163.

179. A method of treating bacterial infection in mammals excluding humans which comprises administering thereto a composition as claimed in any of claims 90, 91 and 96 to 103.

180. A method of treating bacterial infection in mammals excluding humans which comprises administering thereto a composition as claimed in any of claims 104, 105, 110 to 120 or 130.

181. A method of treating bacterial infection in mammals excluding humans which comprises administering thereto a composition as claimed in any of claims 142 to 146.

182. A method of treating bacterial infection in mammals excluding humans which comprises administering thereto a composition as claimed in any of claims 147 to 149.

183. A method as claimed in any of claims 180 or 181 wherein the composition to be administered is as claimed in claims 150 or 151.

184. A method as claimed in claim 179 wherein the composition is administered orally or by injection.

185. A method as claimed in claim 180 wherein the composition is administered orally or by injection.

186. A method as claimed in claim 181 wherein the composition is administered orally or by injection.

187. A method as claimed in claim 182 wherein the composition is administered orally or by injection.

188. A method as claimed in claim 183 wherein the composition is administered orally or by injection.

189. A method as claimed in claims 179 or 184 wherein the infection is mastitis in cattle.

190. A method as claimed in claims 180 or 185 wherein the infection is mastitis in cattle.

191. A method as claimed in claims 181 or 186 wherein the infection is mastitis in cattle.

192. A method as claimed in claims 182 or 187 wherein the infection is mastitis in cattle.

193. A method as claimed in claims 183 or 188 wherein the infection is mastitis in cattle.

194. A process for the preparation of a compound of the formula (II) or a salt thereof as claimed in claim 1 which comprises the de-esterification of an ester of the compound of the formula (II).

195. Methyl 9-O-methylclavulanate substantially as described in Example 1.

196. Benzyl 9-O-methylclavulanate substantially as described in Example 2.

197. Sodium 9-O-methylclavulanate substantially as described in Example 3.

198. Methyl 9-O-benzylclavulanate substantially as described in Example 4.

199. Sodium 9-O-benzylclavulanate substantially as described in Example 5.

200. Benzyl 9-O-(ethoxycarbonylmethyl)clavulanate substantially as described in Example 6.

201. Sodium 9-O-(ethoxycarbonylmethyl)clavulanate substantially as described in Example 7.

202. Sodium 9-O-(ethoxycarbonylmethyl)clavulanate substantially as described in Example 8.

203. Benzyl 9-O-(benzyloxycarbonylmethyl)clavulanate substantially as described in Example 9.

204. Di-sodium 9-O-(carboxymethyl)clavulanate substantially as described in Example 10.

205. Benzyl 9-O-(cyanomethyl)clavulanate substantially as described in Example 11.

206. Sodium 9-O-(cyanomethyl)clavulanate substantially as described in Example 12.

207. Benzyl 9-O-(4-nitrobenzyl)clavulanate substantially as described in Example 13.

208. Benzyl 9-O-allylclavulanate substantially as described in Example 14.

209. Benzyl 9-O-(2-benzyloxycarbonylethyl)clavulanate substantially as described in Example 15.

210. Disodium 9-O-(2-carboxyethyl)clavulanate substantially as described in Example 16.

211. Benzyl 9-O-acetonylclavulanate substantially as described in Example 17.

212. Sodium 9-O-acetonylclavulanate substantially as described in Example 18.

213. Benzyl 9-O-(4-chlorophenacyl)clavulanate substantially as described in Example 19.

214. Benzyl 9-O-[2-(N-Benzyloxycarbonyl-N-methylamino)ethyl]clavulanate substantially as described in Example 20.

215. Sodium 9-O-(4-nitrobenzyl)clavulanate substantially as described in Example 21.

216. Benzyl 9-O-(3-hydroxypropyl)clavulanate substantially as described in Example 22.

217. Sodium 9-O-(3-hydroxypropyl)clavulanate substantially as described in Example 23.

W. G. COLE,
Chartered Patent Agent,
Agent for the Applicants.

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