

6- β -Substituted Penicillanic Acids as β -Lactamase Inhibitors

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 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, cephalexin and cefazolin.

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 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 antibacterial activities of the individual components.

5 The present invention relates to 6- β -iodo-penicillanic acid and salts and readily hydrolyzable in vivo esters thereof, which are potent inhibitors of microbial β -lactamases and enhance the effectiveness of β -lactam antibiotics.

10 The invention also relates to a process for the preparation of 6- β -iodo-penicillanic acid, and its salts and readily hydrolyzable in vivo esters.

Pharmaceutical compositions comprising the above-mentioned 6- β -iodo-penicillanic acid, salts and hydrolyzable esters with certain β -lactam antibiotics are also part of the present invention.

15 6-Substituted penicillanic acids and certain esters have been prepared through 6-diazopenicillanic acid (Helv. Chim. Acta, 50, 1327 (1967)), but the orientation of the substituent is in the α -position. 6- α -Hydroxypenicillanic acid is also prepared from 6-diazopenicillanic acid and esters thereof (J. Org. Chem., 39,
20 1444 (1974)).

6- α -Benzyloxypenicillanic acid methyl ester is reported by Manhas, et al., J. Heterocycl. Chem., 15, 601 (1978).

Certain 6,6-dihalo- and 6-halopenicillanic acids, methyl
5 esters and sulfoxides are reported by Harrison et al., J. Chem. Soc. (Perkin I), 1772 (1976). In each instance of a 6-mono-substituted compound only the 6- α epimer is described.

More recently Loosemore, et al., J. Org. Chem., 43, 3611 (1978) reported that treatment of a 6- α -bromopenicillanic acid with base epimerized a portion of the com-
10 pound to give a mixture of 6- α - and 6- β -bromopenicillanic acid which was comprised of about 12% of the β -epimer. A similar mixture was achieved through a hydrogenation of 6,6-dibromopenicillanic acid in which the β -epimer com-
15 prised about 30% of the total. It was also shown by Pratt, et al., Proc. Natl. Acad. Sci., 75, 4145 (1978) that the β -lactamase inhibitory characteristic of a mixture of 6- α - and 6- β -bromopenicillanic acid was related to the amount of 6- β -bromopenicillanic acid in said mixture. The
20 findings of Pratt, et al., are corroborated by Knott-Hunziker, et al., Biochem. J., 177, 365 (1979) by the demonstration that a mixture of 5% 6- β -bromopenicillanic acid and 95% 6- α -bromopenicillanic acid inhibits β -lactamase while the α -epimer alone is essentially inactive.

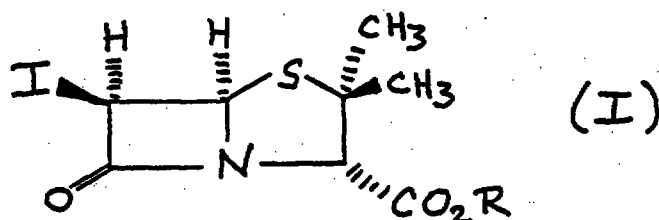
25 U.S. Patent #4,093,625 claims the preparation of 6- β -mercaptopenicillanic acid and derivatives thereof as antibacterial agents.

Cartwright et al., Nature 278, 360 (1979) reports that while 6 α -chloropenicillanic acid is a poor inhibitor of β -lactamase, the corresponding sulfone is a moderately good inhibitor.

Roets, et al., J. Chem. Soc., (Perkin I) 704 (1976)

5 identifies benzyl 6 β -chloropenicillanate as a by-product in the reduction of benzyl 6-oxopenicillanate following hydrochloric acid treatment of the product.

The 6- β -iodo-penicillanic acid, salts and esters of this invention are of the formula



wherein R is hydrogen or an ester-forming residue readily hydrolyzable in vivo, or a pharmaceutically acceptable base salt thereof when R is hydrogen.

Preferably R is hydrogen.

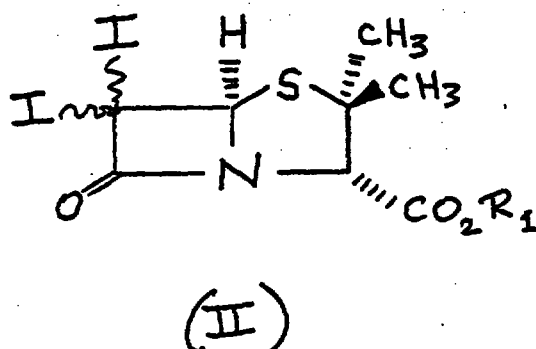
When R is an ester-forming residue readily hydrolyzable in vivo, it is preferably alkanoyloxymethyl of three to six carbon atoms, 1-(alkanoyloxy)ethyl of four to seven carbon atoms, 1-methyl-1-(alkanoyloxy)ethyl of five to eight carbon atoms, 5 alkoxy-carbonyloxymethyl of three to six carbon atoms, 1-(alkoxy-carbonyloxy)ethyl of four to seven carbon atoms, 1-methyl-1-(alkoxy-carbonyloxy)ethyl of five to eight carbon atoms, 3-phthalidyl, 4-crotonolactonyl or γ -butyrolacton-4-yl. Especially preferred among these is pivaloyloxymethyl.

10 The present invention also relates to a pharmaceutical composition useful for treating bacterial infections in mammals comprising a pharmaceutically-acceptable carrier, a β -lactam antibiotic and a compound of the formula (I).

15 Preferably, said β -lactam antibiotic is a penicillin or a cephalosporin.

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The present invention also includes a process for preparing a compound of the formula (I) which comprises reacting a compound of the formula:



5 where R_1 is an ester-forming residue readily hydrolyzable in vivo or a conventional penicillin carboxy-protecting group, with an organotin monohydride at from 0 to 110°C, followed by removal of R_1 when it is a conventional penicillic carboxy-protecting group.

10 A preferred feature of this process is the use of compounds where R_1 is a conventional penicillin carboxy-protecting group selected from

- a) $-PR_2R_3$ where R_2 and R_3 are each alkyl of one to three carbon atoms, alkoxy of one to three carbon atoms or phenyl;
- b) 3,5-di- t-butyl-4-hydroxybenzyl;
- 15 c) $-CH_2-Y$ where Y is $-C(O)R_4$ wherein R_4 is phenyl or alkyl of one to three carbon atoms, cyano or carbo-alkoxy of two to four carbon atoms;

d) $-N=CHR_5$ where R_5 is phenyl or alkyl of one to three carbon atoms;

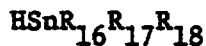
e) $-CH(COCH_3)CO_2R_6$ wherein R_6 is alkyl of one to four carbon atoms;

5 f) $-CR_7R_8R_9$ where R_7 and R_8 are each hydrogen, phenyl or methyl and R_9 is phenyl, 4-methoxyphenyl, or methyl with the proviso that when R_7 and R_8 are each methyl, R_9 is methyl;

g) $-Si(CH_3)_3$ and $Si(CH_3)_2t-C_4H_9$;

10 h) $-SnR_{16}R_{17}R_{18}$ where R_{16} , R_{17} and R_{18} are each alkyl of one to five carbon atoms, phenyl or benzyl;

A further preferred feature of the present process is the use of an organotin monohydride of the formula



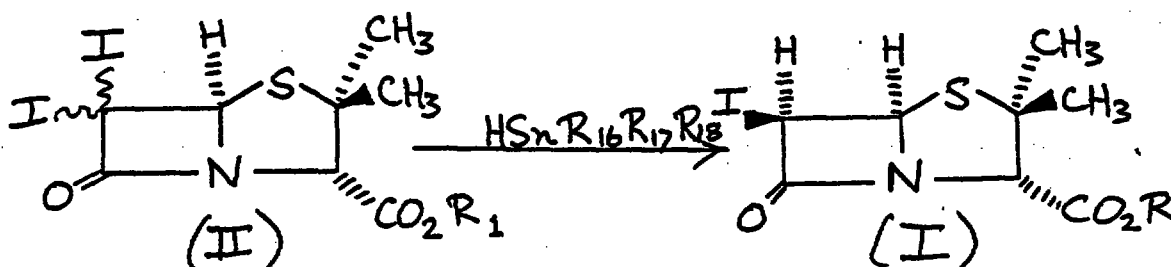
where R_{16} , R_{17} and R_{18} are as previously defined.

15 Especially preferred is the process wherein R_1 is $-CR_7R_8R_9$, wherein R_7 and R_8 are each hydrogen and R_9 is 4-methoxyphenyl, and the organotin monohydride is tri- n-butyltin hydride, said protecting group being removed by hydrolysis.

An additional preferred feature of this process is the use of compounds where R_1 is one of the preferred ester-forming residues readily hydrolyzable in vivo already referred to. Especially preferred is the process wherein R_1 is pivaloyloxymethyl and the organotin monohydride is tri- n-butyltin hydride.

The process of the present invention allows for the synthesis of 6- β -iodo-penicillanic acid and derivatives thereof substantially free of the corresponding 6- α -epimer, and gives products which are comprised of at least 75% of the β -epimer. In many instances the content of the desired β -epimer is as high as 99.5%. Since the α -epimer is essentially inactive as a β -lactamase inhibitor, it is essential for this utility that the products have as high a β -epimer content as possible. Products containing large amounts of the α -epimer must be used in larger doses to achieve inhibition of the β -lactamase enzymes and potentiation of the β -lactam antibiotics. The larger doses of these materials may lead to toxicity problems for the mammalian host.

Thus all the biologically active compounds of the present invention are preferably prepared by the process of the instant invention which is depicted in the following scheme:



5 wherein R_1 is as previously defined, followed by removal of R_1 when it is a penicillin carboxy-protecting group.

In general, the reduction can be carried out neat, without the use of a solvent or can be conducted in a solvent provided that said solvent is a reaction-inert solvent which appreciably
 10 solubilizes the reactant without reacting to any great extent with the reactants or the product under reaction conditions. When a solvent is employed, it is preferred that said solvent be an aprotic solvent, immiscible with water and with a boiling and freezing point compatible with reaction temperatures. Such
 15 solvents or mixtures thereof include aromatic solvents such as benzene or toluene.

When the aforementioned reaction is conducted without a solvent the reactants are mixed thoroughly and heated to the prescribed reaction temperature.

20 The molar ratio of the starting penicillanic acid derivative and the organotin monohydride is not critical to the claimed process. The use of a slight excess of the tin hydride, up to as much as ten percent above an equimolar quantity, aids in the completion of the reaction and offers no serious problem in
 25 isolating the desired product in purified form.

Reaction time is inherently dependent on concentration, reaction temperature and reactivity of the starting reagents. When the present process is conducted without a solvent a reaction temperature of 60-100°C. is employed. Under these temperature conditions the reaction is usually complete in 5-8 hours. When a solvent is employed a reaction temperature of 80-100°C. is used, the reaction requiring 4-6 hours for completion.

The reaction time and temperature can be markedly reduced by carrying out the process under ultraviolet irradiation. Under these conditions the reaction is initiated with a free radical initiator, such as azobisisobutyronitrile, and conducted under cooling such that the temperature is maintained at about 15-25°C. The reaction time under these conditions is about 15 minutes to several hours.

The preferred reaction temperatures are those which allow the reaction to proceed at a practical rate without resulting in thermal degradation of the starting reagents or products of said process. Accordingly, temperatures of 0-100°C. are operable.

While the order of addition of the reactants is not critical, it is preferred that the organotin monohydride be added to the 6,6-disubstituted penicillanic acid derivative. In this preferred manner the bis dehalogenation when a 6,6-dihalopenicillanic acid derivative is employed is reduced to a minimum.

In the compounds of the aforementioned formulae, the broken line attachment of a substituent to the bicyclic penicillanic acid nucleus indicates that the substituent is below the plane of said nucleus, and is said to be in the α -configuration. Solid line attachment of a substituent to said nucleus indicates that the substituent is attached above the plane, and is referred to as the β -configuration. The wavy line is intended to denote the two epimers or mixtures thereof.

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The organotin monohydrides used as reactants in the present process are prepared by methods known to those skilled in the art. Those which are not available commercially can be prepared by the methods as taught by Hayashi, et al., J. Organometal. Chem., 10, 81(1967).

10 ~~in addition~~ ^T the reaction of 6,6-disubstituted penicillanic acids to the corresponding 6- β -substituted compounds is not carried out on the free acids, but is ~~also~~ conducted with a derivative of said acid as defined by R_1 . These types of derivatives at the 3-carboxy group
15 of the penicillanic acid are known to those skilled in the art and are relatively easy to prepare. Subsequent to the process of the present invention certain of these derivatives, i.e. those which are conventional penicillin carboxy protecting groups, can be removed from the carboxy
20 moiety and provide for the generation of the free acid of formula I ~~4~~ /wherein R_1 is hydrogen/. As one skilled in the art can readily appreciate, the removal of a specific protecting group must be compatible with the reactivity of the substituent at the 6- β -position. Accordingly, the
25 removal of a benzyl protecting group from a penicillanic acid containing a 6- β -halo ~~or halo-methyl~~ substituent by hydrogenolysis may provide a lower than optimum yield of the desired product because of the tendency of halogens to dehalogenate under such reaction conditions.

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The first of these conventional penicillin carboxy protecting groups is the phosphine ester. Following the procedure of West German application 2,218,209, the appropriate ~~6- β -hydroxymethyl~~ or 6,6-disubstituted penicillanic acid, as a triethylamine salt, is reacted with a dialkyl- or dialkoxy-chlorophosphine to yield a desired starting reagent for the instant process. On completion of the reaction of said reagent with a halogenating agent or an organotin monohydride, the protecting group is removed from the 6- β -substituted penicillin by addition of water to provide those products wherein R_{13} is hydrogen.

The second protecting group is the 3,5-di-t-butyl-4-hydroxybenzyl ester. This is conveniently prepared from the requisite ~~6- β -hydroxymethyl~~ or 6,6-disubstituted penicillanic acid following the procedure of West German application 2,033,493, which comprises reacting the aforementioned penicillanic acid, as a triethylamine salt, with ethyl chloroformate and the subsequent reaction of the resultant mixed anhydride with 3,5-di-t-butyl-4-hydroxybenzyl alcohol. Subsequent to the reaction of the starting reagent with a halogenating reagent or an organotin monohydride by the present process, the protecting group is removed by aqueous hydrolysis at pH 8.0.

The third type of protecting group suitable in the process of the present invention are those where R_{13} is $-\text{CH}_2\text{Y}$ wherein Y is previously defined. These ~~6- β -hydroxymethyl~~ and 6,6-disubstituted penicillanic acid esters are prepared by alkylation of the corresponding penicillanic acid triethylamine salt with the appropriate halide following the procedure as taught

in Acta. Chem. Scand., 21, 2210(1967). Following the reaction of the aforementioned reagent with a halogenating reagent or an organotin monohydride, the protecting group is removed, preferably, with
 5 potassium thiophenoxide.

The fourth type of protecting group in this series, wherein $R_{1\beta}$ is $-N=CHR_5$ and where R_5 is as previously defined, is incorporated into the ~~6- β -hydroxymethyl~~ or 6,6-disubstituted penicillanic acid following the procedure
 10 taught in J. Chem. Soc., 1917 (1971c), which comprises reacting the mixed anhydride, formed from the requisite ~~6- β -hydroxymethyl~~ or 6,6-disubstituted penicillanic acid and ethyl chloroformate, with the appropriate aldehyde oxime. Subsequent to the reaction of ~~6- β -hydroxymethyl~~
 15 ~~compounds with a halogenating agent~~ of the 6,6-disubstituted compounds with tin hydride in the present process, the protecting group is removed from the 6- β -substituted penicillanic acid by treatment with potassium thiophenoxide.

20 The fifth type of protecting group is an ester derived from acetoacetic acid esters. Methods for introducing this type of protecting group onto a penicillin carboxy group are described by Ishimaru, et al. Chemistry Letters, 1313 and 1317(1977) and comprise the treatment of
 25 the sodium salt of a ~~6- β -hydroxymethyl~~ or 6,6-disubstituted penicillanic acid with an appropriate alkyl α -haloacetoacetate. Following the reaction of this product with a halogenating reagent or an organotin monohydride, the protecting group is removed from the
 30 6- β -penicillanic derivative by treatment with an aqueous solution of sodium nitrite.

The sixth type of protecting group where $R_{1\beta}$ is $-CR_7R_8R_9$ can be prepared by a number of routes, the details of which are all represented in the chemical literature. The preferred synthesis comprises starting with known 6- β -aminopenicillanic acid esters followed by replacement of the 6-amino moiety through the 6-diazo group as taught,

The eighth protecting group contemplated by the present invention and discussed previously is that wherein R_{14} is $-\text{SnR}_{16}\text{R}_{17}\text{R}_{18}$, and where R_{16} , R_{17} and R_{18} are as previously defined. The tin ester protecting group is formed by adding a molar amount of bis(tin)oxide to two moles of the free 6,6-disubstituted penicillanic acid according to Chem. Ind. 1025 (1976). On completion of said process, the protecting group is removed by aqueous hydrolysis.

~~A penicillin carboxy protecting group which is particularly useful in the synthesis of compounds of formula II, wherein R_{11} is said alkylthio, n is 0 or 1 and R_{13} is hydrogen, is trichloroethyl. This group is introduced onto the carboxy group of the appropriate 6,6-disubstituted penicillanic acid using the procedure as taught in West German application 1,937,962. Conversion of said disubstituted penicillanic acid trichloroethyl ester to a 6-halo-6-alkylthiopenicillanic acid trichloroethyl ester or sulfoxide following the hereinafter described procedure, followed by treatment with an organotin monohydride results in the preparation of a 6- β -alkylthiopenicillanic acid trichloroethyl ester or sulfoxide. Removal of the protecting trichloroethyl moiety to provide a compound of formula II wherein R_{13} is hydrogen is achieved by treatment with zinc dust in a buffered solution.~~

As one skilled in the art recognizes, there are numerous, other unmentioned penicillin carboxy protecting groups which are applicable to the operation of the present process for preparing compounds of the aforementioned formula wherein R_{11} is hydrogen. The concept of the use of such protecting groups for the instant process, while not being exhaustively illustrated, is considered within the broad purview of the present invention. The 6- β -substitu-

for example, by Cama, et al., J. Am. Chem. Soc., 94, 1408 (1972) and Harrison, et al., J. Chem. Soc. (Perkin I), 1772 (1976). Following the reaction of the 6,6-disubstituted penicillanic acid having at the 3-carboxy group a conventional penicillin carboxy protecting group with an organotin monohydride, the protecting group is removed. When two or more of the substituents R_7 , R_8 and R_9 are phenyl or if R_9 is 4-methoxyphenyl or if R_7 , R_8 and R_9 are each methyl, the protecting group can be removed by treatment with trifluoroacetic acid. This method of removal is compatible with all possible substituents at the 6- β position. When R_7 or R_8 is methyl or R_7 and R_8 are hydrogen and R_9 is phenyl said protecting groups can be removed by treatment with trimethylsilyl iodide as taught by Jung, et al., J. Am. Chem. Soc., 99, 968(1977). ~~alternately these groups can be removed by hydrogenolysis~~ provided that the 6- β -substituent is not a halogen or an alkylthio moiety.

The preparation of the requisite 6 β -hydroxymethyl-penicillantes can be carried out by reaction of the corresponding 6 β -hydroxymethylpenicillanic acid as an activated anhydride with the appropriate alcohol $\text{HOR}_7\text{R}_8\text{R}_9$ or by the alkylation of a salt of said acid with $\text{R}_7\text{R}_8\text{R}_9\text{C-halide}$. Following the treatment of the 6 β -hydroxymethylpenicillanate with the appropriate halogenating reagent the protecting group is removed as indicated above.

*The seventh type of protecting group is a trimethylsilyl or dimethyl-t-butylysilyl ester which is generated in situ through the reaction of a triethylamine salt of a 6,6-disubstituted penicillanic acid and appropriate silyl chloride, according to the procedure as taught in Ann., 673, 166(1964). Following the reaction of the protected 6,6-disubstituted penicillanic acid with the organotin monohydride, the protecting group is removed by aqueous hydrolysis.

ted penicillanic acid containing conventional penicillin carboxy protecting groups are valuable intermediates leading to the corresponding free acids.

When R_{12} is an ester-forming residue readily hydrolyzable in vivo in a compound of formula (I), it is a group which is notionally derived from an alcohol of the formula $R_{12}-OH$, such that the moiety $COOR_{12}$ in such a compound of formula (I) represents an ester grouping. Moreover, R_{12} is of such a nature that the grouping $COOR_{12}$ is readily cleaved in vivo to liberate a free carboxy group ($COOH$). That is to say, R_{12} is a group of the type that when a compound of formula (II), wherein R_{12} is an ester-forming residue readily hydrolyzed in vivo, is exposed to mammalian blood or tissue, the compound of formula (I), wherein R_{13} is hydrogen, is readily produced. The groups R_{12} are known in the penicillin art. In most instances they improve the absorption characteristics of the penicillin compound. Additionally, R_{12} should be of such a nature that it imparts pharmaceutically-acceptable properties to a compound of formula (I), and it liberates pharmaceutically-acceptable fragments when cleaved in vivo.

As indicated above, the groups R_{12} are known and are readily identified by those skilled in the penicillin art, as taught in West German application 2,517,316. Typical groups for R_{12} are 3-phthalidyl, 4-crotonlactonyl, γ -butyrolacton-4-yl, alkanoyloxyalkyl and alkoxycarbonyloxyalkyl. However, preferred groups for R_{12} are alkanoyloxymethyl having from 3 to 6 carbon atoms, 1-(alkanoyloxy)ethyl having from 4 to 7 carbon atoms, 1-methyl-1-(alkanoyloxy)ethyl having from 5 to 8 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.

- 5 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 R_1 is hydrogen, by esterification. The specific method chosen will depend naturally upon the precise structure
- 10 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, alkanoyloxyalkyl and alkoxycarbonyloxyalkyl they can be
- 15 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, an alkanoyloxyalkyl halide or an alkoxycarbonyloxyalkyl halide. The term "halide" is intended to mean deriva-
- 20 tive 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.
- 25 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
- 30 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 to an alkali metal iodide. This has the effect of speeding up the reaction. With full regard for the foregoing factors, reaction times of from 1 to about 24 hours are commonly used.

Alternately, compounds of the present invention of formula (I) wherein R_2 is an ester-forming residue readily hydrolyzable in vivo can be prepared from compounds of formula II wherein R_1 is comprised of said ester-forming groups by the process of the present invention.

The starting reagents of formula II wherein R_1 is an ester-forming residue readily hydrolyzable in vivo selected from the group consisting of 3-phthalidyl, 4-crotonolactonyl, γ -butyrolacton-4-yl, alkanoyloxyalkyl and alkoxycarbonyloxyalkyl can be prepared by alkylation of the compound of formula II wherein ~~R_1 , X and n are as defined and~~ wherein R_1 is hydrogen with a phthalidyl halide, a crotonolactonyl halide, a γ -butyrolacton-4-yl halide, an alkanoyloxyalkyl halide or an alkoxycarbonyloxyalkyl halide. The reaction is carried out by dissolving a salt of the compound of formula II, 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

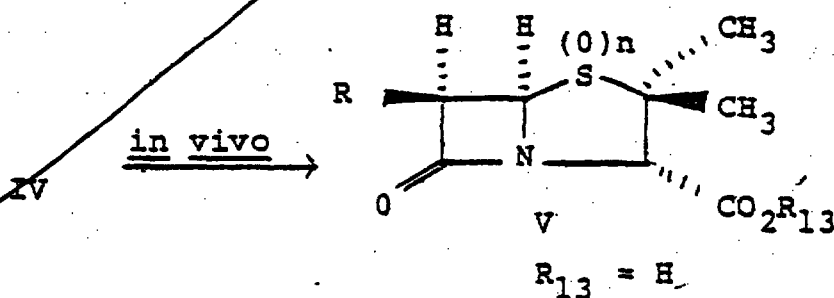
standard techniques. It is often sufficient simply to dilute the reaction medium with an excess of water, and then extract the product into a water-immiscible organic solvent and then recover same by solvent evaporation. Salts of the starting material which are commonly used are alkali metal salts, such 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 halide 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.

An alternate method for preparing the starting reagents for the instant process of formula(II) wherein R_1 is said ester-forming residue is by the diazotization of the appropriate 6- β -aminopenicillanic acid ester and the reaction of the resulting diazopenicillanic acid ester to give the desired 6,6-disubstituted penicillanic acid ester as herein-after described.

~~Compounds of formula I, wherein R_1 , X and n are as defined and R is said alkylthio, are most conveniently prepared from the corresponding 6,6-dihalopenicillanic acid ester, preferably the 6,6-dibromopenicillanic~~

veniently prepared by treatment of the appropriate 6 β -hydroxymethyl penicillanate ester with the requisite halogenating reagent as previously described. The said 6 β -hydroxymethylpenicillanate ester, in turn, is synthesized through the alkylation of the corresponding 6 β -hydroxymethylpenicillanic acid, sulfoxide or sulfone in a manner also previously described.

As hereinbefore indicated, compounds of formula IV wherein R₁₃ is an ester-forming residue readily hydrolyzable in vivo are converted in vivo into compounds wherein R₁₃ is hydrogen as follows:



~~R, n as previously indicated~~

The compounds of formulae (I) and V wherein R₁₃ 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 bases 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-methyldmorpholine 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 formulae (I) and ~~V~~ wherein R_{13} is hydrogen are the sodium, potassium and triethylamine salts.

As indicated hereinbefore, the compounds of formulae (I) ~~II and V~~, wherein R_{13} 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 formulae II and V~~ 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 formulae (I) ~~or V~~ 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 formulae (I) ~~or V~~. 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.

5 The MIC values of combinations are measured using the method described by Barry and Sabath in "Manual of Clinical Microbiology", edited by Lenette, Spaulding and Truant, 2nd edition, 1974, American Society for Microbiology.

10 The compounds of formulae (I) ~~IV and V wherein R₁₃ is hydrogen or an ester-forming residue readily hydrolyzable in vivo~~ enhance the antibacterial effectiveness of β -lactam antibiotics in vivo. That is, they lower the amount of the antibiotic which is needed to
15 protect mice against an otherwise lethal inoculum of certain β -lactamase producing bacteria.

The ability of the compounds of formulae (I) ~~IV or V, wherein R₁₃ is hydrogen or an ester-forming residue readily hydrolyzable in vivo~~ to enhance the effectiveness
20 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 a bacterial infection, the said compound
25 of the formulae (I) ~~IV or V~~ can be comingled with the β -lactam antibiotic, and the two agents thereby administered simultaneously. Alternatively, the said compound of the formulae (I) ~~IV or V~~ can be administered as a separate agent during a course of treatment with a β -lactam
30 antibiotic. In some instances it will be advantageous to pre-dose the subject with the compound of the formulae (I) ~~IV or V~~ before initiating treatment with a β -lactam antibiotic.

When using compounds of formulae ~~(I) IV or V~~ wherein ~~R₁₃ is hydrogen or an ester thereof readily hydrolyzable in vivo~~ to enhance the effectiveness of β -lactam antibiotic, it is administered preferably in formulation with standard pharmaceutical carriers or diluents. A pharmaceutical composition comprising a pharmaceutically-acceptable carrier, a β -lactam antibiotic and a compound of formulae ~~(I) IV or V~~ wherein ~~R₁₃ is hydrogen 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 the compounds of formulae ~~(I) IV or V~~ wherein ~~R₁₃ is hydrogen or an ester thereof readily hydrolyzable in vivo~~ in combination with another β -lactam antibiotic, said compounds 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 ratio of the daily dosages of the compounds of formulae ~~(I) IV or V~~ and the β -lactam antibiotic will normally be in the range from about 1:3 to 3:1. Additionally, when using the compounds of formulae ~~(I) IV or V~~ 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 case it may be necessary to use dosages outside these limits.

Typical β -lactam antibiotics with which the compounds of formulae ~~(I) IV or V~~ 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
 5 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,
 10 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]-
 15 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-phenylacet-
 amido)penicillanic acid,
 20 6-(D-2-[3-methylsulfonylimidazolidin-2-one-1-carboxamido]-
 2-phenylacetamido)penicillanic acid,
 6-([hexahydro-1H-azepin-1-yl]methyleneamino)penicillanic
 acid,
 25 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,
 30 pivaloyloxymethyl 6-(D-2-amino-2-phenylacetamido)penicil-
 lanate,

- pivaloyloxymethyl 6-(D-2-amino-2-[4-hydroxyphenyl]acet-
amido)penicillanate,
1-(ethoxycarbonyloxy)ethyl 6-(2-phenylacetamido)penicil-
lanate,
5 1-(ethoxycarbonyloxy)ethyl 6-(D-2-amino-2-phenylacet-
amido)penicillanate,
1-(ethoxycarbonyloxy)ethyl 6-(D-2-amino-2-[4-hydroxy-
phenyl]acetamido)penicillanate,
3-phthalidyl 6-(2-phenylacetamido)penicillanate,
10 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,
15 6-(2-tolyloxycarbonyl-2-phenylacetamido)penicillanic
acid
6-(2-[5-indanyloxycarbonyl]-2-phenylacetamido)penicil-
lanic acid,
6-(2-phenoxy-carbonyl-2-[3-thienyl]acetamido)penicillanic
acid,
20 6-(2-tolyloxycarbonyl-2-[3-thienyl]acetamido)penicillanic
acid,
6-(2-[5-indanyloxycarbonyl]-2-[3-thienyl]acetamido)peni-
cillanic acid,
25 6-(2,2-dimethyl-5-oxo-4-phenyl-1-imidazolidinyl)penicil-
lanic acid,
7-(2-[2-thienyl]acetamido)cephalosporanic acid,
7-(2-[1-tetrazolyl]acetamido-3-(2-[5-methyl-1,3,4-
thiadiazolyl]thiomethyl)-3-desacetoxymethylcephalo-
30 sporanic acid,

7-(D-2-formyloxy-2-phenylacetamido)-3-(5-[1-methyltetra-
zoly]thiomethyl)-3-desacetoxymethylcephalosporanic
acid,

5 7-(D-2-amino-2-phenylacetamido)desacetoxyccephalosporanic
acid,

7-alpha-methoxy-7-(2-[2-thienyl]acetamido)-3-carbamoyloxy-
methyl-3-desacetoxymethylcephalosporanic acid,

7-(2-cyanoacetamido)cephalosporanic acid,

10 7-(D-2-hydroxy-2-phenylacetamido)-3-(5-[1-methyltetra-
zoly]thiomethyl)-3-desacetoxymethylcephalosporanic
acid,

7-(D-2-amino-2-p-hydroxyphenylacetamido)desacetox-
cephalosporanic acid,

7-(2-[4-pyridylthio]acetamido)cephalosporanic acid,

15 7-(D-2-amino-2[1,4-cyclohexadienyl]acetamido)cephalo-
sporanic acid,

7-(D-2-amino-2-phenylacetamido)cephalosporanic acid,

20 7-[D-(-)-alpha-(4-ethyl-2,3,-dioxo-1-piperazinecarboxamido)-
alpha-(4-hydroxyphenyl)acetamido]-3-[[1-methyl-1,2-
3,4-tetrazol-5-yl]thiomethyl]-3-cephem-4-carboxylic
acid,

7-(D-2-amino-2-phenylacetamido)-3-chloro-3-cephem-4-
carboxylic acid,

25 7-[2-(2-amino-4-thiazolyl)-2-(methoximino)acetamido]cep-
halosporanic acid,

[6R,7R-3-carbamoyloxymethyl-7(2Z)-2-methoxyimino(fur-2-
yl)acetamido-ceph-3-em-4-carboxylate]

30 7-[2-(2-aminothiazol-4-yl)acetamido]-3-[[[1-2-dimethyl-
aminoethyl)-1H-tetrazol-5-yl]thio)methyl]ceph-3-em-
4-carboxylic acid, and a pharmaceutically acceptable
salt 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.

5 When compounds of formulae (I) ~~IV or V~~ wherein R_{13} is ~~hydrogen or an ester thereof readily hydrolyzable in vivo~~ ^{are} to be used simultaneously (i.e. co-mingled) with a β -lactam antibiotic which is effective only on parenteral administration, a combination formulation suitable

10 for parenteral use will be required. When the compounds of formulae (I) ~~IV or V~~ wherein R_{13} is ~~hydrogen or ester thereof~~ ^{are} to be used simultaneously (co-mingled) with a β -lactam antibiotic which is effective orally or

15 or parenteral administration can be prepared. Additionally, it is possible to administer preparations of the compounds of formulae (I) ~~IV or V~~ orally, while at the same time administering a further β -lactam antibiotic parenterally; and it is also possible to administer

20 preparations of the compounds of formulae (I) ~~IV or V~~ parenterally, while at the same time administering the further β -lactam antibiotic orally. of the invention

The following ^{preparations and} examples are provided solely for the purpose of further illustration. Nuclear magnetic

25 resonance spectra (NMR) were measured at 60 MHz for solutions in deuteriochloroform ($CDCl_3$), perdeutero dimethyl sulfoxide ($DMSO-d_6$) or deuterium oxide (D_2O) or are noted otherwise, and peak positions are expressed in parts per million (ppm) downfield from tetramethyl-

30 silane 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.

PREPARATION A

6-8-Chloropenicillanic Acid

A 2.95 g. sample of sodium 6-chloro-6-iodopenicillanic acid was converted to the free acid, and then it was dissolved in 125 ml. of benzene under nitrogen. To the solution was added 1.08 ml. of triethylamine, and the mixture was cooled to 0-5°C. To the cooled mixture was then added 0.977 ml. of trimethylsilyl chloride, and the reaction mixture was stirred at 0-5°C. for 5 min., at 25°C. for 60 min. and at 50°C. for 30 min. The reaction mixture was cooled to 25°C. and the triethylamine hydrochloride was removed by filtration. The filtrate was heated to reflux and 15 mg. of azobisisobutyronitrile, and 2.02 ml. of tri-n-butyltin hydride were added. The refluxing mixture was irradiated with ultraviolet light for 5 min. The solvent was then removed by evaporation in vacuo, and the residue was dissolved in a 1:1 mixture of tetrahydrofuran-water. The pH was adjusted to 7.0 and the tetrahydrofuran was removed by evaporation in vacuo. The aqueous phase was washed with ether, and then an equal volume of ethyl acetate was added. The pH was adjusted to 1.8 and the ethyl acetate layer removed. The aqueous phase was extracted with further ethyl acetate, and then the combined ethyl acetate solutions were dried and evaporated in vacuo. This afforded 980 mg. of 6-8-chloropenicillanic acid.

The above product was dissolved in tetrahydrofuran, and an equal volume of water was added. The pH was adjusted to 6.8, and the tetrahydrofuran was removed by evaporation in vacuo. The aqueous phase remaining was freeze-dried to give 850 mg. of sodium 6-8-chloropenicillanic acid. The NMR spectrum (D_2O) showed absorption at 5.70 (d, 1H, $J = 4\text{Hz}$), 5.50 (d, 1H, $J = 4\text{Hz}$), 4.36 (s, 1H), 1.60 (s, 3H) and 1.53 (s, 3H) ppm.

A mixture of 5.94 g. of sodium nitrite in 260 ml. of water and 2.63 g. of 6- β -aminopenicillanic acid pivaloyloxymethyl ester in 260 ml. of methylene chloride was stirred with cooling in an ice bath. *p*-Toluenesulfonic acid (1.2 g.) was added in three portions over a period of 30 min. and the mixture was allowed to stir for one hour at room temperature. The organic phase was separated and dried over sodium sulfate. Iodine (1.3 g.) was added to the organic phase and the resulting solution allowed to stir at room temperature for 4 hrs. The solution was washed with aqueous sodium thiosulfate, separated and concentrated in vacuo to a low volume. The residue was chromatographed on silica gel using petroleum ether (b.p. 60-80°) containing an increasing proportion of ethyl acetate as the eluent. The fractions containing the product were combined, dried over sodium sulfate and concentrated under vacuum to dryness to give 1.43 g., m.p. 136-138°C. The NMR spectrum (CDCl₃) showed absorption at 5.79 (bs, 2H), 5.71 (s, 1H), 4.52 (s, 1H), 1.65 (s, 3H), 1.44 (s, 3H) and 1.21 (s, 9H) ppm.

(6) 6-8-iodopenicillanic acid pivaloyloxymethyl ester

To a solution of 1.29 g. of 6,6-diiodopenicillanic acid pivaloyloxymethyl ester in 8 ml. of benzene under a nitrogen atmosphere was added 500 mg. of triphenyltin hydride and a few crystals (~10 mg.) of azobisisobutyronitrile, and resulting reaction mixture was warmed to 50°C. for one hour. An additional 500 mg. of hydride and 10 mg. of nitrile were added and the heating continued with stirring for 3 hrs. Column chromatographing on silica gel using petroleum ether (b.p. 60-80°C.) with an increasing proportion of methylene chloride as the eluent gave 140 mg. of the desired product, m.p. 73-77°C. The NMR spectrum (CDCl₃) showed absorption at 5.9 (d, AB, J=5.8Hz), 5.82 (d, AB, J=5.8Hz), 5.66 (d, 1H, AB, J=4.1Hz), 5.42 (d, 1H, AB, J=4.1Hz), 4.59 (s, 1H), 1.71 (s, 3H), 1.50 (s, 3H) and 1.24 (s, 9H) ppm.

EXAMPLE 36-8-Iodopenicillanic Acid Benzyl Ester

In a manner similar to Example 2, 6-8-aminopenicillanic acid benzyl ester was converted to 6,6-diiodopenicillanic acid benzyl ester. The NMR spectrum (CDCl₃) showed absorption at 7.40 (m, 5H), 5.77 (s, 1H), 5.21 (s, 2H), 4.59 (s, 1H), 1.67 (s, 3H) and 1.37 (s, 3H) ppm.

The isolated 6,6-diiodopenicillanic acid benzyl ester was converted to 6-8-iodopenicillanic acid benzyl ester using the appropriate portion of the Example 2 procedure. The NMR spectrum (CDCl₃) showed absorption at 7.42 (m, 5H), 5.64 (d, 1H, AB, J=4.0Hz), 5.42 (d, 1H, AB, J=4.0Hz), 4.59 (s, 1H), 1.69 (s, 3H) and 1.40 (s, 3H) ppm.

EXAMPLE 24

Starting with the appropriate 6- β -aminopenicillanic acid ester and employing the procedure of Example 2, ~~the~~ the following 6- β -iodopenicillanic acid esters are prepared:

6- β -iodopenicillanic acid 3-phthalidyl ester; 6- β -iodopenicillanic acid 1-(acetoxymethyl)ethyl ester; 6- β -iodopenicillanic acid 4-crotonolactonyl ester; 6- β -iodopenicillanic acid γ -butyrolacton-4-yl ester; 6- β -iodopenicillanic acid acetoxymethyl ester; 6- β -iodopenicillanic acid hexanoyloxymethyl ester; 6- β -iodopenicillanic acid 1-(isobutyryloxy)ethyl ester; 6- β -iodopenicillanic acid methoxycarbonyloxymethyl ester; 6- β -iodopenicillanic propoxycarbonyloxymethyl ester; 6- β -iodopenicillanic acid 1-(ethoxycarbonyloxy)ethyl ester; 6- β -iodopenicillanic acid 1-(butoxycarbonyl)ethyl ester; 6- β -iodopenicillanic acid 1-methyl-1-(methoxycarbonyloxy)ethyl ester; and 6- β -iodopenicillanic acid 1-methyl-1-(isopropoxycarbonyl)ethyl ester.

EXAMPLE 136- β -Chloropenicillanic Acid Acetoxymethyl Ester6-Chloro-6-iodopenicillanic acid acetoxymethyl ester

To a solution of 5.03 g. of 6-chloro-6-iodopenicillanic acid in 50 ml. of acetone and 50 ml. of acetonitrile is added 900 mg. of di-isopropylethylamine followed by 0.7 ml. of acetoxymethyl bromide. The resulting solution is allowed to stir at room temperature for 48 hrs. An additional 0.7 ml. of bromide and 900 mg. of amine are added and the stirring is continued for an additional 48 hrs. The solution is concentrated in vacuo to dryness and the residue suspended in ethyl acetate. The

PREPARATION BPivaloyloxymethyl 6- β -Bromopenicillanate

To a solution of 280 mg. of 6- β -bromopenicillanic acid in 2 ml. of N,N-dimethylformamide is added 260 mg. of diisopropylethylamine followed by 155 mg. of chloromethyl pivalate and 15 mg. of sodium iodide. The reaction mixture is stirred at room temperature for 24 hours, and then it is diluted with ethyl acetate and water. The pH is adjusted to 7.5, and then the ethyl acetate layer is separated and washed three times with water and once with saturated sodium chloride solution. The ethyl acetate solution is then dried using anhydrous sodium sulfate, and evaporated in vacuo to give the title compound.

EXAMPLE 5

Reaction of 6- β -iodo-penicillanic acid with 4-crotonolactonyl chloride, or 1-(ethoxycarbonyloxy)ethyl chloride, according to the procedure of Preparation B affords 4-crotonolactonyl 6- β -iodopenicillanate and 1-(ethoxycarbonyloxy)ethyl 6- β -iodopenicillanate.

~~6- β -fluoropenicillanic acid, 6- β -chloropenicillanic acid, 6- β -bromopenicillanic acid, 6- β -iodopenicillanic acid,; 6- β -methoxypenicillanic acid; 6- β -methylthiopenicillanic acid; 6- β -bromopenicillanic acid sulfoxide; 6- β -methoxypenicillanic acid sulfoxide; 6- β -fluoropenicillanic acid sulfoxide; and 6- β -chloropenicillanic acid sulfoxide.~~

EXAMPLE #6

6- β -Iodopenicillanic Acid

10 A. 6,6-diiodopenicillanic acid benzhydryl ester

To a solution of 5.94 g. of sodium nitrite in 250 ml. of water at 5°C. was added with stirring 2.9 g. of 6- β -aminopenicillanic acid benzhydryl ester tosylate salt in 250 ml. of methylene chloride. p-Toluene sulfonic acid (1.2 g.) was added in three portions over a period of 30 min. and the mixture allowed to stir for one hour at room temperature. The organic phase was separated, dried over sodium sulfate and treated with 1.3 g. of iodine. The resulting solution was stirred at room temperature for 4 hrs. and was then washed with an aqueous sodium thiosulfate solution and concentrated to a low volume. The residual was chromatographed on silica gel using petroleum ether with increasing proportions of ethyl acetate as the eluent. The fractions containing the product were combined and concentrated in vacuo to give the desired product.

B. 6- β -iodopenicillanic acid benzhydryl ester

To a solution of 1.92 g. of 6,6-diiodopenicillanic acid benzhydryl ester in 8 ml. of benzene was added 30 500 mg. of triphenyltin hydride and 10 mg. of azobisisobutyronitrile, and the resulting reaction mixture allowed to stir under a nitrogen atmosphere at 50°C. for one hour.

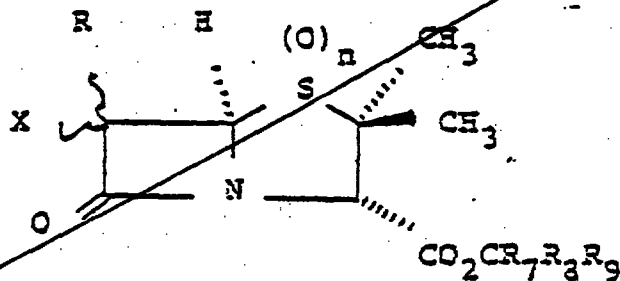
An additional amount of hydride (500 mg.) and nitrile (10 mg.) was added and heating at 50°C. continued for 3 hrs. The solvent was removed under vacuum and the residue chromatographed over silica gel using petroleum ether with increasing proportions of ethyl acetate as the eluent. The fractions containing the product were combined and concentrated to dryness. The NMR spectrum (CDCl₃) showed absorption at 7.50 (bs, 10H), 6.97 (s, 1H), 5.66 (d, 1H, AB, J=4.0Hz), 5.44 (d, 1H, AB, J=4.0Hz), 4.67 (s, 1H), 1.70 (s, 3H) and 1.40 (s, 3H) ppm.

C. 6- β -iodopenicillanic acid

Trifluoroacetic acid (0.5 ml.) was added to 80 mg. of 6- β -iodopenicillanic acid benzhydryl ester in 1 ml. of methylene chloride and the reaction mixture stirred for 30 min. at room temperature. The mixture was evaporated to dryness to yield 76 mg. of crude product. Purification is affected by chromatography on silica gel.

EXAMPLE 49

A. Starting with the appropriate penicillanic acid ester and employing the procedure of Example 48A, the following compounds are prepared:



EXAMPLE 76- β -Iodopenicillanic AcidA. 6,6-diiodopenicillanic acid 4-methoxybenzyl ester

5 The title compound was prepared from 6- β -aminopenicillanic acid 4-aminopenicillanic acid 4-methoxybenzyl ester following the procedure of Example ~~4A~~ 6A

B. 6- β -iodopenicillanic acid 4-methoxybenzyl ester

10 The title compound was prepared from 6,6-diiodopenicillanic acid 4-methoxybenzyl ester using the procedure of Example ~~4B~~ ^{6B}. The NMR (CDCl₃) spectrum shows absorption at 7.36 (d, 2H, AA', XX', J=9Hz), 6.95 (d, 2H, AA', XX', J=9.0Hz), 5.65 (d, 1H, AB, J=4.2Hz), 5.42
15 (d, 1H, AB, J=4.2Hz), 4.58 (s, 1H), 3.89 (s, 3H), 1.71 (s, 3H), 1.70 (s, 3H) and 1.39 (s, 3H) ppm.

C. 6- β -iodopenicillanic acid

6- β -Iodopenicillanic acid 4-methoxybenzyl ester (90 mg.) was dissolved in 2 mL. of methylene chloride to which
20 was then added 1 mL. of trifluoroacetic acid and 3 drops of anisole. The mixture was stirred at room temperature for 5 hrs. and was then evaporated to dryness. The residue was chromatographed on silica using petroleum ether and then ethyl acetate as the eluent. The fractions con-
25 taining the product were combined and concentrated to give 40 mg. of the desired product. The NMR (CDCl₃) spectrum showed absorption ca. 9 (bs, 1H), 5.65 (d, 1H, AB, J4.0Hz), 5.39 (d, 1H, AB, J=4.0Hz), 4.57 (s, 2H), 1.74 (s, 3H) and 1.57 (s, 3H).

PREPARATION C6- β -Bromopenicillanic Acid Sodium SaltA. 6,6-dibromopenicillanic acid tri- n -butyltin ester

To a slurry of 35.9 g. of 6,6-dibromopenicillanic acid in 700 ml. of toluene was added 29.5 g. of bis(tri- n -butyltin)oxide and the resulting mixture heated to reflux. Over a period of about 45 min. the toluene was allowed to distill from the reaction mixture, water being azeotropically removed during that period of time.

The remainder of the solvent was removed at room temperature in vacuo, to give 78.7 g. of the desired intermediate.

B 6- β -bromopenicillanic acid sodium salt

To 1.0 g. of 6,6-dibromopenicillanic acid tri- n -butyltin ester in 5 ml. of toluene at 55°C. was added 0.4 ml. of tri- n -butyltin hydride dropwise. Heating was continued for 3.5 hours, after which the solvent was removed and the residue dissolved in 25 ml. of chloroform. The chloroform was washed with a saturated sodium bicarbonate solution (2 x 50 ml.). The aqueous washes were combined, the pH adjusted to 1.5 with 6 N hydrochloric acid and the product extracted with ethyl acetate. The ethyl acetate extracts were combined, dried over magnesium sulfate and 1.24 ml. (1.24 mmol./CC.) of ethyl acetate containing sodium 2-ethylhexanoate was added. After stirring in the cold for one hour the product was filtered and dried, 114 mg.

EXAMPLE 7

Starting with 6,6-iodo penicillanic acid and bis(triphenyltin)oxide, and employing the procedure of Preparation C, 6,6-diiodopenicillanic acid triphenyltin ester and 6- β -iodopenicillanic acid are prepared.

PREPARATION D.

6-8-Bromopenicillanic AcidA. 6,6-dibromopenicillanic acid methyl
acetoacetate ester

5 To 5.0 g. of 6,6-dibromopenicillanic acid sodium
salt in 100 ml. of dimethylformamide was added 1.6 ml.
of methyl 2-chloroacetoacetate, and the resulting re-
action mixture allowed to stir over night at room
temperature. The mixture was poured into 400 ml. of
10 ice and water and extracted with ethyl acetate. The
organic phase was separated and washed successively
with water, a saturated aqueous sodium bicarbonate solu-
tion and a brine solution. The organic phase was then
dried over magnesium sulfate and concentrated to a dark
15 oil (5.0 g.), which was chromatographed on 300 g. of
silica gel. The fractions of eluate, which was comprised
of toluene/ethyl acetate (2:1, V:V), containing the pro-
duct were combined and concentrated in vacuo to give 4.0
g. of the desired product.

B. 6- β -bromopenicillanic acid

Under anhydrous conditions and a nitrogen atmosphere 2.0 g. of 6,6-dibromopenicillanic acid methyl acetoacetate ester in 140 ml. of dry benzene was treated with 1.1 ml. of tri- n-butyltin hydride, and the resulting reaction mixture allowed to stir overnight at room temperature. The benzene solvent was removed in vacuo and the residue slurried in hexane. The undissolved material was chromatographed on 250 g. of silica gel using toluene/ethyl acetate (5:1, V:V) as the eluent. The fractions containing the desired product were combined and concentrated under reduced pressure to dryness.

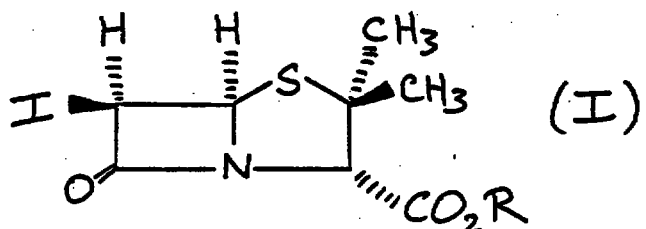
To 3.9 g. of 6- β -bromopenicillanic acid methyl acetoacetate ester, prepared by the above procedure, in 50 ml. of acetone is added 2.1 g. of sodium nitrite in 10 ml. of water with stirring. After stirring for three hours at room temperature the solvent is removed in vacuo and the residue aqueous extracted once with ether. The aqueous is then made acid to pH 1.5 with 6 N hydrochloric and extracted with ethyl acetate. The organic phase is dried over sodium sulfate and concentrated under reduced pressure to give the desired product.

EXAMPLE 8

Employing the procedure of Preparation D and starting with the 6,6-diiodo penicillanic acid sodium salt, 6- β -iodopenicillanic acid is prepared.

CLAIMS

1. A 6 β -iodo-penicillanic acid compound of the formula



wherein:

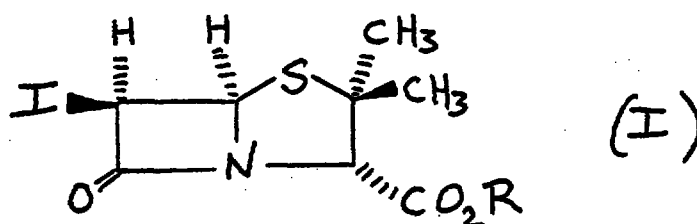
- 5 R is hydrogen or an ester-forming residue readily hydrolyzable in vivo, or a pharmaceutically acceptable base salt thereof when R is hydrogen.
2. 6 β -Iodo-penicillanic acid or a pharmaceutically acceptable base salt thereof.
- 10 3. A compound as claimed in claim 1, wherein R is alkanoyloxymethyl having from 3 to 6 carbon atoms, 1-(alkanoyloxy)ethyl having from 4 to 7 carbon atoms, 1-methyl-1(alkanoyloxy)ethyl having from 5 to 8 carbon atoms, alkoxycarbonyloxymethyl having from 3 to 6 carbon atoms,
- 15 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 or gamma-butyrolacton-4-yl.
4. 6 β -Iodo-penicillanic acid pivaloyloxymethyl ester.



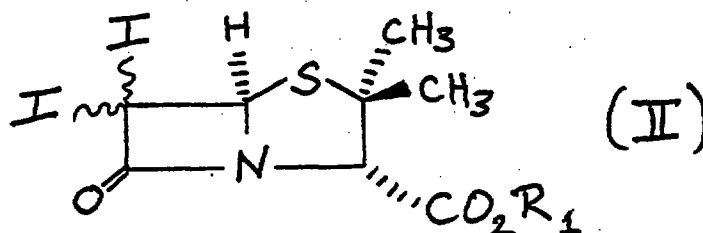
5. A pharmaceutical composition comprising a compound as claimed in any of claims 1 to 4 and a pharmaceutically acceptable carrier.

6. A composition as claimed in claim 5 which also comprises a beta-lactam antibiotic.

7. A process for the preparation of a compound of the formula



wherein R is as defined in claim 1, which comprises reacting a compound of the formula:



wherein R_1 is an ester-forming residue readily hydrolyzable in vivo or a penicillin carboxy-protecting group, with an organotin monohydride at from 0 to 110°C, followed by removal of R_1 when it is a penicillin carboxy-protecting group.



8. A process as claimed in claim 7, wherein R_1 is

(a) $-PR_2R_3$ wherein R_2 and R_3 are each alkyl having from one to three carbon atoms, alkoxy having from one to three carbon atoms or phenyl;

5 (b) 3,5-di-t-butyl-4-hydroxybenzyl;

(c) $-CH_2-Y$ wherein Y is $-CO-R_4$ (wherein R_4 is phenyl or alkyl having from one to three carbon atoms), cyano or carboalkoxy having from two to four carbon atoms;

10 (d) $-N=CH-R_5$ wherein R_5 is phenyl or alkyl having from one to three carbon atoms;

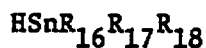
(e) $-CH(COCH_3)CO_2R_6$ wherein R_6 is alkyl having from one to four carbon atoms;

15 (f) $-CR_7R_8R_9$ wherein R_7 and R_8 are each hydrogen, phenyl or methyl, and R_9 is phenyl, 4-methoxyphenyl or methyl, with the proviso that when R_7 and R_8 are each methyl, R_9 is also methyl;

(g) $-Si(CH_3)_3$ or $-Si(CH_3)_2$ t- C_4H_9 ; or

(h) $-SnR_{16}R_{17}R_{18}$ wherein R_{16} , R_{17} and R_{18} are each alkyl having from one to five carbon atoms, phenyl or benzyl.

20 9. A process as claimed in claim 7 or claim 8, wherein the organotin monohydride is of the formula:



wherein R_{16} , R_{17} and R_{18} are as defined in claim 8.



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10. A process as claimed in claim 9, wherein R_1 is p-methoxybenzyl and the organotin monohydride is tri- n-butyltin hydride.

11. A process as claimed in any of claims 7 to 10, wherein the R_1 group is removed by hydrolysis.

12. A process as claimed in claim 7, wherein R_1 is an ester-forming residue readily hydrolyzable in vivo as defined for R is claim 3.

13. A process as claimed in claim 12, wherein R_1 is pivaloyloxymethyl and the organotin monohydride is tri- n-butyltin hydride.

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