

SPECIFICATION

Penicillanic Acid S-oxide Derivatives

5 This invention relates to penicillanic acid 1,1-dioxide and esters thereof readily hydrolyzable *in vitro* which are useful as antibacterial agents and for enhancing the effectiveness of β -lactam antibiotics against β -lactamase producing bacteria. The invention also relates to derivatives of penicillanic acid 1,1-dioxide having the carboxy group protected by a conventional penicillin carboxy protecting group which are useful intermediates to the penicillanic acid 1,1-dioxides. Penicillanic acid 1-oxides and certain esters thereof which
10 are useful chemical intermediates to penicillanic acid 1,1-dioxide and its esters are also within the scope of the invention.

One of the most well-known and widely used class of antibacterial agents are the so-called β -lactam antibiotics. These compounds are characterized in that they have a nucleus consisting of a 2-azetidinone (β -lactam) ring fused to either a thiazolidine or a dihydro-1,3-thiazine ring. When the nucleus contains a
15 thiazolidine ring, the compounds are usually referred to generically as penicillins, whereas when the nucleus contains a dihydrothiazine ring, the compounds are referred to as cephalosporins. Typical examples of penicillins which are commonly used in clinical practice are benzylpenicillin (penicillin G), phenoxymethylpenicillin (penicillin V), ampicillin and carbenicillin; typical examples of common cephalosporins are cephalothin, cephalixin and cefazolin.

20 However, despite the wide use and wide acceptance of the β -lactam antibiotics as valuable chemotherapeutic agents, they suffer from the major drawback that certain members are not active against certain microorganisms. It is thought that in many instances this resistance of a particular microorganism to a given β -lactam antibiotic results because the microorganism produces a β -lactamase. The latter substances are enzymes which cleave the β -lactam ring of penicillins and cephalosporins to give products which are devoid
25 of antibacterial activity. However, certain substances have the ability to inhibit β -lactamases, and when a β -lactamase inhibitor is used in combination with a penicillin or cephalosporin it can increase or enhance the antibacterial effectiveness of the penicillin or cephalosporin against certain microorganisms. It is considered that there is an enhancement of antibacterial effectiveness when the antibacterial activity of a combination of a β -lactamase inhibiting substance and a β -lactam antibiotic is significantly greater than the sum of the
30 antibacterial activities of the individual components.

Thus, according to the invention, there are provided certain new chemical compounds which are new members of the class of antibiotics known as the penicillins, and which are useful as antibacterial agents. More specifically, these new penicillin compounds are penicillanic acid 1,1-dioxide, and esters thereof readily hydrolyzable *in vitro*.

35 Additionally, penicillanic acid 1,1-dioxide and its esters readily hydrolyzable *in vitro* are potent inhibitors of microbial β -lactamases. Accordingly, there is also provided a method for enhancing the effectiveness of β -lactam antibiotics, using penicillanic acid 1,1-dioxide and certain readily hydrolyzable esters thereof.

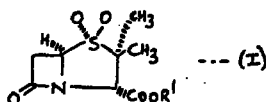
Still further, there are provided derivatives of penicillanic acid 1,1-dioxide having a carboxy protecting group, said compounds being useful as chemical intermediates for penicillanic acid 1,1-dioxide.

40 Yet further, there are provided penicillanic acid 1-oxides, and certain esters thereof, as chemical intermediates to penicillanic acid 1,1-dioxide.

1,1-Dioxides of benzylpenicillin, phenoxymethylpenicillin and certain esters thereof have been disclosed in United States Patents 3,197,466 and 3,536,698, and in an article by Guddal *et al.*, in *Tetrahedron Letters*, No. 0, 381 (1962). Harrison *et al.*, in the *Journal of the Chemical Society* (London), Perkin I, 1772 (1976), have
45 disclosed a variety of penicillin 1,1-dioxides and 1-oxides, including methyl phthalimidopenicillanate 1,1-dioxide, methyl 6,6-dibromopenicillanate 1,1-dioxide, methyl penicillanate 1 α -oxide, methyl penicillanate 1 β -oxide, 6,6-dibromopenicillanic acid 1 α -oxide and 6,6-dibromopenicillanic acid 1 β -oxide.

According to the invention there are provided novel compounds of the formula

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and the pharmaceutically-acceptable base salts thereof, wherein R¹ is selected from the group consisting of hydrogen, ester-forming residues readily hydrolyzable *in vitro*, and conventional penicillin carboxy protecting groups. The term "ester-forming residues readily hydrolyzable *in vitro*" is here intended to refer to non-toxic ester residues which are rapidly cleaved in mammalian blood or tissue, to release the corresponding free acid (i.e. the compound of formula I, wherein R¹ is hydrogen). Typical examples of such readily hydrolyzable ester-forming residues which can be used for R¹ are alkanoyloxymethyl having from 3
60 to 8 carbon atoms, 1-(alkanoyloxy)ethyl having from 4 to 9 carbon atoms, 1-methyl-1-(alkanoyloxy)ethyl having from 5 to 10 carbon atoms, alkoxycarbonyloxymethyl having from 3 to 6 carbon atoms, 1-(alkoxycarbonyloxy)ethyl having from 4 to 7 carbon atoms, 1-methyl-1-(alkoxycarbonyloxy)ethyl having from 5 to 8 carbon atoms, 3-phthalidyl, 4-crotonolactonyl and γ -butyrolacton-4-yl.

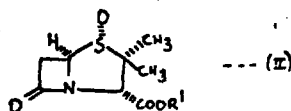
The compounds of the formula I, wherein R¹ is hydrogen or an ester-forming residue readily hydrolyzable
65 *in vitro*, are useful as antibacterial agents and for enhancing the antibacterial activity of β -lactam antibiotics.

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Said compounds of the formula I, wherein R¹ is a penicillin carboxy protecting group, are useful as chemical intermediates to the compound of the formula I, wherein R¹ is hydrogen or an ester-forming residue readily hydrolyzable *in vitro*. Typical carboxy protecting groups are benzyl and substituted benzyl, e.g. 4-nitrobenzyl.

5 It will be appreciated that the terms "ester-forming residue readily hydrolysable *in vivo*", and "conventional penicillin carboxy protecting group", do not include methyl.

Also according to the invention there are provided novel compounds of the formula

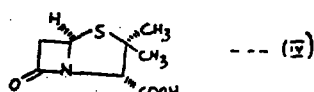


and



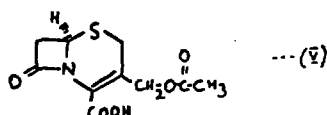
and the salts thereof, wherein R¹ is as defined previously. Said compounds of the formulas II and III are intermediates to said compounds of the formula I.

20 This invention relates to the novel compounds of formulas I, II and III, and throughout this specification they are referred to as derivatives of penicillanic acid, which is represented by the structural formula

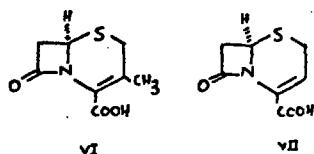


In formula IV, broken line attachment of a substituent to the bicyclic nucleus indicates that the substituent is below the plane of the bicyclic nucleus. Such a substituent is said to be in the α-configuration. Conversely, solid line attachment of a substituent to the bicyclic nucleus indicates that the substituent is attached above the plane of the nucleus. This latter configuration is referred to as the β-configuration.

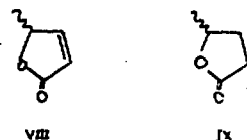
Also in this specification reference is made to certain derivatives of cephalosporanic acid, which has the formula



40 In formula V, the hydrogen at C-6 is below the plane of the bicyclic nucleus. The derived terms desacetoxycephalosporanic acid and 3-desacetoxymethylcephalosporanic acid are used to refer to the structures VI and VII, respectively.



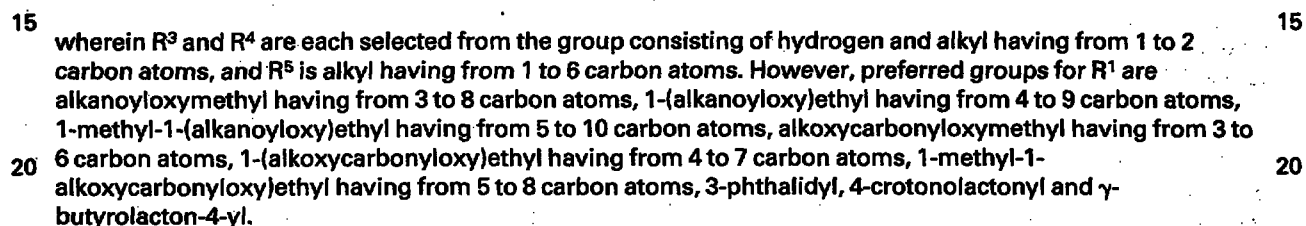
50 4-Crotonolactonyl and γ-butyrolacton-4-yl refer to structures VIII and IX, respectively. The wavy lines are intended to denote each of the two epimers and mixtures thereof.



When R¹ is an ester-forming residue readily hydrolyzable *in vivo* in a compound of formula I, it is a grouping which is notionally derived from an alcohol of the formula R¹-OH, such that the moiety COOR¹ in such a compound of formula I represents an ester grouping. Moreover, R¹ is of such a nature that the grouping COOR¹ is readily cleaved in *in vivo* to liberate a free carboxy group (COOH). That is to say, R¹ is a group of the type that when a compound of formula I, wherein R¹ is an ester-forming residue readily hydrolyzed *in vivo*, is exposed to mammalian blood or tissue, the compound of formula I, wherein R¹ is hydrogen, is readily produced. The groups R¹ are well-known in the penicillin art. In most instances they

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When a compound of the Formula II or III, wherein R¹ is as defined previously, is oxidized to the corresponding compound of the formula I using a metal permanganate, the reaction is usually carried out by treating the compound of the formula II or III with from about 0.5 to about 5 molar equivalents of the permanganate, and preferably about 1 molar equivalent of the permanganate, in an appropriate solvent system. An appropriate solvent system is one that does not adversely interact with either the starting materials or the product, and water is commonly used. If desired, a co-solvent which is miscible with water but will not interact with the permanganate, such as tetrahydrofuran, can be added. The reaction is normally carried out at a temperature in the range from about -20° to about 50°C., and preferably at about 0°C. At about 0°C. the reaction is normally substantially complete within a short period, e.g. within one hour. Although the reaction can be carried out under neutral, basic or acid conditions, it is preferable to operate under substantially neutral conditions in order to avoid decomposition of the β -lactam ring system of the compound of the formula I. Indeed, it is often advantageous to buffer the pH of the reaction medium in the vicinity of neutrality. The product is recovered by conventional techniques. Any excess permanganate is usually decomposed using sodium bisulfite, and then if the product is out of solution, it is recovered by filtration. It is separated from manganese dioxide by extracting it into an organic solvent and removing the solvent by evaporation. Alternatively, if the product is not out of solution at the end of the reaction, it is isolated by the usual procedure of solvent extraction.

When oxidizing a compound of the formula II or III to a compound of the formula I using an organic peroxy acid, it is sometimes advantageous to add a catalyst such as a manganese salt, e.g. manganic acetylacetonate.

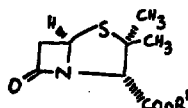
60 The compound of the formula I, wherein R¹ is hydrogen, can also be obtained by removal of the protecting group R¹ from a compound of the formula I, wherein R¹ is a penicillin carboxy protecting group. In this context, R¹ can be any carboxy protecting group conventionally used in the penicillin art to protect carboxy groups at the 3-position. The identity of the carboxy protecting group is not critical. The only requirements for the carboxy protecting group R¹ are that: (i) it must be stable during oxidation of the compound of formula II or III; and (ii) it must be removable from the compound of formula I, using conditions under which

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the β -lactam remains substantially intact. Typical examples which can be used are the tetrahydropyranyl group, substituted benzyl groups (e.g. 4-nitrobenzyl), the benzylhydryl group, the 2,2,2-trichloroethyl group, the t-butyl group and the phenacyl group. See further: United States Patents 3,632,850 and 3,197,466; British Patent No. 1,041,985, Woodward *et al.*, *Journal of the American Chemical Society*, **88**, 852 (1966); Chauvette, *Journal of Organic Chemistry*, **36**, 1259 (1971); Sheehan *et al.*, *Journal of Organic Chemistry*, **29**, 2006 (1964); and "Cephalosporin and Penicillins, Chemistry and Biology", edited by H. E. Flynn, Academic Press, Inc., 1972. The penicillin carboxy protecting group is removed in conventional manner, having due regard for the lability of the β -lactam ring system.

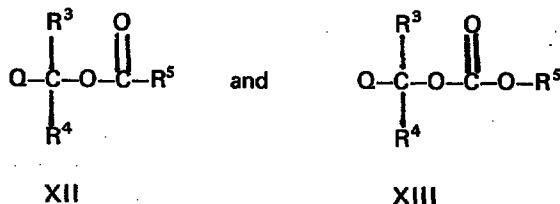
In like manner, compounds of the formula I, wherein R^1 is as previously defined, can be prepared by

oxidation of a compound of the formula



wherein R^1 is as previously defined. This is carried out in exactly the same manner as described hereinbefore for oxidation of a compound of the formula II or III, except that twice as much oxidant is usually used.

Compounds of the formula I, wherein R^1 is an ester-forming residue readily hydrolyzable *in vivo*, can be prepared directly from the compound of formula I, wherein X is hydrogen, by esterification. The specific method chosen will depend naturally upon the precise structure of the ester-forming residue, but an appropriate method will be readily selected by one skilled in the art. In the case wherein R^1 is selected from the group consisting of 3-phthalidyl, 4-crotonolactonyl, γ -butyrolacton-4-yl and groups of the formula X and XI, wherein R^3 , R^4 and R^5 are as defined previously, they can be prepared by alkylation of the compound of formula I, wherein R^1 is hydrogen, with a 3-phthalidyl halide, a 4-crotonolactonyl halide, a γ -butyrolacton-4-yl halide or a compound of the formula



wherein Q is halo, and R^3 , R^4 and R^5 are as previously defined. The terms "halide" and "halo" are intended to mean derivatives of chlorine, bromine and iodine. The reaction is conveniently carried out by dissolving a salt of the compound of formula I, wherein R^1 is hydrogen, in a suitable, polar, organic solvent, such as *N,N*-dimethylformamide, and then adding about one molar equivalent of the halide. When the reaction has proceeded essentially to completion, the product is isolated by standard techniques. It is often sufficient simply to dilute the reaction medium with an excess of water, and then extract the product into a water-immiscible organic solvent and then recover same by solvent evaporation. Salts of the starting material which are commonly used are alkali metal salts, such as sodium and potassium salt, and tertiary amine salts, such as triethylamine, *N*-ethylpiperidine, *N,N*-dimethylaniline and *N*-methylmorpholine salts. The reaction is run at a temperature in the range from about 0 to 100°C., and usually at about 25°C. The length of time needed to reach completion varies according to a variety of factors, such as the concentration of the reactants and the reactivity of the reagents. Thus, when considering the halo compound, the iodide reacts faster than the bromide, which in turn reacts faster than the chloride. In fact, it is sometimes advantageous, when utilizing a chloro compound, to add up to one molar equivalent of an alkali metal iodide. This has the effect of speeding up the reaction. With full regard for the foregoing factors, reaction times of from about 1 to about 24 hours are commonly used.

Penicillanic acid 1 α -oxide, the compound of the formula II, wherein R^1 is hydrogen, can be prepared by debromination of 6,6-dibromopenicillanic acid 1 α -oxide. The debromination can be carried out using a conventional hydrogenolysis technique. Thus, a solution of 6,6-dibromopenicillanic acid 1 α -oxide is stirred or shaken under an atmosphere of hydrogen, or hydrogen mixed with an inert diluent such as nitrogen or argon, in the presence of a catalytic amount of palladium-on-calcium carbonate catalyst. Convenient solvents for this debromination are lower-alkanols, such as methanol; ethers, such as tetrahydrofuran and dioxan; low molecular weight esters, such as ethyl acetate and butyl acetate; water; and mixtures of these solvents. However, it is usual to choose conditions under which the dibromo compound is soluble. The hydrogenolysis is usually carried out at room temperature and at a pressure from about atmospheric pressure to about 50 p.s.i. The catalyst is usually present in an amount from about 10 percent by weight based on the dibromo compound, up to an amount equal in weight to the dibromo compound, although larger amounts can be used. The reaction commonly takes about one hour, after which the compound of the formula II, wherein R^1 is hydrogen, is recovered simply by filtration followed by removal of the solvent *in vacuo*.

6,6-Dibromopenicillanic acid 1 α -oxide is prepared by oxidation of 6,6-dibromopenicillanic acid with 1

equivalent of 3-chloroperbenzoic acid in tetrahydrofuran at 0–25°C. for ca. 1 hour, according to the procedure of Harrison *et al.*, *Journal of the Chemical Society* (London) Perkin I, 1772 (1976). 6,6-Dibromopenicillanic acid is prepared by the method of Clayton, *Journal of the Chemical Society* (London), (C) 2123 (1969).

Penicillanic acid 1 β -oxide, the compound of the formula III, wherein R¹ is hydrogen, can be prepared by controlled oxidation of penicillanic acid. Thus, it can be prepared by treating penicillanic acid with one molar equivalent of 3-chloroperbenzoic acid in an inert solvent at about 0°C. for about one hour. Typical solvents which can be used include chlorinated hydrocarbons, such as chloroform and dichloromethane; ethers, such as diethyl ether and tetrahydrofuran; and low molecular weight esters such as ethyl acetate and butyl acetate. The product is recovered by conventional techniques.

Penicillanic acid is prepared as described in British patent No. 1,072,108.

Compounds of the formula II and III, wherein R¹ is an ester-forming residue readily hydrolyzable *in vivo*, can be prepared directly from the compound of formula II or III, wherein R¹ is hydrogen, by esterification, using standard procedures. In the case wherein R¹ is selected from the group consisting of 3-phthalidyl, 4-crotonolactonyl, γ -butyrolacton-4-yl and groups of the formula X, and XI, wherein R³, R⁴ and R⁵ are as defined previously, they can be prepared by alkylation of the appropriate compound of the formula II or III, wherein R¹ is hydrogen, with a 3-phthalidyl halide, 4-crotonolactonyl halide, a γ -butyrolacton-4-yl halide, or a compound of the formula XII or XIII. The reaction is carried out in exactly the same manner as described previously for esterification of penicillanic acid 1,1-dioxide with a 3-phthalidyl halide, a 4-crotonolactonyl halide, a γ -butyrolacton-4-yl halide, or a compound of the formula XII or XIII.

Alternatively, the compounds of the formula II, wherein R¹ is an ester-forming residue readily hydrolyzable *in vivo*, can be prepared by oxidation of the appropriate ester of 6,6-dibromopenicillanic acid, followed by debromination. The esters of 6,6-dibromopenicillanic acid are prepared from 6,6-dibromopenicillanic acid by standard methods. The oxidation is carried out, for example, by oxidation with one molar equivalent of 3-chloroperbenzoic acid, as described previously for the oxidation of 6,6-dibromopenicillanic acid to 6,6-dibromopenicillanic acid 1 α -oxide; and the debromination is carried out as described previously for the debromination of 6,6-dibromopenicillanic acid 1 α -oxide.

In like manner, the compounds of the formula III, wherein R¹ is an ester-forming residue readily hydrolyzable *in vivo* can be prepared by oxidation of the appropriate ester of penicillanic acid. The latter compounds are readily prepared by esterification of penicillanic acid using standard methods. The oxidation is carried out, for example, by oxidation with one molar equivalent of 3-chloroperbenzoic acid, as described previously for the oxidation of penicillanic acid to penicillanic acid 1 β -oxide.

The compounds of the formula II, wherein R¹ is a carboxy protecting group can be obtained in one of two ways. They can be obtained simply by taking penicillanic acid 1 α -oxide and attaching a carboxy protecting group thereto. Alternatively, they can be obtained by: (a) attaching a carboxy protecting group to 6,6-dibromopenicillanic acid; (b) oxidizing the protected 6,6-dibromopenicillanic acid to a protected 6,6-dibromopenicillanic acid 1 α -oxide using 1 molar equivalent of 3-chloroperbenzoic acid; and (c) debrominating the protected 6,6-dibromopenicillanic acid 1 α -oxide by hydrogenolysis.

The compounds of the formula III, wherein R¹ is a carboxy protecting group can be obtained simply by attaching a protecting group to penicillanic acid 1 β -oxide. Alternatively, they can be obtained by: (a) attaching a carboxy protecting group to penicillanic acid; and (b) oxidizing the protected penicillanic acid using 1 molar equivalent of 3-chloroperbenzoic acid as previously described.

The compounds of formulas I, II and III, wherein R¹ is hydrogen, are acidic and will form salts with basic agents. Such salts are considered to be within the scope of this invention. These salts can be prepared by standard techniques, such as contacting the acidic and basic components, usually in a 1:1 molar ratio, in an aqueous, non-aqueous or partially aqueous medium, as appropriate. They are then recovered by filtration, by precipitation with a non-solvent followed by filtration, by evaporation of the solvent, or in the case of aqueous solutions, by lyophilization, as appropriate. Basic agents which are suitably employed in salt formation belong to both the organic and inorganic types, and they include ammonia, organic amines, alkali metal hydroxides, carbonates, bicarbonates, hydrides and alkoxides, as well as alkaline earth metal hydroxides, carbonates, hydrides and alkoxides. Representative examples of such basis are primary amines, such as *n*-propylamine, *n*-butylamine, aniline, cyclohexylamine, benzylamine and octylamine; secondary amines, such as diethylamine, morpholine, pyrrolidine and piperidine; tertiary amines, such as triethylamine, *N*-ethylpiperidine, *N*-methylmorpholine and 1,5-diazabicyclo[4.3.0]non-5-ene; hydroxides, such as sodium hydroxide, potassium hydroxide, ammonium hydroxide and barium hydroxide; alkoxides, such as sodium ethoxide and potassium ethoxide; hydrides, such as calcium hydride and sodium hydride; carbonates, such as potassium carbonate and sodium carbonate; bicarbonates, such as sodium bicarbonate and potassium bicarbonate; and alkali metal salts of long-chain fatty acids, such as sodium 2-ethylhexanoate.

Preferred salts of the compounds of the formulas I, II and III are sodium, potassium and triethylamine salts.

As indicated hereinbefore, the compounds of formula I, wherein R¹ is hydrogen or an ester-forming residue readily hydrolyzable *in vivo*, are antibacterial agents of medium potency. In *in vitro* activity of the compound of the formula I, wherein R¹ is hydrogen, can be demonstrated by measuring its minimum inhibitory concentration (MIC's) in mcg-ml against a variety of microorganisms. The procedure which is followed is the one recommended by the International Collaborative Study on Antibiotic Sensitivity Testing (Ericsson and Sherris, *Acta. Pathologica et Microbiologia Scandinav*, Supp. 217, Sections A and B: 1–90 [1970]), and

employs brain heart infusion (BHI) agar and the inocula replicating device. Overnight growth tubes are diluted 100 fold for use as the standard inoculum (20,000-10,000 cells in approximately 0.002 ml. are placed on the agar surface; 20 ml. of BHI agar-dish). Twelve 2 fold dilutions of the test compound are employed, with initial concentration of the test drug being 200 mcg.-ml. Single colonies are disregarded when reading plates after 18 hrs. at 37°C. The susceptibility (MIC) of the test organism is accepted as the lowest concentration of compound capable of producing complete inhibition of growth as judged by the naked eye. MIC values for penicillanic acid 1,1-dioxide against several microorganisms are shown in Table I.

Thus the invention also includes a pharmaceutical composition comprising a compound of the formula (I) as defined above or a pharmaceutically acceptable base salt thereof wherein R¹ is hydrogen or an ester-forming residue readily hydrolyzable *in vivo*, together with a pharmaceutically acceptable carrier.

TABLE I

**In Vitro Antibacterial Activity of
Penicillanic Acid 1,1-Dioxide**

Microorganism	MIC (mcg./ml.)
Staphylococcus aureus	100
Streptococcus faecalis	> 200
Streptococcus pyogenes	100
Escherichia coli	50
Pseudomonas aeruginosa	200
Klebsiella pneumoniae	50
Proteus mirabilis	100
Proteus morgani	100
Salmonella typhimurium	50
Pasteurella multocida	50
Serratia marcescens	100
Enterobacter aerogenes	25
Enterobacter cloacae	100
Citrobacter freundii	50
Providencia	100
Staphylococcus epidermis	200
Pseudomonas putida	> 200
Hemophilus influenzae	> 50
Neisseria gonorrhoeae	0.312

The compounds of the formula I, wherein R¹ is hydrogen or an ester-forming residue readily hydrolyzable *in vivo*, are active as antibacterial agents *in vivo*. In determining such activity, acute experimental infections are produced in mice by the intraperitoneal inoculation of the mice with a standardized culture of the test organism suspended in 5 percent weight per volume hog gastric mucin. Infection severity is standardized so that the mice receive one to ten times the LD₁₀₀ dose of the organism (LD₁₀₀: the minimum inoculum of organism required to consistently kill 100 percent of the infected, non-treated control mice). The test compound is administered to the infected mice using a multiple dosage regimen. At the end of the test, the activity of a compound is assessed by counting the number of survivors among the treated animals and expressing the activity of a compound as the percentage of animals which survive.

The *in vitro* antibacterial activity of the compound of the formula I wherein R¹ is hydrogen makes it useful as an industrial antimicrobial, for example in water treatment, slime control, paint preservation and wood preservation, as well as for topical application as a disinfectant. In the case of use of this compound for topical application, it is often convenient to admix the active ingredient with a non-toxic carrier, such as vegetable or mineral oil or an emollient cream. Similarly, it can be dissolved or dispersed in liquid diluents or solvents such as water, alkanols, glycols or mixtures thereof. In most instances it is appropriate to employ concentrations of the active ingredient of from about 0.1 percent to about 10 percent by weight, based on total composition.

The *in vivo* activity of the compounds of formula I, wherein R¹ is hydrogen or an ester-forming-residue readily hydrolyzable *in vivo*, makes them suitable for the control of bacterial infections in mammals, including man, by both the oral and parenteral modes of administration. The compounds will find use in the control of infections caused by susceptible bacteria in human subjects, e.g. infections caused by strains of *Neisseria gonorrhoeae*.

When considering therapeutic use of a compound of the formula I, or a salt thereof, in a mammal, particularly man, the compound can be administered alone, or it can be mixed with pharmaceutically acceptable carriers or diluents. They can be administered orally or parenterally, i.e. intramuscularly, subcutaneously or intraperitoneally. The carrier or diluent is chosen on the basis of the intended mode of administration. For example, when considering the oral mode of administration, an antibacterial penam

compound of this invention can be used in the form of tablets, capsules, lozenges, troches, powders syrups, elixirs, aqueous solutions and suspensions, and the like, in accordance with standard pharmaceutical practice. The proportional ratio of active ingredient to carrier will naturally depend on the chemical nature, solubility and stability of the active ingredient, as well as the dosage contemplated. However, pharmaceutical compositions containing an antibacterial agent of the formula I will likely contain from about 20% to about 95% by weight of active ingredient. In the case of tablets for oral use, carriers which are commonly used include lactose, sodium citrate and salts of phosphoric acid. Various disintegrants such as starch, and lubricating agents, such as magnesium stearate, sodium lauryl sulfate and talc, are commonly used in tablets. For oral administration in capsule form, useful diluents are lactose and high molecular weight polyethylene glycols. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening and-or flavoring agents can be added. For parenteral administration, which includes intramuscular, intraperitoneal, subcutaneous and intravenous use, sterile solutions of the active ingredient are usually prepared, and the pH of the solutions are suitably adjusted and buffered. For intravenous use, the total concentration of solutes should be controlled to render the preparation isotonic.

As indicated earlier, the antibacterial agents of this invention are of use in human subjects against susceptible organisms. The prescribing physician will ultimately determine the appropriate dose for a given human subject, and this can be expected to vary according to the age, weight, and response of the individual patient, as well as the nature and the severity of the patient's symptoms. The compounds of this invention will normally be used orally at dosages in the range from about 10 to about 200 mg. per kilogram of body weight per day, and parenterally at dosages from about 10 to about 400 mg. per kilogram of body weight per day. These figures are illustrative only, however, and in some cases it may be necessary to use dosages outside these limits.

However, as indicated hereinbefore, the compounds of the formula I, wherein R¹ is hydrogen or an ester-forming residue readily hydrolyzable *in vivo*, are potent inhibitors of microbial β -lactamases, and they increase the antibacterial effectiveness of β -lactam antibiotics (penicillins and cephalosporins) against many microorganisms, particularly those which produce a β -lactamase. The manner in which the said compounds of the formula I increase the effectiveness of a β -lactam antibiotic can be appreciated by reference to experiments in which the MIC of a given antibiotic alone, and a compound of the formula I alone, are measured. These MIC's are then compared with the MIC values obtained with a combination of the given antibiotic and the compound of the formula I. When the antibacterial potency of the combination is significantly greater than would have been predicted from the potencies of the individual compounds, this is considered to constitute enhancement of activity. The MIC values of combinations are measured using the method described by Barry and Sabath in "Manual of Clinical Microbiology", edited by Lennette, Spaulding and Truant, 2nd edition, 1974, American Society for Microbiology.

Results of experiments illustrating that penicillanic acid 1,1-dioxide enhances the effectiveness of ampicillin are reported in Table II. From Table II, it can be seen that against 19 ampicillin-resistant strains of *Staphylococcus aureus*, that mode MIC of ampicillin, and of penicillanic acid 1,1-dioxide, is 200 mcg.-ml. However, the mode MIC's of ampicillin and penicillanic acid 1,1-dioxide in combination are 1.56 and 3.12 mcg.-ml., respectively. Looked at another way, this means that whereas ampicillin alone has a mode MIC of 200 mcg.-ml. against the 19 strains of *Staphylococcus aureus*, its mode MIC is reduced to 1.56 mcg.-ml. in the presence of 3.12 mcg.-ml. of penicillanic acid 1,1-dioxide. The other entries in Table II show enhancement of the antibacterial effectiveness of ampicillin against 26 ampicillin resistance strains of *Haemophilus influenzae*, 18 ampicillin resistant strains of *Klebsiella pneumoniae*, and 15 strains of the anerobe *Bacteroides fragilis*. Tables III, IV and V show enhancement of the antibacterial potency of benzylpenicillin (penicillin G), carbenicillin (α -carboxybenzylpenicillin) and cefazolin, respectively, against strains of *S. aureus*, *H. influenzae*, *K. pneumoniae* and *B. fragilis*.

TABLE II

Effect of Penicillanic Acid 1,1-Dioxide (PA, 1-Dioxide) on the Antibacterial Activity of Ampicillin

Microorganism	No. of Strains	Mode MIC of ampicillin alone	Mode MIC of PA 1,1-Dioxide alone	Mode MIC's of ampicillin and PA 1,1-Dioxide in combination	
				ampicillin	PA 1,1-Dioxide
<i>Staphylococcus aureus</i>	19	200	200	1.56	3.12
<i>Haemophilus influenzae</i>	26	200	>200	0.78	3.12
<i>Klebsiella pneumoniae</i>	18	>400	50	6.25	6.25
<i>Bacteroides fragilis</i>	15	50	50	1.56	0.78

TABLE III

Effect of Penicillanic Acid 1,1-Dioxide (PA 1,1-Dioxide) on the
Antibacterial Activity of Penicillin G

Microorganism	No. of Strains	Mode MIC of penicillin G alone	Mode MIC of PA 1,1-Dioxide alone	Mode MIC's of penicillin G and PA 1,1-Dioxide in combination	
				penicillin G	PA 1,1-Dioxide
<i>Staphylococcus aureus</i>	20	200	200	3.12	6.25
<i>Haemophilus influenzae</i>	25	50	100	0.78	1.56
<i>Klebsiella pneumoniae</i>	24	400	50	25	12.5
<i>Bacteriodes fragilis</i>	15	25	25	1.56	0.39

TABLE IV

Effect of Penicillanic Acid 1,1-Dioxide (PA 1,1-Dioxide) on the
Antibacterial Activity of Carbenicillin

Microorganism	No. of Strains	Mode MIC of carbenicillin alone	Mode MIC of PA 1,1-Dioxide alone	Mode MIC's of carbenicillin and PA 1,1-Dioxide in combination	
				carbenicillin	PA 1,1-Dioxide
<i>Staphylococcus aureus</i>	20	12.5	200	6.25	6.25
<i>Haemophilus influenzae</i>	25	6.25	100	0.39	0.78
<i>Klebsiella pneumoniae</i>	16	>400	50	50	6.25
<i>Bacteroides fragilis</i>	15	50	50	3.12	0.78

TABLE V

Effect of Penicillanic Acid 1,1-Dioxide (PA 1,1-Dioxide) on the
Antibacterial Activity of Cefazolin

Microorganism	No. of Strains	Mode MIC of Cefazolin alone	Mode MIC of PA 1,1-Dioxide alone	Mode MIC's of Cefazolin and PA 1,1-Dioxide in combination	
				Cefazolin	PA 1,1-Dioxide
<i>Staphylococcus aureus</i>	20	0.78	200	0.2	25
<i>Haemophilus influenzae</i>	25	25	200	3.12	0.20
<i>Klebsiella pneumoniae</i>	3	100	50	6.25	25
<i>Bacteroides fragilis</i>	15	200	50	6.25	6.25

The compounds of the formula I, wherein R¹ is hydrogen or an ester-forming residue readily hydrolyzable *in vivo*, enhance the antibacterial effectiveness of β -lactam antibiotics *in vivo*. This is, they lower the amount of the antibiotic which is needed to protect mice against an otherwise lethal inoculum of certain β -lactamase producing bacteria.

The ability of the compounds of the formula I, wherein R¹ is hydrogen or an ester-forming residue readily hydrolyzable *in vivo*, to enhance the effectiveness of a β -lactam antibiotic against β -lactamase-producing bacteria makes them valuable for co-administration with β -lactam antibiotics in the treatment of bacterial infections in mammals, particularly man. In the treatment of bacterial infections in mammals, particularly man. In the treatment of a bacterial infection, the said compound of the formula I can be comingled with the β -lactam antibiotic, and the two agents thereby administered simultaneously. Alternatively, the said compound of the formula I can be administered as a separate agent during a course of treatment with a β -lactam antibiotic. In some instances it will be advantageous to pre-dose the subject with the compound of the formula I before initiating treatment with a β -lactam antibiotic.

When using penicillanic acid 1,1-dioxide or an ester thereof readily hydrolyzable *in vivo* to enhance the effectiveness of β -lactam antibiotic, it is administered preferably in formulation with standard pharmaceutic-

al carriers or diluents. The methods of formulation discussed earlier for use of penicillanic acid 1,1-dioxide or an ester thereof readily hydrolyzable *in vivo* as a single-entity antibacterial agent can be used when co-administration with another β -lactam antibiotic is intended. A pharmaceutical composition comprising a pharmaceutically-acceptable carrier, a β -lactam antibiotic and penicillanic acid 1,1-dioxide or a readily hydrolyzable ester thereof will normally contain from about 5 to about 80 percent of the pharmaceutically acceptable carrier by weight.

When using penicillanic acid 1,1-dioxide or an ester thereof readily hydrolyzable *in vivo* in combination with another β -lactam antibiotic, the sulfone can be administered orally or parenterally, i.e. intramuscularly, subcutaneously or intraperitoneally. Although the prescribing physician will ultimately decide the dosage to be used in a human subject, the weight ratio of the daily dosages of the penicillanic acid 1,1-dioxide or ester thereof and the β -lactam antibiotic will normally be in the range from about 1:3 to 3:1. Additionally, when using penicillanic acid 1,1-dioxide or an ester thereof readily hydrolyzable *in vivo* in combination with another β -lactam antibiotic, the daily oral dosage of each component will normally be in the range from about 10 to about 200 mg. per kilogram of body weight and the daily parenteral dosage of each component will normally be about 10 to about 400 mg. per kilogram of body weight. These figures are illustrative only, however, and in some cases it may be necessary to use dosages outside these limits.

Typical β -lactam antibiotics with which penicillanic acid 1,1-dioxide and its esters readily hydrolyzable *in vivo* can be co-administered are: 6-(2-phenylacetamido)penicillanic acid, 6-(2-phenoxyacetamido)penicillanic acid, 6-(2-phenylpropionamido)penicillanic acid, 6-(D-2-amino-2-phenylacetamido)penicillanic acid, 6-(D-2-amino-2-[4-hydroxyphenyl]acetamido)penicillanic acid, 6-(D-2-amino-2-[1,4-cyclohexadienyl]acetamido)penicillanic acid, 6-(1-aminocyclohexanecarboxamido)penicillanic acid, 6-(2-carboxy-2-phenylacetamido)penicillanic acid, 6-(2-carboxy-2-[3-thienyl]acetamido)penicillanic acid, 6-(D-2-[4-hydroxy-1,5-naphthyridine-3-carboxamido]-2-phenylacetamido)penicillanic acid, 6-(D-2-sulfo-2-phenylacetamido)penicillanic acid, 6-(D-2-sulfoamino-2-phenylacetamido)penicillanic acid, 6-(D-2-[imidazolidin-2-one-1-carboxamido]-2-phenylacetamido)penicillanic acid, 6-(D-[3-methylsulfonyl-imidazolidin-2-one-1-carboxamido]-2-phenylacetamido)penicillanic acid, 6-[(hexahydro-1H-azepin-1-yl)methyleneamino]penicillanic acid, acetoxymethyl 6-(2-phenylacetamido)penicillanate, acetoxymethyl 6-(D-2-amino-2-phenylacetamido)penicillanate, acetoxymethyl 6-(D-2-amino-2-[4-hydroxyphenyl]acetamido)penicillanate, pivaloyloxymethyl 6-(2-phenylacetamido)penicillanate, pivaloyloxymethyl 6-(D-2-amino-2-[4-hydroxyphenyl]acetamido)penicillanate, 1-(ethoxycarbonyloxy)ethyl 6-(D-2-amino-2-phenylacetamido)penicillanate, 1-(ethoxycarbonyloxy)ethyl 6-(D-2-amino-2-[4-hydroxyphenyl]acetamido)penicillanate, 3-phthalidyl 6-(2-phenylacetamido)penicillanate, 3-phthalidyl 6-(D-2-amino-2-phenylacetamido)penicillanate, 3-phthalidyl 6-(D-2-amino-2-[4-hydroxyphenyl]acetamido)penicillanate, 6-(2-phenoxycarbonyl-2-phenylacetamido)penicillanic acid, 6-(2-tolyloxycarbonyl-2-phenylacetamido)penicillanic acid, 6-(2-[5-indanyloxycarbonyl]-2-phenylacetamido)penicillanic acid, 6-(2-phenoxycarbonyl-2-[3-thienyl]acetamido)penicillanic acid, 6-(2-tolyloxycarbonyl-2-[3-thienyl]acetamido)penicillanic acid, 6-(2-[5-indanyloxy-carbonyl]-2-[3-thienyl]acetamido)penicillanic acid, 6-(2,2-dimethyl-5-oxo-4-phenyl-1-imidazolidinyl)penicillanic acid, 7-(2-[2-thienyl]acetamido)cephalosporanic acid, 7-(2-[1-tetrazolyl]acetamido)-3-(2-[5-methyl-1,3,4-thiazadiazolyl]thiomethyl)-3-desacetoxymethylcephalosporanic acid, 7-(D-2-amino-2-phenylacetamido)desacetoxymethylcephalosporanic acid, 7- α -methoxy-7-(2-[3-thienyl]acetamido)-3-carbamoyloxy-methyl-3-desacetoxymethylcephalosporanic acid, 7-(2-cyanoacetamido)cephalosporanic acid, 7-(D-2-hydroxy-2-phenylacetamido)-3-(5-[1-methyltetrazolyl]thiomethyl)-3-desacetoxymethylcephalosporanic acid, 7-(2-[4-pyridylthio]acetamido)cephalosporanic acid, 7-(D-2-amino-2-[1,4-cyclohexadienyl]acetamido)cephalosporanic acid, 7-(D-2-amino-2-phenylacetamido)cephalosporanic acid, and the pharmaceutically-acceptable salts thereof.

As will be appreciated by one skilled in the art, some of the above β -lactam compounds are effective when administered orally or parenterally, while others are effective only when administered by the parenteral route. When penicillanic acid 1,1-dioxide or an ester thereof readily hydrolyzable *in vivo* is to be used simultaneously (i.e. co-mingled) with a β -lactam antibiotic which is effective only on parenteral administration, a combination formulation suitable for parenteral use will be required. When the penicillanic acid 1,1-dioxide or ester thereof is to be used simultaneously (co-mingled) with a β -lactam antibiotic which is effective orally or parenterally, combinations suitable for either oral or parenteral administration can be prepared. Additionally, it is possible to administer preparations of the penicillanic acid 1,1-dioxide or ester thereof orally, while at the same time administering a further β -lactam antibiotic parenterally; and it is also possible to administer preparations of the penicillanic acid 1,1-dioxide or ester thereof parenterally, while at the same time administering the further β -lactam antibiotic orally.

The following examples are provided solely for the purpose of further illustration. Infrared (IR) spectra were measured as potassium bromide discs (KBr discs) or as Nujol (Registered Trade Mark) mulls, and diagnostic absorption bands are reported in wave numbers (cm^{-1}). Nuclear magnetic resonance spectra (NMR) were measured at 60 MHz for solutions in deuteriochloroform (CDCl_3), perdeutero dimethyl sulfoxide ($\text{DMSO}-d_6$) or deuterium oxide (D_2O), and peak positions are expressed in parts per million (ppm) downfield from tetramethylsilane or sodium 2,2-dimethyl-2-silapentane-5-sulfonate. The following abbreviations for peak shapes are used: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet.

EXAMPLE 1

Penicillanic Acid 1,1-Dioxide

To a solution of 6.51 g. (41 mmole) of potassium permanganate in 130 ml. of water and 4.95 ml. of glacial acetic acid, cooled to ca. 5°C., was added a cold (ca. 5°C.) solution of 4.58 g. (21 mmole) of the sodium salt of penicillanic acid in 50 ml. of water. The mixture was stirred at ca. 5°C. for 20 minutes and then the cooling bath was removed. Solid sodium bisulfite was added until the color of the potassium permanganate had been discharged, and then the mixture was filtered. To the aqueous filtrate was added half its volume of saturated sodium chloride solution, and then the pH was adjusted to 1.7. The acidic solution was extracted with ethyl acetate. The extracts were dried, and then evaporated *in vacuo*, to give 3.47 g. of the title product. The aqueous mother liquor was saturated with sodium chloride, and further extracted with ethyl acetate. The ethyl acetate solution was dried and evaporated *in vacuo*, to give a further 0.28 g. of product. The total yield was therefore 3.75 g. (78% yield). The NMR spectrum (DMSO-*d*₆) of the product showed absorptions at 1.40 (s, 3H), 1.50 (s, 3H), 3.13 (d of d's, 1H, J₁ = 16 Hz, J₂ = 2 Hz), 3.63 (d of d's, 1H, J₁ = 16 Hz, J₂ = 4 Hz), 4.22 (s, 1H) and 5.03 (d of d's, 1H, J₁ = 4 Hz, J₂ = 2 Hz) ppm.

EXAMPLE 2

Benzyl Penicillanate 1,1-Dioxide

To a stirred solution of 6.85 g. (24 mmole) of benzyl penicillanate in 75 ml. of ethanol-free chloroform, under nitrogen, in an ice-bath, was added in two portions, several minutes apart, 4.78 g. of 85% pure by weight 3-chloroperbenzoic acid. Stirring was continued for 30 minutes in the ice-bath, and then for 45 minutes without external cooling. The reaction mixture was washed with aqueous alkali (pH 8.5), followed by saturated sodium chloride, and then it was dried and evaporated *in vacuo* to give 7.05 g. of residue. Examination of this residue showed it to be a mixture of benzyl penicillanate 1-oxide and benzyl penicillanate 1,1-dioxide in a molar ratio of 5.5:1.

To a stirred solution of 4.85 g. of the above 5.5:1 sulfoxide-sulfone mixture in 50 ml. of ethanol-free chloroform, under nitrogen, was added 3.2 g. of 85% pure by weight 3-chloroperbenzoic acid at room temperature. The reaction mixture was stirred for 2.5 hours, and then it was diluted with ethyl acetate. The resultant mixture was added to water at pH 8.0, and then the layers were separated. The organic phase was washed with water at pH 8.0, followed by saturated sodium chloride, and then it was dried using sodium sulfate. Evaporation of the solvent *in vacuo* afforded 3.59 g. of the title compound. The NMR spectrum of the product (in CDCl₃) showed absorptions at 1.28 (s, 3H), 1.58 (s, 3H), 3.42 (m, 2H), 4.37 (s, 1H), 4.55 (m, 1H), 5.18 (q, 2H, J = 12 Hz) and 7.35 (s, 5H) ppm.

EXAMPLE 3

Penicillanic Acid 1,1-Dioxide

To a stirred solution of 8.27 g. of benzyl penicillanate 1,1-dioxide in a mixture of 40 ml. of methanol and 10 ml. of ethyl acetate was slowly added 10 ml. of water, followed by 12 g. of 5% weight per weight palladium-on-calcium carbonate. The mixture was shaken under an atmosphere of hydrogen, at 52 psi, for 40 minutes, and then it was filtered through Supercel (a diatomaceous earth). The filter cake was washed with methanol, and with aqueous methanol, and the washings were added to the filtrate. The combined solution was evaporated *in vacuo* to remove the majority of the organic solvents and then the residue was partitioned between ethyl acetate and water at a pH of 2.8. The ethyl acetate layer was removed and the aqueous phase was further extracted with ethyl acetate. The combined ethyl acetate solutions were washed with saturated sodium chloride solution, dried using sodium sulfate and then evaporated *in vacuo*. The residue was slurried in a 1:2 volume per volume mixture of ethyl acetate-ether, to give 2.37 g. of the title product having a melting point of 148–51°C. The ethyl acetate-ether mixture was evaporated giving a further 2.17 g. of product.

EXAMPLE 4

Pivaloyloxymethyl Penicillanate 1,1-Dioxide

To 0.615 g. (2.41 mmole) of penicillanic acid 1,1-dioxide in 2 ml. of *N,N*-dimethylformamide was added 0.215 g. (2.50 mmole) of diisopropylethylamine followed by 0.365 ml. of chloromethyl pivalate. The reaction mixture was stirred at room temperature for 24 hours, and then it was diluted with ethyl acetate and water. The ethyl acetate layer was separated and washed three times with water and once with saturated sodium chloride solution. The ethyl acetate solution was then dried using anhydrous sodium sulfate, and evaporated *in vacuo* to give 0.700 g. of the title product as a solid, mp 103–4°C. The NMR spectrum of the product (in CDCl₃) showed absorptions at 1.27 (s, 9H), 1.47 (s, 3H), 1.62 (s, 3H), 3.52 (m, 2H), 4.47 (s, 1H), 4.70 (m, 1H), 5.72 (d, 1H, J = 6.0 Hz) and 5.98 (d, 1H, J = 6.0 Hz).

EXAMPLE 5

The procedure of Example 4 is repeated, except that the pivaloyloxymethyl chloride used therein is replaced by an equimolar amount of acetoxymethyl chloride, propionyloxymethyl chloride and hexanoyloxymethyl chloride, respectively, to give: acetoxymethyl penicillanate 1,1-dioxide, propionyloxymethyl penicillanate 1,1-dioxide and hexanoyloxymethyl penicillanate 1,1-dioxide, respectively.

EXAMPLE 6

3-Phthalidyl Penicillanate 1,1-Dioxide

To 0.783 g. (3.36 mmole) of penicillanic acid 1,1-dioxide in 5 ml. of *N,N*-dimethylformamide was added 0.47 ml. of triethylamine followed by 0.715 g. of 3-bromophthalide. The reaction mixture was stirred for 2 hours at room temperature and then it was diluted with ethyl acetate and water. The pH of the aqueous

phase was raised to 7.0 and the layers were separated. The ethyl acetate layer was washed successively with water and saturated sodium chloride solution, and then it was dried using sodium sulfate. The ethyl acetate solution was evaporated *in vacuo* leaving the title product as a white foam. The NMR spectrum of the product (in CDCl₃) showed absorptions at 1.47 (s, 6H), 3.43 (m, 1H), 4.62 (m, 1H), 7.40 and 7.47 (2s's, 1H) and 7.73 (m, 4H) ppm.

When the above procedure is repeated, except that the 3-bromophthalide is replaced by 4-bromocrotonolactone and 4-bromo- γ -butyrolactone, respectively, this affords: 4-crotonolactonyl penicillanate 1,1-dioxide and γ -butyrolacton-4-yl penicillanate, respectively.

EXAMPLE 7

1-(Ethoxycarbonyloxy)ethyl Penicillanate 1,1-Dioxide

A mixture of 0.654 g. of penicillanic acid 1,1-dioxide, 0.42 ml. of triethylamine, 0.412 g. of 1-chloroethyl ethyl carbonate, 0.300 g. of sodium bromide and 3 ml. of *N,N*-dimethylformamide was stirred at room temperature for 6 days. It was then worked up by diluting it with ethyl acetate and water, and then the pH was adjusted to 8.5. The ethyl acetate layer was separated, washed three times with water, washed once with saturated sodium chloride, and then it was dried using anhydrous sodium sulfate. The ethyl acetate was removed by evaporation *in vacuo* leaving 0.390 g. of the title product as an oil.

The above product was combined with an approximately equal weight of similar material from a similar experiment. The combined product was dissolved in chloroform and 1 ml. of pyridine was added. The mixture was stirred at room temperature overnight and then the chloroform was removed by evaporation *in vacuo*. The residue was partitioned between ethyl acetate and water at pH 8. The separated and dried ethyl acetate was then evaporated *in vacuo* to give 150 mg. of the title product (yield ca. 7%). The IR spectrum (film) of the product showed absorptions at 1805 and 1763 cm⁻¹. The NMR spectrum (CDCl₃) showed absorptions at 1.43 (m, 12H), 3.47 (m, 2H), 3.9 (q, 2H, J = 7.5 Hz), 4.37 (m, 1H), 4.63 (m, 1H) and 6.77 (m, 1H) ppm.

EXAMPLE 8

The procedure of Example 7 is repeated, except that the 1-chloroethyl ethyl carbonate is replaced by an equimolar amount of the appropriate 1-chloroalkyl alkyl carbonate, 1-(alkanoyloxy)ethyl chloride or 1-methyl-1-(alkanoyloxy)ethyl chloride, to produce the following compounds: methoxycarbonyloxymethyl penicillanate 1,1-dioxide, ethoxycarbonyloxymethyl penicillanate 1,1-dioxide, isobutoxycarbonyloxymethyl penicillanate 1,1-dioxide, 1-(methoxycarbonyloxy)ethyl penicillanate 1,1-dioxide, 1-(butoxycarbonyloxy)ethyl penicillanate 1,1-dioxide, 1-(acetoxo)ethyl penicillanate 1,1-dioxide, 1-(butyryloxy)ethyl penicillanate 1,1-dioxide, 1-(pivaloyloxy)ethyl penicillanate 1,1-dioxide, 1-(hexanoyloxy)ethyl penicillanate 1,1-dioxide, 1-methyl-1-(acetoxo)ethyl penicillanate 1,1-dioxide and 1-methyl-1-(isobutyryloxy)ethyl penicillanate 1,1-dioxide, respectively.

EXAMPLE 9

The procedure of Example 4 is repeated, except that the chloromethyl pivalate is replaced by an equimolar amount of benzyl bromide and 4-nitrobenzyl bromide, respectively, to produce benzyl penicillanate 1,1-dioxide and 4-nitrobenzyl penicillanate 1,1-dioxide, respectively.

EXAMPLE 10

Penicillanic Acid 1 α -Oxide

To 1.4 g. of prehydrogenated 5% weight per weight palladium-on-calcium carbonate in 50 ml. of water was added a solution of 1.39 g. of benzyl 6,6-dibromopenicillanate 1 α -oxide in 50 ml. of tetrahydrofuran. The mixture was shaken under an atmosphere of hydrogen at ca. 45 p.s.i. and 25°C. for 1 hour, and then it was filtered. The filtrate was evaporated *in vacuo* to remove the bulk of the tetrahydrofuran, and then the aqueous phase was extracted with ether. The ether extracts were evaporated *in vacuo* to give 0.5 g. of material which appeared to be largely benzyl penicillanate 1 α -oxide.

The above benzyl penicillanate 1 α -oxide was combined with a further 2.0 g. of benzyl 6,6-dibromopenicillanate 1 α -oxide and dissolved in 50 ml. of tetrahydrofuran. The solution was added to 4.0 g. of 5% weight per weight palladium-on-calcium carbonate, in 50 ml. of water, and the resulting mixture was shaken under an atmosphere of hydrogen, at ca. 45 p.s.i. and 25°C. overnight. The mixture was filtered, and the filtrate was extracted with ether. The extracts were evaporated *in vacuo*, and the residue was purified by chromatography on silica gel, eluting with chloroform. This afforded 0.5 g. of material.

The latter material was hydrogenated at ca. 45 p.s.i. at 25°C. in water-methanol (1:1 volume per volume) with 0.50 g. of 5% weight per weight palladium-on-calcium carbonate for 2 hours. At this point, an additional 0.50 g. of 5% weight per weight palladium-on-calcium carbonate was added and the hydrogenation was continued at 45 p.s.i. and 25°C. overnight. The reaction mixture was filtered, extracted with ether and the extracts were discarded. The residual aqueous phase was adjusted to pH 1.5 and then extracted with ethyl acetate. The ethyl acetate extracts were dried (Na₂SO₄) and then evaporated *in vacuo* to give 0.14 g. of penicillanic acid 1 α -oxide. The NMR spectrum (CDCl₃-DMSO-d₆) showed absorptions at 1.4 (s, 3H), 1.64 (s, 3H), 3.60 (m, 2H), 4.3 (s, 1H) and 4.54 (m, 1H) ppm. The IR spectrum of the product (KBr disc) showed absorptions at 1795 and 1745 cm⁻¹.

EXAMPLE 11

Penicillanic Acid 1 α -Oxide

To 1.0 g. of prehydrogenated 5% weight per weight palladium-on-calcium carbonate in 30 ml. of water is added a solution of 1.0 g. of 6,6-dibromopenicillanic acid 1 α -oxide. The mixture is shaken under an

atmosphere of hydrogen, at ca. 45 p.s.i. and 25°C., for 1 hour. The reaction mixture is then filtered and the filtrate is concentrated *in vacuo* to remove the methanol. The residual aqueous phase is diluted with an equal volume of water, adjusted to pH 7, and washed with ether. The aqueous phase is then acidified to pH 2 with dilute hydrochloric acid and extracted with ethyl acetate. The ethyl acetate extracts are dried (Na₂SO₄) and

5 evaporated *in vacuo* to give penicillanic acid 1 α -oxide.

EXAMPLE 12

Penicillanic Acid 1 β -Oxide

To a stirred solution of 2.65 g. (12.7 mmole) of penicillanic acid in chloroform at 0°C. was added 2.58 g. of 85% pure by weight 3-chloroperbenzoic acid. After 1 hour, the reaction mixture was filtered and the filtrate was evaporated *in vacuo*. The residue was dissolved in a small amount of chloroform. The solution was concentrated slowly until a precipitate began to appear. At this point the evaporation was stopped and the mixture was diluted with ether. The precipitate was removed by filtration, washed with ether and dried, to give 0.615 g. of penicillanic acid 1 β -oxide, m.p. 140–3°C. The IR spectrum of the product (CHCl₃ solution) showed absorptions at 1175 and 1720 cm⁻¹. The NMR spectrum (CDCl₃-DMSO-d₆) showed absorptions at

15 1.35 (s, 3H), 1.76 (s, 3H), 3.36 (m, 2H), 4.50 (s, 1H) and 5.05 (m, 1H) ppm. From the NMR spectrum, the product appeared to be ca. 90% pure by weight.

Examination of the chloroform-ether mother liquor revealed that it contained further penicillanic acid 1 β -oxide, and also some penicillanic acid 1 β -oxide.

EXAMPLE 13

20 Esterification of penicillanic acid 1 α -oxide or penicillanic acid 1 β -oxide, as appropriate, with the requisite alkanoyloxy chloride, according to Example 5, provides the following compounds: acetoxymethyl penicillanate 1 α -oxide, propionyloxymethyl penicillanate 1 α -oxide, pivaloyloxymethyl penicillanate 1 α -oxide, acetoxymethyl penicillanate 1 β -oxide, propionyloxymethyl penicillanate 1 β -oxide and pivaloyloxymethyl penicillanate 1 β -oxide, respectively.

EXAMPLE 14

25 Reaction of penicillanic acid 1 α -oxide or penicillanic acid 1 β -oxide with 3-bromophthalide, 4-bromocrotonolactone or 4-bromo- γ -butyrolactone, as appropriate, affords the following compounds: 3-phthalidyl penicillanate 1 α -oxide, 4-crotonolactonyl penicillanate 1 α -oxide, 3-phthalidyl penicillanate 1 β -oxide, 4-crotonolactonyl penicillanate 1 β -oxide and γ -butyrolacton-4-yl penicillanate 1 β -oxide, respectively.

EXAMPLE 15

Reaction of penicillanic acid 1 α -oxide or penicillanic acid 1 β -oxide, as appropriate, with the requisite 1-chloroalkyl alkyl carbonate or 1-(alkanoyloxy)ethyl chloride, according to the procedure of Example 7, provides the following compounds: 1-(ethoxycarbonyloxy)ethyl penicillanate 1 α -oxide, methoxycarbonyloxymethyl penicillanate 1 α -oxide, ethoxycarbonyloxymethyl penicillanate 1 α -oxide, isobutoxycarbonyloxymethyl penicillanate 1 α -oxide, 1-(methoxycarbonyloxy)ethyl penicillanate 1 α -oxide, 1-(butoxycarbonyloxy)ethyl penicillanate 1 α -oxide, 1-(acetoxymethyl)ethyl penicillanate 1 α -oxide, 1-(butyryloxy)ethyl penicillanate 1 α -oxide, 1-(pivaloyloxy)ethyl penicillanate 1 α -oxide, 1-(ethoxycarbonyloxy)ethyl penicillanate 1 β -oxide, methoxycarbonyloxymethyl penicillanate 1 β -oxide, ethoxycarbonyloxymethyl penicillanate 1 β -oxide, isobutoxycarbonyloxymethyl penicillanate 1 β -oxide, 1-(methoxycarbonyloxy)ethyl penicillanate 1 β -oxide, 1-(butoxycarbonyloxy)ethyl penicillanate 1 β -oxide, 1-(acetoxymethyl)ethyl penicillanate 1 β -oxide, 1-(butyryloxy)ethyl penicillanate 1 β -oxide and 1-(pivaloyloxy)ethyl penicillanate 1 β -oxide, respectively.

EXAMPLE 16

45 Reaction of penicillanic acid 1 α -oxide and penicillanic acid 1 β -oxide with benzyl bromide, according to the procedure of Example 4, produces benzyl penicillanate 1 α -oxide and benzyl penicillanate 1 β -oxide, respectively.

In like manner, reaction of penicillanic acid 1 α -oxide and penicillanic acid 1 β -oxide with 4-nitrobenzyl bromide, according to the procedure of Example 4, produces 4-nitrobenzyl penicillanate 1 α -oxide and 4-nitrobenzyl penicillanate 1 β -oxide, respectively.

EXAMPLE 17

Penicillanic Acid 1,1-Dioxide

To 2.17 g. (10 mmole) of penicillanic acid 1 α -oxide in 30 ml. of ethanol-free chloroform at ca. 0°C. is added 1.73 g. (10 mmole) of 3-chloroperbenzoic acid. The mixture is stirred for 1 hour at ca. 0°C. and then for an additional 24 hours at 25°C. The filtered reaction mixture is evaporated *in vacuo* to give penicillanic acid 1,1-dioxide.

EXAMPLE 18

The procedure of Example 17 is repeated, except that the penicillanic acid 1 α -oxide used therein is replaced by: penicillanic acid 1 β -oxide, acetoxymethyl penicillanate 1 α -oxide, propionyloxymethyl penicillanate 1 α -oxide, pivaloyloxymethyl penicillanate 1 α -oxide, acetoxymethyl penicillanate 1 β -oxide, propionyloxymethyl penicillanate 1 β -oxide, 3-phthalidyl penicillanate 1 α -oxide, 3-phthalidyl penicillanate 1 β -oxide, 1-(ethoxycarbonyloxy)ethyl penicillanate 1 α -oxide, methoxycarbonyloxymethyl penicillanate 1 α -oxide, ethoxycarbonyloxymethyl penicillanate 1 α -oxide, isobutoxycarbonyloxymethyl penicillanate 1 α -oxide, 1-(methoxycarbonyloxy)ethyl penicillanate 1 α -oxide, 1-(butoxycarbonyloxy)ethyl penicillanate 1 α -oxide, 1-(acetoxymethyl)ethyl penicillanate 1 α -oxide, 1-(butyryloxy)ethyl penicillanate 1 α -oxide, 1-(pivaloyloxy)ethyl

22

penicillanate 1 α -oxide, 1-(ethoxycarbonyloxy)ethyl penicillanate 1 β -oxide, methoxycarbonyloxymethyl penicillanate 1 β -oxide, ethoxycarbonyloxymethyl penicillanate 1 β -oxide, isobutoxycarbonyloxymethyl penicillanate 1 β -oxide, 1-(methoxycarbonyloxy)ethyl penicillanate 1 β -oxide, 1-(butoxycarbonyloxy)ethyl penicillanate 1 β -oxide, 1-(acetoxo)ethyl penicillanate 1 β -oxide, 1-(butyryloxy)ethyl penicillanate 1 β -oxide and 1-(pivaloyloxy)ethyl penicillanate 1 β -oxide, respectively. This affords: penicillanic acid 1,1-dioxide, acetoxymethyl penicillanate 1,1-dioxide, propionyloxymethyl penicillanate 1,1-dioxide, pivaloyloxymethyl penicillanate 1,1-dioxide, acetoxymethyl penicillanate 1,1-dioxide, propionyloxymethyl penicillanate 1,1-dioxide, pivaloyloxymethyl penicillanate 1,1-dioxide, 3-phthalidyl penicillanate 1,1-dioxide, 3-phthalidyl penicillanate 1,1-dioxide, 1-(ethoxycarbonyloxy)ethyl penicillanate 1,1-dioxide, methoxycarbonyloxymethyl penicillanate 1,1-dioxide, ethoxycarbonyloxymethyl penicillanate 1,1-dioxide, isobutoxycarbonyloxymethyl penicillanate 1,1-dioxide, 1-(methoxycarbonyloxy)ethyl penicillanate 1,1-dioxide, 1-(butoxycarbonyloxy)ethyl penicillanate 1,1-dioxide, 1-(acetoxo)ethyl penicillanate 1,1-dioxide, 1-(butyryloxy)ethyl penicillanate 1,1-dioxide, 1-(pivaloyloxy)ethyl penicillanate 1,1-dioxide, 1-(ethoxycarbonyloxy)ethyl penicillanate 1,1-dioxide, methoxycarbonyloxymethyl penicillanate 1,1-dioxide, ethoxycarbonyloxymethyl penicillanate 1,1-dioxide, isobutoxycarbonyloxymethyl penicillanate 1,1-dioxide, 1-(methoxycarbonyloxy)ethyl penicillanate 1,1-dioxide, 1-(butoxycarbonyloxy)ethyl penicillanate 1,1-dioxide, 1-(acetoxo)ethyl penicillanate 1,1-dioxide, 1-(butyryloxy)ethyl penicillanate 1,1-dioxide and 1-(pivaloyloxy)ethyl penicillanate 1,1-dioxide, respectively.

EXAMPLE 19

Oxidation of benzyl penicillanate 1 α -oxide and benzyl penicillanate 1 β -oxide with 3-chloroperbenzoic acid, according to the procedure of Example 17, produces, in each case, benzyl penicillanate 1,1-dioxide.

In like manner, oxidation of 4-nitrobenzyl penicillanate 1 α -oxide and 4-nitrobenzyl penicillanate 1 β -oxide with 3-chloroperbenzoic acid, according to the procedure of Example 17, produces 4-nitrobenzyl penicillanate 1,1-dioxide.

EXAMPLE 20

Penicillanic Acid 1,1-Dioxide

Hydrogenolysis of 4-nitrobenzyl penicillanate 1,1-dioxide, according to the procedure of Example 3, affords penicillanic acid 1,1-dioxide.

EXAMPLE 21

Sodium Penicillanate 1,1-Dioxide

To a stirred solution of 32.75 g. (0.14 mmole) of penicillanic acid 1,1-dioxide in 450 ml. of ethyl acetate was added a solution of 25.7 g. of sodium 2-ethylhexanoate (0.155 mmole) in 200 ml. of ethyl acetate. The resulting solution was stirred for 1 hour and then an additional 10% molar excess of sodium 2-ethylhexanoate in a small volume of ethyl acetate was added. Product immediately began to precipitate. Stirring was continued for 30 minutes and then the precipitate was removed by filtration. It was washed sequentially with ethyl acetate, with 1:1 volume per volume ethyl acetate-ether and with ether. The solid was then dried over phosphorus pentoxide, at ca. 0.1 mm of Hg for 16 hours at 25°C., giving 36.8 g. of the title sodium salt, contaminated with a small amount of ethyl acetate. The ethyl acetate content was reduced by heating to 100°C. for 3 hours under vacuum. The IR spectrum of this final product (KBr disc) showed absorptions at 1786 and 1608 cm⁻¹. The NMR spectrum (D₂O) showed absorptions at 1.48 (s, 3H), 1.62 (s, 3H), 3.35 (d of d's, 1H, J₁ = 16 Hz, J₂ = 2 Hz), 3.70 (d of d's, 1H, J₁ = 16 Hz, J₂ = 4 Hz), 4.25 (s, 1H) and 5.03 (d of d's, 1H, J₁ = 4 Hz, J₂ = 2 Hz) ppm.

The title sodium salt can also be prepared using acetone in place of ethyl acetate.

EXAMPLE 22

Penicillanic Acid 1,1-Dioxide

To a mixture of 7,600 ml. of water and 289 ml. of glacial acetic acid was added, portionwise, 379.5 g. of potassium permanganate. This mixture was stirred for 15 minutes, and then it was cooled to 0°C. To it was then added, with stirring, a mixture which had been prepared from 270 g. of penicillanic acid, 260 ml. of 4N sodium hydroxide and 2,400 ml. of water (pH 7.2), and which had then been cooled to 8°C. The temperature rose to 15°C. during this latter addition. The temperature of the resulting mixture was reduced to 5°C. and the stirring was continued for 30 minutes. To the reaction mixture was then added 142.1 g. of sodium bisulfite, in portions, during 10 minutes. The mixture was stirred for 10 minutes at 10°C., and then 100 g. of supercel (a diatomaceous earth) was added. After a further 5 minutes of stirring, the mixture was filtered. To the filtrate was added 4.0 liters of ethyl acetate, and then the pH of the aqueous phase was lowered to 1.55 using 6N hydrochloric acid. The ethyl acetate layer was removed and combined with several further ethyl acetate extracts. The combined organic layer was washed with water, dried (MgSO₄) and evaporated almost to dryness *in vacuo*. The slurry thus obtained was stirred with 700 ml. of ether at 10°C., for 20 minutes, and then the solid was collected by filtration. This afforded 82.6 g. (26% yield) of the title compound having a melting point of 154–155.5°C. (dec.).

EXAMPLE 23

Pivaloyloxymethyl Penicillanate 1,1-Dioxide

To a solution of 1.25 g. pivaloyloxymethyl penicillanate in 40 ml. of chloroform, cooled to ca. -15°C., was added 0.8 g. of 3-chloroperbenzoic acid. The mixture was stirred at ca. -15°C. for 20 minutes and then it was allowed to warm to room temperature. Analysis of the resulting solution by NMR indicated that it contained both the 1 α - and 1 β -oxide.

The chloroform solution was concentrated to about 20 ml. and a further 0.8 g. of 3-chloroperbenzoic acid was added. This mixture was stirred overnight at room temperature, and then all the solvent was removed by evaporation *in vacuo*. The residue was redissolved in *ca.* 4 ml. of dichloromethane and 0.4 g. of 3-chloroperbenzoic acid was added. The mixture was stirred for 3 hours and then the solvent was removed by evaporation *in vacuo*. The residue was partitioned between ethyl acetate and water at pH 6.0, and sodium bisulfite was added until a test for the presence of peroxides was negative. The pH of the aqueous phase was raised to 8.0 and the layers were separated. The organic layer was washed with brine, dried using anhydrous sodium sulfate and evaporated *in vacuo*. The residue was dissolved in ether and reprecipitated by the addition of hexane. The resulting solid was recrystallized from ether to give 0.357 g. of the title compound.

The NMR spectrum of the product (CDCl₃) showed absorptions at 1.23 (s, 9H), 1.50 (s, 3H), 1.67 (s, 3H), 3.28 (m, 2H), 4.45 (s, 1H), 5.25 (m, 1H) and 5.78 (m, 2H) ppm.

EXAMPLE 24

3-Phthalidyl Penicillanate 1,1-Dioxide

To a solution of 713 mg. of 3-phthalidyl penicillanate in 3 ml. of chloroform was added 0.430 g. of 3-chloroperbenzoic acid at *ca.* 10°C. The mixture was stirred for 30 minutes and then a further 0.513 g. of 3-chloroperbenzoic acid was added. The mixture was stirred for 4 hours at room temperature, and then the solvent was removed by evaporation *in vacuo*. The residue was partitioned between ethyl acetate and water at pH 6.0, and sodium bisulfite was added to decompose any remaining peracid. The pH of the aqueous phase was raised to 8.8. The layers were separated and the organic phase was evaporated *in vacuo*. This afforded the title compound as a foam. The NMR spectrum (CDCl₃) showed absorptions at 1.62 (m, 6H), 3.3 (m, 2H), 4.52 (p, 1H), 5.23 (m, 1H) and 7.63 (m, 5H) ppm.

EXAMPLE 25

2,2,2-Trichloroethyl Penicillanate 1,1-Dioxide

To 100 mg. of 2,2,2-trichloroethyl penicillanate in a small volume of chloroform was added 50 mg. of 3-chloroperbenzoic acid and the mixture was stirred for 30 minutes. Examination of the reaction product at this point revealed that it was mostly sulfoxide. (The NMR spectrum (CDCl₃) showed absorptions at 1.6 (s, 3H), 1.77 (s, 3H), 3.38 (m, 2H), 4.65 (s, 1H), 4.85 (m, 2H) and 5.37 (m, 1H) ppm.) A further 100 mg. of 3-chloroperbenzoic acid was added and the mixture was stirred overnight. The solvent was then removed by evaporation *in vacuo*, and the residue was partitioned between ethyl acetate and water at pH 6.0. Sufficient sodium bisulfite was added to decompose the excess peracid and then the pH was raised to 8.5. The organic phase was separated, washed with brine and dried. Evaporation *in vacuo* afforded 65 mg. of the title product. The NMR spectrum (CDCl₃) showed absorptions at 1.53 (s, 3H), 1.72 (s, 3H), 3.47 (m, 2H), 4.5 (s, 1H), 4.6 (m, 1H) and 4.8 (m, 2H) ppm.

EXAMPLE 26

4-Nitrobenzyl Penicillanate 1,1-Dioxide

A solution of 4-nitrobenzyl penicillanate in chloroform was cooled to about 15°C. and 1 equivalent of 3-chloroperbenzoic acid was added. The reaction mixture was stirred for 20 minutes. Examination of the reaction mixture at this point by nuclear magnetic resonance spectroscopy revealed that it contained 4-nitrobenzyl penicillanate 1-oxide. A further 1 equivalent of 3-chloroperbenzoic acid was added and the reaction mixture was stirred for 4 hours. At this point a further 1 equivalent of 3-chloroperbenzoic acid was added and the reaction mixture was stirred overnight. The solvent was removed by evaporation, and the residue was partitioned between ethyl acetate and water at pH 8.5. The ethyl acetate layer was separated, washed with water, dried and evaporated to give the crude product. The crude product was purified by chromatography on silica gel, eluting with a 1:4 volume per volume mixture of ethyl acetate-chloroform.

The NMR spectrum of the product (CDCl₃) showed absorptions at 1.35 (s, 3H), 1.58 (s, 3H), 3.45 (m, 2H), 4.42 (s, 1H), 4.58 (m, 1H), 5.30 (s, 2H) and 7.83 (q, 4H) ppm.

EXAMPLE 27

Penicillanic Acid 1,1-Dioxide

To 0.54 g. of 4-nitrobenzyl penicillanate 1,1-dioxide in 30 ml. of methanol and 10 ml. of ethyl acetate was added 0.54 g. of 10% weight per weight palladium-on-carbon. The mixture was then shaken under an atmosphere of hydrogen at a pressure of about 50 psig. until hydrogen uptake ceased. The reaction mixture was filtered, and the solvent removed by evaporation. The residue was partitioned between ethyl acetate and water at pH 8.5, and the water layer was removed. Fresh ethyl acetate was added and the pH was adjusted to 1.5. The ethyl acetate layer was removed, washed with water and dried, and then it was evaporated *in vacuo*. This afforded 0.168 g. of the title compound as a crystalline solid.

EXAMPLE 28

Penicillanic Acid 1,1-Dioxide

A stirred solution of 512 mg. of 4-nitrobenzyl penicillanate 1,1-dioxide in a mixture of 5 ml. of acetonitrile and 5 ml. of water was cooled to 0°C. and then a solution of 484 mg. of sodium dithionite in 1.4 ml. of 1.0N sodium hydroxide was added portionwise over several minutes. The reaction mixture was stirred for an additional 5 minutes and then it was diluted with ethyl acetate and water at pH 8.5. The ethyl acetate layer was removed and evaporated *in vacuo* giving 300 mg. of starting material. Fresh ethyl acetate was added to the aqueous phase and the pH was adjusted to 1.5. The ethyl acetate was removed, dried and evaporated *in vacuo* giving 50 mg. of the title compound.

EXAMPLE 29

1-Methyl-1-(acetoxy)ethyl Penicillanate 1,1-Dioxide

To 2.33 g. of penicillanic acid 1,1-dioxide in 5 ml. of *N,N*-dimethylformamide was added 1.9 ml. of ethyldiisopropylamine, followed by the dropwise addition of 1.37 g. of 1-methyl-1-(acetoxy)ethyl chloride, at ca. 20°C. The mixture was stirred at ambient temperature overnight and then the mixture was diluted with ethyl acetate and with water. The layers were separated and the ethyl acetate layer was washed with water at pH 9. The ethyl acetate solution was then dried (Na₂SO₄) and evaporated *in vacuo* leaving 1.65 g. of crude product as an oil. The oil solidified on standing in the refrigerator, and it was then recrystallized from a mixture of chloroform and ether giving material having a melting point of 90–92°C.

The NMR spectrum of the crude product (CDCl₃) showed absorptions at 1.5 (s, 3H), 1.62 (s, 3H), 1.85 (s, 3H), 1.83 (s, 3H), 2.07 (s, 3H), 3.43 (m, 2H), 4.3 (s, 1H) and 4.57 (m, 1H) ppm.

EXAMPLE 30

The procedure of Example 29 is repeated, except that the 1-methyl-1-(acetoxy)ethyl chloride is replaced by the appropriate 1-methyl-1-(alkanoyloxy)ethyl chloride, to produce the following compounds: 1-methyl-1-(propionyloxy)ethyl penicillanate 1,1-dioxide, 1-methyl-1-(pivaloyloxy)ethyl penicillanate 1,1-dioxide and 1-methyl-1-(hexanoyloxy)ethyl penicillanic acid 1,1-dioxide, respectively.

EXAMPLE 31

Penicillanic Acid 1,1-Dioxide

To a stirred solution of 1.78 g. of penicillanic acid in water, at pH 7.5, was added 1.46 ml. of 40% peracetic acid, followed by an additional 2.94 ml. of 40% volume per volume peracetic acid 30 minutes later. The reaction mixture was stirred for 3 days at room temperature and then it was diluted with ethyl acetate and water. Solid sodium bisulfite was added to decompose excess peracid, and then the pH was adjusted to 1.5. The ethyl acetate layer was removed, dried (Na₂SO₄) and evaporated *in vacuo*. The residue was a 3:2 mixture of penicillanic acid 1,1-dioxide and penicillanic acid 1-oxide.

EXAMPLE 32

Pivaloyloxymethyl Penicillanate 1,1-Dioxide

A stirred solution of 595 mg. of pivaloyloxymethyl penicillanate 1-oxide in 5 ml. of ethyl acetate was cooled to ca. –15°C., and 5 mg. of manganic acetylacetonate was added. To the dark brown mixture thus obtained was added, during several minutes, 0.89 ml. of 40% volume per volume peracetic acid in small amounts over several minutes. After 40 minutes the cooling bath was removed, and the mixture was stirred at ambient temperature for 3 days. The mixture was diluted with ethyl acetate and water at pH 8.5, and the ethyl acetate layer was removed, dried and evaporated *in vacuo*. This afforded 178 mg. of material which was shown by NMR spectroscopy to be a mixture of pivaloyloxymethyl penicillanate 1,1-dioxide and pivaloyloxymethyl penicillanate 1-oxide.

The above material was redissolved in ethyl acetate and reoxidized using 0.9 ml. of peracetic acid and 5 mg. of manganic acetylacetonate, as described above, using a reaction time of 16 hours. The reaction mixture was worked up as described above. This afforded 186 mg. of pivaloyloxymethyl penicillanate 1,1-dioxide.

PREPARATION A

6,6-Dibromopenicillanic Acid 1 α -Oxide

The title compound is prepared by oxidation of 6,6-dibromopenicillanic acid with 1 equivalent of 3-chloroperbenzoic acid in tetrahydrofuran at 0–25°C. for ca. 1 hour, according to the procedure of Harrison *et al.*, *Journal of the Chemical Society* (London) Perkin I, 1772 (1976).

PREPARATION B

Benzyl 6,6-Dibromopenicillanate

To a solution of 54 g. (0.165 mmole) of 6,6-dibromopenicillanic acid in 350 ml. of *N,N*-dimethylacetamide was added 22.9 ml. (0.165 mmole) of triethylamine and the solution was stirred for 40 minutes. Benzyl bromide (19.6 ml., 0.165 mmole) was added and the resulting mixture was stirred at room temperature for 48 hours. The precipitated triethylamine hydrobromide was filtered off, and the filtrate was added to 1,500 ml. of ice-water, adjusted to pH 2. The mixture was extracted with ether, and the extracts were washed successively with saturated sodium bicarbonate, water and brine. The dried (MgSO₄) ether solution was evaporated *in vacuo* to give an off-white solid, which was recrystallized from isopropanol. This afforded 70.0 g. (95% yield) of the title compound m.p. 75–76°C. The IR spectrum (KBr disc) showed absorptions at 1795 and 1740 cm^{–1}. The NMR spectrum (CDCl₃) showed absorptions at 1.53 (s, 3H), 1.58 (s, 3H), 4.50 (s, 1H), 5.13 (s, 2H), 5.72 (s, 1H) and 7.37 (s, 5H) ppm.

PREPARATION C

Benzyl 6,6-Dibromopenicillanate 1 α -Oxide

To a stirred solution of 13.4 g. (0.03 mmole) of benzyl 6,6-dibromopenicillanate in 200 ml. of dichloromethane was added a solution of 6.12 g. (0.03 mmole) of 3-chloroperbenzoic acid in 100 ml. of dichloromethane, at ca. 0°C. Stirring was continued for 1.5 hours at ca. 0°C. and then the reaction mixture was filtered. The filtrate was washed successively with 5% sodium bicarbonate and water, and then it was dried (Na₂SO₄). Removal of the solvent by evaporation *in vacuo* gave 12.5 g. of the title product as an oil. The oil was induced to solidify by trituration under ether. Filtration then afforded 10.5 g. of benzyl 6,6-dibromopenicillanate 1 α -oxide as a solid. The IR spectrum (CHCl₃) showed absorptions at 1800 and 1750 cm^{–1}. The NMR spectrum of the product (CDCl₃) showed absorptions at 1.3 (s, 3H), 1.5 (s, 3H), 4.5 (s, 1H), 5.18

(s, 2H), 5.2 (s, 1H) and 7.3 (s, 5H) ppm.

PREPARATION D

4-Nitrobenzyl Penicillanate

Reaction of the triethylamine salt of penicillanic acid with 4-nitrobenzyl bromide; according to the procedure of Preparation B, affords 4-nitrobenzyl penicillanate.

PREPARATION E

2,2,2-Trichloroethyl Penicillanate

To 403 mg. of penicillanic acid in 10 ml. of dichloromethane was added 25 mg. of diisopropylcarbodiimide followed by 0.19 ml. of 2,2,2-trichloroethanol. The mixture was stirred overnight and then the solvent was removed by evaporation *in vacuo*. The crude product was purified by column chromatography using silica gel as the adsorbent and chloroform as the eluant.

PREPARATION F

3-Phthalidyl Penicillanate

To a solution of 506 mg. of penicillanic acid in 2 ml. of *N,N*-dimethylformamide was added 0.476 ml. of diisopropylethylamine followed by 536 mg. of 3-phthalidyl bromide. The mixture was stirred overnight and then it was diluted with ethyl acetate and water. The pH was adjusted to 3.0 and the layers were separated. The organic layer was washed with water, and then with water at pH 8.0, and then it was dried using anhydrous sodium sulfate. The dried ethyl acetate solution was evaporated *in vacuo* giving 713 mg. of the title ester as an oil. The NMR spectrum (CDCl₃) showed absorptions at 1.62 (m, 6H), 3.3 (m, 2H), 4.52 (s, 1H), 5.23 (m, 1H) and 7.63 (m, 5H).

PREPARATION G

Pivaloyloxymethyl Penicillanate

To 3.588 g. of 6,6-dibromopenicillanic acid in 10 ml. of *N,N*-dimethylformamide was added 1.8 ml. of diisopropylethylamine, followed by 1.40 ml. of chloromethyl pivalate. The mixture was stirred overnight and then it was diluted with ethyl acetate and water. The organic layer was removed and washed successively with water at pH 3.0 and water at pH 8.0. The ethyl acetate solution was dried (Na₂SO₄) and then evaporated *in vacuo* to give pivaloyloxymethyl 6,6-dibromopenicillanate as an amber oil (3.1 g.) which slowly crystallized.

The above ester was dissolved in 100 ml. of methanol, and then 3.1 g. of 10% weight per weight palladium-on-carbon and 1.31 g. of potassium bicarbonate in 20 ml. of water were added. The mixture was shaken under hydrogen at atmospheric pressure until hydrogen uptake ceased. The reaction mixture was filtered and the methanol was removed by evaporation *in vacuo*. The residue was partitioned between water and ethyl acetate at pH 8, and then the organic layer was removed. The latter was dried (Na₂SO₄) and evaporated *in vacuo* to give 1.25 g. of the title compound. The NMR spectrum (CDCl₃) showed absorptions at 1.23 (s, 9H), 1.5 (s, 3H), 1.67 (s, 3H), 3.28 (m, 2H), 4.45 (s, 1H), 5.25 (m, 1H) and 5.78 (m, 2H) ppm.

PREPARATION H

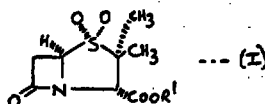
4-Nitrobenzyl Penicillanate

To a stirred solution of 2.14 g. of penicillanic acid and 2.01 ml. of ethyldiisopropylamine in 10 ml. of *N,N*-dimethylformamide was added dropwise 2.36 g. of 4-nitrobenzyl bromide, at ca. 20°C. The mixture was stirred at ambient temperature overnight, and then it was diluted with ethyl acetate and water. The layers were separated and the ethyl acetate layer was washed with water at pH 2.5, followed by water at pH 8.5. The ethyl acetate solution was then dried (Na₂SO₄) and evaporated *in vacuo* leaving 3.36 g. of the title compound.

The NMR spectrum of the product (in CDCl₃) showed absorptions at 1.45 (s, 3H), 1.68 (s, 3H), 3.32 (s, 2H), 4.50 (s, 1H), 5.23 (m, 1H), 5.25 (s, 2H) and 7.85 (q, 4H) ppm.

CLAIMS

1. A compound of the formula:



or a pharmaceutically acceptable base salt thereof, wherein R¹ is hydrogen, an ester-forming residue readily hydrolyzable *in vivo*, or a conventional penicillin carboxy protecting group.

2. A compound according to claim 1 wherein R¹ is alkanoyloxymethyl having from 3 to 8 carbon atoms, 1-(alkanoyloxy)ethyl having from 4 to 9 carbon atoms, alkoxycarbonyloxymethyl having from 3 to 6 carbon atoms, 1-(alkoxycarbonyloxy)ethyl having from 4 to 7 carbon atoms, 3-phthalidyl, 4-crotonolactonyl or γ -butyrolacton-4-yl.

3. A compound according to claim 1, wherein R¹ is 1-methyl-1-(alkanoyloxy)ethyl having from 5 to 10 carbon atoms, or 1-methyl-1-(alkoxycarbonyloxy)ethyl having from 5 to 8 carbon atoms.

4. A compound according to claim 2 wherein R¹ is pivaloyloxy methyl.

5. A compound according to claim 2 wherein R¹ is 1-(ethoxycarbonyloxy)ethyl.

6. A compound according to claim 1 wherein R¹ is hydrogen.



(s, 2H), 5.2 (s, 1H) and 7.3 (s, 5H) ppm.

PREPARATION D

4-Nitrobenzyl Penicillanate

Reaction of the triethylamine salt of penicillanic acid with 4-nitrobenzyl bromide; according to the procedure of Preparation B, affords 4-nitrobenzyl penicillanate.

PREPARATION E

2,2,2-Trichloroethyl Penicillanate

To 403 mg. of penicillanic acid in 10 ml. of dichloromethane was added 25 mg. of diisopropylcarbodiimide followed by 0.19 ml. of 2,2,2-trichloroethanol. The mixture was stirred overnight and then the solvent was removed by evaporation *in vacuo*. The crude product was purified by column chromatography using silica gel as the adsorbent and chloroform as the eluant.

PREPARATION F

3-Phthalidyl Penicillanate

To a solution of 506 mg. of penicillanic acid in 2 ml. of *N,N*-dimethylformamide was added 0.476 ml. of diisopropylethylamine followed by 536 mg. of 3-phthalidyl bromide. The mixture was stirred overnight and then it was diluted with ethyl acetate and water. The pH was adjusted to 3.0 and the layers were separated. The organic layer was washed with water, and then with water at pH 8.0, and then it was dried using anhydrous sodium sulfate. The dried ethyl acetate solution was evaporated *in vacuo* giving 713 mg. of the title ester as an oil. The NMR spectrum (CDCl_3) showed absorptions at 1.62 (m, 6H), 3.3 (m, 2H), 4.52 (s, 1H), 5.23 (m, 1H) and 7.63 (m, 5H).

PREPARATION G

Pivaloyloxymethyl Penicillanate

To 3.588 g. of 6,6-dibromopenicillanic acid in 10 ml. of *N,N*-dimethylformamide was added 1.8 ml. of diisopropylethylamine, followed by 1.40 ml. of chloromethyl pivalate. The mixture was stirred overnight and then it was diluted with ethyl acetate and water. The organic layer was removed and washed successively with water at pH 3.0 and water at pH 8.0. The ethyl acetate solution was dried (Na_2SO_4) and then evaporated *in vacuo* to give pivaloyloxymethyl 6,6-dibromopenicillanate as an amber oil (3.1 g.) which slowly crystallized.

The above ester was dissolved in 100 ml. of methanol, and then 3.1 g. of 10% weight per weight palladium-on-carbon and 1.31 g. of potassium bicarbonate in 20 ml. of water were added. The mixture was shaken under hydrogen at atmospheric pressure until hydrogen uptake ceased. The reaction mixture was filtered and the methanol was removed by evaporation *in vacuo*. The residue was partitioned between water and ethyl acetate at pH 8, and then the organic layer was removed. The latter was dried (Na_2SO_4) and evaporated *in vacuo* to give 1.25 g. of the title compound. The NMR spectrum (CDCl_3) showed absorptions at 1.23 (s, 9H), 1.5 (s, 3H), 1.67 (s, 3H), 3.28 (m, 2H), 4.45 (s, 1H), 5.25 (m, 1H) and 5.78 (m, 2H) ppm.

PREPARATION H

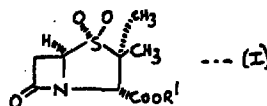
4-Nitrobenzyl Penicillanate

To a stirred solution of 2.14 g. of penicillanic acid and 2.01 ml. of ethyldiisopropylamine in 10 ml. of *N,N*-dimethylformamide was added dropwise 2.36 g. of 4-nitrobenzyl bromide, at ca. 20°C. The mixture was stirred at ambient temperature overnight, and then it was diluted with ethyl acetate and water. The layers were separated and the ethyl acetate layer was washed with water at pH 2.5, followed by water at pH 8.5. The ethyl acetate solution was then dried (Na_2SO_4) and evaporated *in vacuo* leaving 3.36 g. of the title compound.

The NMR spectrum of the product (in CDCl_3) showed absorptions at 1.45 (s, 3H), 1.68 (s, 3H), 3.32 (s, 2H), 4.50 (s, 1H), 5.23 (m, 1H), 5.25 (s, 2H) and 7.85 (q, 4H) ppm.

CLAIMS

1. A compound of the formula:



or a pharmaceutically acceptable base salt thereof, wherein R^1 is hydrogen, an ester-forming residue readily hydrolyzable *in vivo*, or a conventional penicillin carboxy protecting group.

2. A compound according to claim 1 wherein R^1 is alkanoyloxymethyl having from 3 to 8 carbon atoms, 1-(alkanoyloxy)ethyl having from 4 to 9 carbon atoms, alkoxycarbonyloxymethyl having from 3 to 6 carbon atoms, 1-(alkoxycarbonyloxy)ethyl having from 4 to 7 carbon atoms, 3-phthalidyl, 4-crotonolactonyl or γ -butyrolacton-4-yl.

3. A compound according to claim 1, wherein R^1 is 1-methyl-1-(alkanoyloxy)ethyl having from 5 to 10 carbon atoms, or 1-methyl-1-(alkoxycarbonyloxy)ethyl having from 5 to 8 carbon atoms.

4. A compound according to claim 2 wherein R^1 is pivaloyloxy methyl.

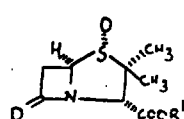
5. A compound according to claim 2 wherein R^1 is 1-(ethoxycarbonyloxy)ethyl.

6. A compound according to claim 1 wherein R^1 is hydrogen.



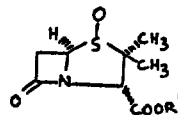
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7. A compound according to claim 1, which is in the form of a sodium, potassium or triethylamine salt.
8. A pharmaceutical composition which comprises a compound of the formula (I) as claimed in claim 1 wherein R¹ is hydrogen or an ester-forming residue readily hydrolyzable *in vivo*, or a pharmaceutically acceptable base salt thereof, together with a pharmaceutically acceptable carrier.
9. A pharmaceutical composition according to claim 8, wherein R¹ is pivaloyloxymethyl.
10. A pharmaceutical composition according to claim 8, wherein R¹ is hydrogen.
11. A pharmaceutical composition which comprises a sodium salt as claimed in claim 7, together with a pharmaceutically acceptable carrier.
12. A pharmaceutical composition according to any of claims 8 to 11, which further comprises a β -lactam antibiotic.
13. A pharmaceutical composition according to claim 12 wherein said β -lactam antibiotic is: 6-(2-phenylacetamido)penicillanic acid, 6-(2-phenoxyacetamido)penicillanic acid, 6-(2-phenylpropionamido)penicillanic acid, 6-(D-2-amino-2-phenylacetamido)penicillanic acid, 6-(D-2-amino-2-[4-hydroxyphenyl]acetamido)penicillanic acid, 6-(D-2-amino-2-[1,4-cyclohexadienyl]acetamido)penicillanic acid, 6-(1-aminocyclohexanecarboxamido)penicillanic acid, 6-(2-carboxy-2-phenylacetamido)penicillanic acid, 6-(2-carboxy-2-[3-thienyl]acetamido)penicillanic acid, 6-(D-2-[4-ethylpiperazin-2,3-dione-1-carboxamido]-2-phenylacetamido)penicillanic acid, 6-(D-2-[4-hydroxy-1,5-naphthyridine-3-carboxamido]-2-phenylacetamido)penicillanic acid, 6-(D-2-sulfo-2-phenylacetamido)penicillanic acid, 6-(D-2-sulfonamino-2-phenylacetamido)penicillanic acid, 6-(D-2-[imidazolidin-2-one-1-carboxamido]-2-phenylacetamido)penicillanic acid, 6-(D-2-[3-methylsulfonylimidazolidin-2-one-1-carboxamido]-2-phenylacetamido)penicillanic acid, 6-[(hexahydro-1H-azepin-1-yl)methyleneamino]penicillanic acid, acetoxymethyl 6-(2-phenylacetamido)penicillanate, acetoxymethyl 6-(D-2-amino-2-phenylacetamido)penicillanate, acetoxymethyl 6-(D-2-amino-2-[4-hydroxyphenyl]acetamido)penicillanate, pivaloyloxymethyl 6-(2-phenylacetamido)penicillanate, pivaloyloxymethyl 6-(D-2-amino-2-phenylacetamido)penicillanate, pivaloyloxymethyl 6-(D-2-amino-2-[4-hydroxyphenyl]acetamido)penicillanate, 1-(ethoxycarbonyloxy)ethyl 6-(2-phenylacetamido)penicillanate, 1-(ethoxycarbonyloxy)ethyl 6-(D-2-amino-2-phenylacetamido)penicillanate, 1-(ethoxycarbonyloxy)ethyl 6-(D-2-amino-2-[4-hydroxyphenyl]acetamido)penicillanate, 3-phthalidyl 6-(2-phenylacetamido)penicillanate, 3-phthalidyl 6-(D-2-amino-2-phenylacetamido)penicillanate, 3-phthalidyl 6-(D-2-amino-2-[4-hydroxyphenyl]acetamido)penicillanate, 6-(2-phenoxy-carbonyl-2-phenylacetamido)penicillanic acid, 6-(2-tolylloxycarbonyl-2-phenylacetamido)penicillanic acid, 6-(2-[5-indanyloxycarbonyl]-2-phenylacetamido)penicillanic acid, 6-(2-phenoxy-carbonyl-2-[3-thienyl]acetamido)penicillanic acid, 6-(2-tolylloxycarbonyl-2-[3-thienyl]acetamido)penicillanic acid, 6-(2-[5-indanyloxycarbonyl]-2-[3-thienyl]acetamido)penicillanic acid, 6-(2,2-dimethyl-5-oxo-4-phenyl-1-imidazolidinyl)penicillanic acid, 7-(2-[2-thienyl]acetamido)cephalosporanic acid, 7-(2-[1-tetrazolyl]acetamido-3-(2-[5-methyl-1,3,4-thiadiazolyl]thiomethyl)-3-desacetoxymethylcephalosporanic acid, 7-(D-2-amino-2-phenylacetamido)desacetoxyccephalosporanic acid, 7- α -methoxy-7-(2-[2-thienyl]acetamido)-3-carbamoyloxymethyl-3-desacetoxymethylcephalosporanic acid, 7-(2-cyanoacetamido)cephalosporanic acid, 7-(D-2-hydroxy-2-phenylacetamido)-3-(5-[1-methyltetrazolyl]thiomethyl)-3-desacetoxymethylcephalosporanic acid, 7-(2-[4-pyridylthio]acetamido)cephalosporanic acid, 7-(D-2-amino-2-[1,4-cyclohexadienyl]acetamido)cephalosporanic acid, or 7-(D-2-amino-2-phenylacetamido)cephalosporanic acid, or a pharmaceutically-acceptable salt thereof.
14. A pharmaceutical composition according to claim 13, wherein said β -lactam antibiotic is 6-(2-phenoxyacetamido)penicillanic acid or a pharmaceutically-accepted salt thereof.
15. A pharmaceutical composition according to claim 13, wherein said β -lactam antibiotic is 6-(D-2-amino-2-phenylacetamido)penicillanic acid or a pharmaceutically-acceptable salt thereof.
16. A pharmaceutical composition according to claim 13, wherein said β -lactam antibiotic is 1-(ethoxycarbonyloxy)ethyl 6-(D-2-amino-2-phenylacetamido)penicillanate or a pharmaceutically-acceptable salt thereof.
17. A pharmaceutical composition according to claim 13, wherein said β -lactam antibiotic is 6-(2-phenylacetamido)penicillanic acid or a pharmaceutically-acceptable salt thereof.
18. A pharmaceutical composition according to claim 13, wherein said β -lactam antibiotic is 6-(2-carboxy-2-phenylacetamido)penicillanic acid or a pharmaceutically-acceptable salt thereof.
19. A pharmaceutical composition according to claim 13, wherein said β -lactam antibiotic is 6-(D-2-amino-2-[4-hydroxyphenyl]acetamido)penicillanic acid or a pharmaceutically-acceptable salt thereof.
20. A pharmaceutical composition according to claim 13, wherein said β -lactam antibiotic is 7-(2-[1-tetrazolyl]acetamido-3-(2-[5-methyl-1,3,4-thiadiazolyl]thiomethyl)-3-desacetoxymethylcephalosporanic acid or a pharmaceutically-acceptable salt thereof.
21. A compound of the formula:



--- (I)

or



--- (II)

or a pharmaceutically acceptable base salt thereof, wherein R¹ is as defined in claim 1.

22. A compound according to claim 21, wherein R¹ is as defined in claim 2.
23. A compound according to claim 21, wherein R¹ is as defined in claim 3.
24. A compound according to claim 22, wherein R¹ is pivaloyloxymethyl.
- 5 25. A compound according to claim 21, wherein R¹ is hydrogen. 5
26. A process for preparing a compound of the formula (I) as claimed in claim 1 or a pharmaceutically acceptable base salt thereof, substantially as described herein.
27. A process according to claim 26 substantially as hereinbefore described in any one of Examples 1 to 9 and 17 to 32.
- 10 28. A compound of the formula (I) as claimed in claim 1 or a pharmaceutically acceptable base salt thereof which has been prepared by a process as claimed in claim 26 or 27. 10
29. A process for preparing a compound of the formula (II) or (III) as claimed in claim 21 or a pharmaceutically-acceptable base salt thereof, substantially as described herein.
30. A process according to claim 29, substantially as hereinbefore described in any one of Examples 10 to 16.
- 15 31. A compound of the formula (II) or (III) as claimed in claim 21 or a pharmaceutically-acceptable base salt thereof which has been prepared by a process as claimed in claim 29 or 30. 15

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