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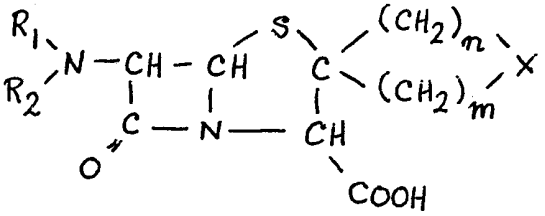
(54) AMINO-SPIRO [OXA(OR THIA)CYCLOALKANEPENAM]-CARBOXYLIC ACID DERIVATIVES

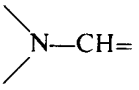
(71) We, UCB, of 4, Chaussée de Charleroi, Saint-Gilles-lez-Bruxelles, Belgium, a Body Corporate organised under the laws of Belgium, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention is concerned with new anti-bacterially active amino-spiro[oxa(or thia)cycloalkanepenam]-carboxylic acid derivatives and with the preparation thereof.

The amino-spiro[oxa(or thia)cycloalkane-penam]-carboxylic acid derivatives of the present invention are compounds of the general formula:—

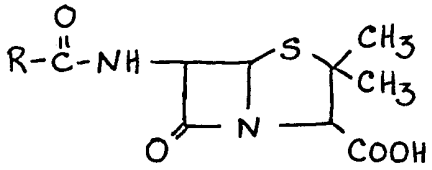


wherein X is a sulphur or oxygen atom or a sulphinyl group, *n* and *m*, which can be the same or different, are 1 or 2 and are preferably 2, R_1 is a hydrogen atom and R_2 is a monocarboxylic acyl, radical selected from those known in the chemistry of the penicillins, preferably a 2-phenylacetyl, 2-amino-2-phenyl-acetyl, 5-methyl-3-phenyl-4-isoxazolecarbonyl or 2,6-dimethoxybenzoyl radical, or R_1 and R_2 together represent a bivalent aminomethylene radical



and preferably a (hexahydro-1H-azepin-1-yl)-methylene radical; as well as the pharmaceutically acceptable non-toxic salts thereof and preferably the sodium and potassium salts theroef.

The penicillins are a group of compounds having the following general formula:—

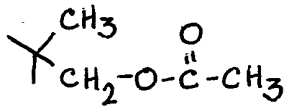


wherein R may represent one of a multitude of substituents, those most currently used being mentioned in Ullmann's Encyklopädie der Technischen Chemie, 4th Edition, *7*, 651—652/1974.

Most of the investigation hitherto carried out in this field were based on the search for new substituents R, while the basic ring system of the molecule remained unchanged. Nevertheless, a number of attempts have been made to study the influence of some variations in the ring system on the activity of the compounds thus obtained. Thus, compounds analogous to penicillins have been proposed but in which the gem-dimethyl group situated in the α-position to the sulphur atom was replaced by other groups. The following groups have been proposed:—

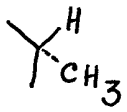
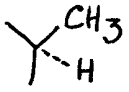
(Belgian Patent Specification No. 738,131)

and



(D.H.R. Barton *et al.*, Chem. Commun. *13*, 1683—1684/1970)

However, it was concluded at the time of these different attempts that the nature of the substituents on the carbon atom in the α-position to the sulphur atom was not essential for the antibacterial activity of the penicillins (see, for example, R. J. Stoodley, Progress in Organic Chemistry, *8*, 106/1973). More recently, the following groups have also been proposed:—

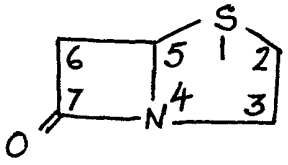
 and 

(P. J. Claes *et al.*, Eur. J. Med. Chem. Chimica Therapeutica, *10*, 573—577/1975)

The conclusion with regard to antibacterial activity was the same.

We have now found that by suitably substituting the carbon atom on the α-position to the sulphur atom, it is possible to obtain compounds, the antibacterial activities of which are at least equal to those of the corresponding penicillins. Furthermore, these new compounds are particularly interesting in the treatment of infections caused by Gram-negative bacteria which produce β-lactamases. These new compounds and the preparation and use thereof form the subject matter of the present invention.

The nomenclature used hereinafter is that proposed by R. J. Stoodley, *loc. cit.*, 102—103. In particular, the following ring system is designated "penam":



The new compounds of the present invention are amino-spiro[oxa(or thia)-cycloalkane-penam]-carboxylic acids in which the amino group is substituted by a substituent known in the chemistry of penicillins.

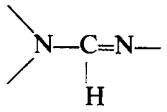
These compounds are analogous to penicillins, wherein the carbon atom in the 2-position of the "penam" ring is substituted by an oxa- or thia-alkylene chain, which forms a heterocycle with this carbon atom. Thus, these compounds have the structure of a spiro heterocycle, constituted by the penam ring and by a saturated monocyclic heterocycle having an oxygen or sulphur atom. In addition, this sulphur atom may carry an oxygen atom.

Among the substituents known in the chemistry of penicillins, mention is

made, in particular, of those mentioned in Ullmann's Encyklopädie der Technischen Chemie, *loc. cit.*

Thus, when substituent R_2 is a 2-phenylacetyl radical, the compounds of the present invention are similar to benzylpenicillin (penicillin G); when R_2 is a 2-amino-2-phenylacetyl radical, the compounds of the present invention are similar to ampicillin; when R_2 is a 5-methyl-3-phenyl-4-isoxazolecarbonyl radical, the compounds of the present invention are similar to oxacillin and when R_2 is a 2,6-dimethoxybenzoyl radical, the compounds of the present invention are similar to methicillin.

When R_1 and R_2 together represent a (hexahydro-1H-azepin-1-yl)-methylene radical, the compounds of the present invention are similar to the penicillins which are the subject matter of British Patent Specification No. 1,293,590. Therefore, in this case, the compounds have side chains connected to the penam ring system by a

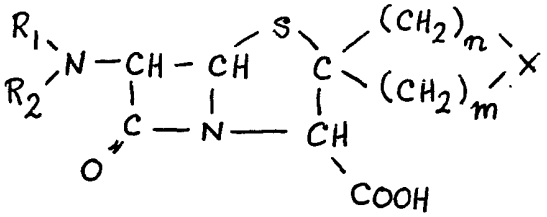


group and these compounds are referred to as "amidinopenicillins". This type of side chain has been introduced recently into the chemistry of penicillins and it leads to compounds the activity of which at the bacterial wall is due to a mechanism which is different from that of the penicillins having conventional side chains, such as those mentioned above. The difference in their mode of action on the bacteria imparts a novel antibacterial spectrum to the amidinopenicillins.

As regards stereochemistry, the existence of three asymmetric carbon atoms at C_3, C_5 and C_6 should lead to the formation of 8 isomers which can be grouped into 4 racemic diastereoisomers. The kinetics of the reactions leads, in fact, to the formation of only three α, β and γ racemates. The α racemate, the relative configuration of which corresponds to that of natural penicillin, i.e. the configuration S at C_3 and the configuration R at C_5 and C_6, is preferably isolated from this mixture.

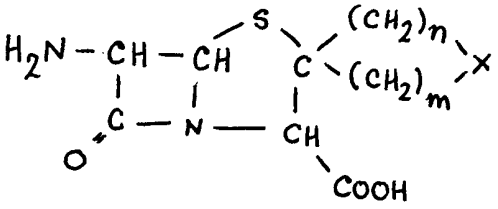
The compounds of general formula (I) according to the present invention, in which X is a sulphur or oxygen atom, *n* is 1 or 2, *m* is 1 or 2, R_1 is a hydrogen atom and R_2 is a monocarboxylic acyl radical selected from those known in the chemistry of penicillins, preferably a 2-phenylacetyl, 2-amino-2-phenylacetyl, 5-methyl-3-phenyl-4-isoxazolecarbonyl or 2,6-dimethoxybenzoyl radical, are obtained by subjecting an amino-spiro[oxa(or thia)cycloalkane-penam]-carboxylic acid or an ester or salt thereof, to an acylation reaction.

Thus, the present invention also provides a process for the preparation of an amino-spiro[oxa(or thia)cyclo-alkane-penam]-carboxylic acid derivative of the general formula:—



(I)

wherein X is a sulphur or oxygen atom, *n* is 1 or 2, *m* is 1 or 2, R_1 is a hydrogen atom and R_2 is a monocarboxylic acyl radical selected from those known in the chemistry of penicillins, which comprises reacting an amino-spiro[oxa(or thia)cycloalkane-penam]-carboxylic acid of the general formula:—



(II)

in which *n*, *m* and X have the meanings given above, or an ester or salt thereof, with a halide of a monocarboxylic acid of the general formula R_2OH or with a functional equivalent thereof, R_2 having the same meaning as above, this acid halide preferably being phenylacetyl chloride, 2-phenylglycyl chloride, 5-methyl-3-phenyl-4-isoxazole-carbonyl chloride or 2,6-dimethoxybenzoyl chloride.

The functional equivalents of the above-mentioned acid halides used as acylating agents for the primary amino radical of the amino-spiro[oxa(or thia)cycloalkane-penam]-carboxylic acids of general formula (II) are, in particular, the acid anhydrides, including the mixed anhydrides and especially the mixed anhydrides formed with stronger acids, such as the lower aliphatic monoesters of carbonic acid, alkylsulphonic and arylsulphonic acids, and the acids which have a more pronounced hindrance, such as diphenylacetic acid. Furthermore, an acid azide or an active ester or thioester (for example with *p*-nitrophenol, 2,4-dinitrophenol, thiophenol or thioacetic acid) may be used but, as a variant, the free acid itself may be condensed with the amino-spiro[oxa(or thia)cycloalkane-penam]-carboxylic acids of general formula (II), after the free acid has first been activated by reacting it, for example, with (chloromethylene)-dimethylammonium chloride (see British Patent Specification No. 1,008,170 and Novak and Weichnet, Experientia, *XXI*, 6, 360/1965) or by means of enzymes, or with an N,N'-carbonyldiimidazole or an N,N'-carbonyl-ditriazole (see British Patent Specification No. 967,108), or with a carbodiimide, for example dicyclohexyl-carbodiimide (see Example II.1.a) and b)), diisopropyl-carbodiimide or N-cyclohexyl-N'-(2-morpholinoethyl)-carbodiimide (see Sheehan and Hess, J.A.C.S., *77*, 1067/1955) or an alkynylamine (see Buijle and Viehe, Agnew. Chem. International Edition, *3*, 582/1964) or a ketone-imine (see Stevens and Munk, J.A.C.S., *80*, 4065/1958) or an isoxazolium salt (see Woodward *et al.*, J.A.C.S., *83*, 1010/1961). It is also possible to use the corresponding azolides instead of the acid halides.

When the starting compound is an ester of an amino-spiro[oxa(or thia)cycloalkane-penam]-carboxylic acid of general formula (II), preferably the benzyl ester, the process according to the present invention includes a second stage which comprises a hydrogenolysis of the resulting esters to the corresponding acids. These esters have a weak antibacterial activity and are essentially useful as intermediates in the synthesis of the corresponding acids or salts.

The starting compounds may be, as desired, the amino spiro[oxa(or thia)cycloalkane-penam]-carboxylic acids of general formula (II) or the salts or esters thereof. Nevertheless, we have found that it is preferable, with regard to yield, to use, in certain cases, the esters and, in other cases, the acids themselves. In particular, when the substituent R_2 is a 2-phenylacetyl, 2-amino-2-phenyl-acetyl or 2,6-dimethoxybenzoyl radical, it is preferable to start from an ester, for example a benzyl ester, of the corresponding amino-spiro[oxa(or thia) cycloalkane-penam]-carboxylic acid and to subject the resulting compound to a subsequent debenzylation reaction to give the free acid. When, on the other hand, the substituent R_2 is a 5-methyl-3-phenyl-4-isoxazolecarbonyl radical, it is preferable to start from the acid itself. Nevertheless, it must be remembered that the amino-spiro[oxa(or thia)-cycloalkane-penam]-carboxylic acids are themselves always obtained from the corresponding esters, because it is necessary temporarily to protect the acid function. In other words, the stage of conversion from ester into acid is not a supplementary stage because it is, in fact, simply displaced in the general synthesis process: in certain cases it is carried out prior to the acylation reaction and in certain cases it is carried out after this acylation reaction.

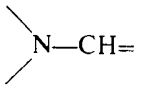
The amino-spiro[oxa(or thia)cycloalkane-penam]-carboxylic acids of general formula (II) and the alkali metal salts and esters thereof, which are the starting materials for the preparation of the compounds according to the present invention, as well as the preparation thereof form the subject matter of our co-pending Patent Application No. 1905/77. (Serial No. 1567561).

By "pharmaceutically acceptable non-toxic salts" are to be understood, in particular, the metal salts, such as sodium, potassium, calcium and aluminium salts, the ammonium salts and the salts of amines, such as the trialkylamines, particularly triethylamine, procaine, dibenzylamine, N-benzyl-B-phenethylamine, L-ephenamine, N,N'-dibenzyl-ethylenediamine, dehydroabietylamine, N,N'-bis-dehydroabietyl-ethylenediamine and N-(lower alkyl)-piperidines, such as N-ethylpiperidine, and, generally speaking, the salts already known for penicillins G and V (see Ullmann's Encyklopädie, *loc. cit.* p.653). These salts can be prepared from the corresponding acids by known methods.

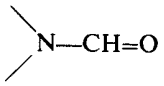
In the particular case in which R_2 is a 2-amino-2-phenylacetyl radical, the

products of the present invention can be converted into their acid addition salts, for example with pharmaceutically-acceptable non-toxic acids, such as acetic acid, citric acid, succinic acid, ascorbic acid, hydrochloric acid, hydrobromic acid, sulphuric acid or phosphoric acid.

The compounds of general formula (I) in which X is a sulphur or oxygen atom, *n* is 1 or 2, *m* is 1 or 2 and R_1 and R_2 together represent a bivalent aminomethylene radical

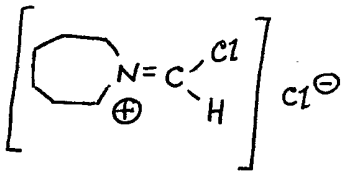


preferably the (hexahydro-1H-azepin-1-yl)-methylene radical, can be prepared by reacting an amino-spiro[oxa(or thia)cycloalkanepenam]-carboxylic acid of general formula (II) or an ester or salt thereof with an activated derivative of an amino-carboxaldehyde containing the grouping



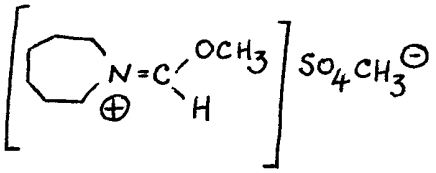
and preferably with an activated derivative of hexahydro-1H-azepin-1-carboxaldehyde.

The activated derivative of the aminocarboxaldehyde can be, for example and preferably, the chloride of the corresponding amide obtained by reaction with oxalyl chloride or a complex obtained by reaction with dimethyl sulphate. Preferably, there are used the following compounds, which are activated derivatives of hexahydro-1H-azepin-1-carboxaldehyde:



1-(chloromethylene)-hexahydro-1H-azepinium chloride

and



1-(methoxymethylene)-hexahydro-1H-azepinium methylsulphate

The compounds of general formula (I), in which X is a sulphinyl group (S → O) and *n*, *m*, R_1 and R_2 have the same meanings as above, are obtained by subjecting an amino-spiro[oxa(or thia)cycloalkane-penam]-carboxylic acid derivative of general formula (I), wherein X is a sulphur atom, or an ester or a salt thereof, to an oxidation by means of an organic peracid, such as peracetic, perbenzoic or preferably *m*-chloroperbenzoic acid. When the starting compound used is an ester of a derivative of general formula (I), for example a benzyl ester, the ester obtained should, of course, subsequently be converted into the corresponding acid by hydrogenolysis.

The present invention relates also to the use of the compounds of general formula (I), as well as of their pharmaceutically acceptable non-toxic salts, as antibacterial agents, as dietetic supplements for animal foodstuffs and as therapeutic agents for man and animals in the treatment of infectious diseases caused by Gram-positive and Gram-negative bacteria.

The compounds of the present invention possess a very broad spectrum of antibacterial activity, both against Gram-positive and against Gram-negative bacteria but they are particularly interesting for combating Gram-negative strains which produce β-lactamases.

The following Examples are given for the purpose of illustrating the present invention:—

Example I.

Preparation of (2″ - phenylacetamido) - spiro[oxa(or thia) - cycloalkane-penam]-carboxylic acids and of their derivatives.

I.l. Preparation of benzyl (2″ - phenylacetamido) - spiro[oxa(or thia)cycloalkane-penam] - carboxylate.

a) Benzyl 6 - (2″ - phenylacetamido) - 2′, 3′, 5′, 6′ - tetrahydrospiro[penam - 2,4′-[4H]pyran] - 3 - carboxylate:

303 mg. (0.003 mole) triethylamine in 10 ml. anhydrous dichloromethane are added all at once at ambient temperature and with stirring to a suspension of 1.56 g. (0.003 mole) benzyl 6-amino-2′, 3′, 5′, 6′-tetrahydro-spiro[penam-2,4′-[4H]pyran]-3-carboxylate *p*-toluenesulphonate (prepared according to the method described in Example 5.1 of our co-pending Patent Application No. 1905/77 (Serial No. 1567561) in 100 ml. anhydrous dichloromethane. The *p*-toluenesulphonate dissolves instantaneously. The reaction mixture is cooled to between 0 and –5°C. and then there are added alternately and in small portions, over the course of about 1 hour, 510 mg. (0.0033 mole) phenylacetyl chloride in 10 ml. dichloromethane and 335 mg. (0.0033 mole) triethylamine in 10 ml. dichloromethane. Stirring is continued for 2 hours, whilst the reaction mixture gradually returns to ambient temperature.

After washing the organic phase successively with 1/20 N hydrochloric acid, 5% aqueous sodium bicarbonate solution and water and then drying over anhydrous sodium sulphate, it is evaporated to dryness. The residue obtained is recrystallised either from ethyl acetate or from a diethyl ether/hexane mixture (1:3 v/v) to give 1.32 g. (yield ;4.4% of theory) benzyl 6-(2″-phenylacetamido)-2′,3′,5′, 6′-tetrahydro-spiro[penam-2,4′-[4H]pyran]-3-carboxylate; m.p. 130°C.

Analysis for $C_{25}H_{26}N_2O_5S$ (M.W. = 466)

calculated:	C 64.37%;	H 5.58%;	N 6.00%
found:	64.70%;	5.81%;	6.30%;

Infra-red spectrum (KBr) in cm^{-1}:

3240	NH
3040, 2940, 2830	CH and CH_2
1772	CO β-lactam
1743	CO ester
1635	CO amide
725, 688	monosubstituted phenyl

N.M.R. ($CDCl_3$-tetramethylsilane (TMS)):

ppm	multiplicity	integration	attribution
1 to 2.10	m	4 H	two $H_{2'}$ and two $H_{6'}$
3 to 4.10	m	4 H	two $H_{3'}$ and two $H_{5'}$
3.64	s	2 H	CH_2 of phenylacetyl group
4.53	s	1 H	H_3
5.17	s	2 H	CH_2 of benzyl group
5.57	q (J = 4 cps)	2 H	H_5 and H_6
6	m	1 H	NH
7.35	s	10 H	two phenyl groups

(m = multiplet, s = singlet, q = quartet)

b) Benzyl 6 - (2" - phenylacetamido) - 2', 3', 5', 6' - tetrahydrospiro[penam - 2,4'-[4H]thiopyrany] - 3 - carboxylate:

This compound is prepared in the same manner as the preceding one but starting with benzyl 6-amino-2', 3', 5', 6'-tetrahydro-spiro[penam-2, 4'-[4H]thiopyran]-3-carboxylate *p*-toluenesulphonate (prepared according to the method described in Example 5.2 of our co-pending Patent Application No. 1905/77). (Serial No. 1567561). After recrystallisation of the compound obtained from diethyl ether, the yield is 56% of theory; m.p. 138—141°C.

Analysis for $C_{25}H_{28}N_2O_4S_2$ (M.W. = 482)

calculated:	C 62.20%;	H 5.40%;	N 5.81%
found:	62.01%;	5.45%;	5.80%

Infra-red spectrum (KBr) in cm^{-1}:

3260	NH
3035, 2900	CH and CH_2
1785	CO β-lactam
1760	CO ester
1650	CO amide
1530	NH amide
730, 695	monosubstituted phenyl

N.M.R. ($CDCl_3$–TMS):

ppm	multiplicity	integration	attribution
1.5 to 3	m	8 H	twice: H_2, H_3, H_5, and H_5,
3.65	s	2 H	CH_2 of phenylacetyl group
4.48	s	1 H	H_3
5.2	s	2 H	CH_2 of benzyl group
5.2 to 5.8	m	2 H	H_5 and H_6
5.9 to 6.7	m	1 H	NH
7 to 7.7	m	10 H	two phenyl groups

c) The following compounds are prepared in the same manner: benzyl 6'-(2"-phenylacetamido) - 4,5 - dihydro - spiro[furan - 3(2H), 2' - penam] - 3' - carboxylate; benzyl 6-(2"-phenylacetamido)-4',5'-dihydro-spiro[penam-2,3'(2'H)-thiophene]-3-carboxylate; benzyl 6'-(2"-phenylacetamido)-spiro[oxetane-3,2'-penam]-3'-carboxylate; benzyl 6-(2"-phenylacetamido)-spiro[penam-2,3'-thietane]-3'-carboxylate.

d) Benzyl 6 - (2"-phenylacetamido) - 2', 3', 5', 6' - tetrahydrospiro[penam - 2,4'-[4H] - thiopyran] - 3 - carboxylate 1' - oxide.

1.6 g. (0.0033 mole) benzyl 6-(2"-phenylacetamido)-2', 3', 5', 6'-tetrahydro-spiro[penam-2,4'-[4H]thiopyran]-3-carboxylate (prepared as described in 1.b.) above) are dissolved in 15 ml. chloroform. The solution is cooled in an ice-bath and a solution of 685 mg. (0.0031 mole) *m*-chloroperbenzoic acid (85%) in 10 ml. chloroform is added dropwise within the course of 25 minutes. Stirring is continued for 30 minutes. The solution is washed successively with a 5% aqueous sodium bicarbonate solution and water. It is then dried over anhydrous magnesium sulphate and evaporated to dryness. A solid residue of 1.6 g. benzyl 6-(2"-phenylacetamido)-2', 3', 5', 6'-tetrahydro-spiro-[penam-2,4'-[4H]thiopyran]-3-carboxylate 1'-oxide is obtained. This product gives a single spot in a thin layer chromatogram on silica (eluent: benzene, ethyl acetate, acetic acid: 20:10:5 v/v/v; Rf = 0.45).

Infra-red spectrum ($CHCl_3$) in cm^{-1}:

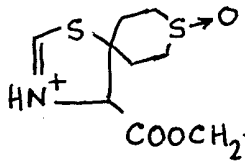
3400	NH
300	CH_2
1785	CO β-lactam
1740	CO ester
1680	CO amide
1500	phenyl
1040	SO

N.M.R. ($CDCl_3$—TMS):

ppm	multiplicity	integration	attribution
1.4 to 3.4	m	8 H	twice: $H_{2'}$, $H_{3'}$, $H_{5'}$ and $H_{6'}$
3.65	s	2 H	CH_2 of phenylacetyl group
4.6	s	1 H	H_3
5.2	s	2 H	CH_2 of benzyl group
5.4 to 5.8	m	2 H	H_5 and H_6
6 to 6.5	m	1 H	NH
7.4	d	10 H	two phenyl groups

(d = doublet)

Mass spectrum: M^+ at m/e 498



at m/e 324

I.2. Preparation of (2" - phenylacetamido) - spiro[oxa(or thia) - cycloalkane-penam]-carboxylic acids.

a) 6 - (2" - Phenylacetamido) - 2', 3', 5', 6' - tetrahydro - spiro - [penam - 2,4' - [4H]-pyran] - 3 - carboxylic acid (potassium salt).

466 mg. (0.001 mole) Benzyl 6-(2"-phenylacetamido)-2', 3', 5', 6'-tetrahydro-spiro[penam-2,4'-[4H]pyran]-3-carboxylate are dissolved in 50 ml. ethyl acetate. Hydrogenolysis is carried out under a pressure of 3 kg. hydrogen in the presence of 700 mg. Pd/C catalyst containing 10% of palladium. The catalyst is filtered off and rinsed with a little ethanol and then the filtrate is evaporated to dryness. The residue is dissolved in 50 ml. dichloromethane, followed by the addition of 50 ml. water. The aqueous phase is brought to pH 7.2 with a dilute aqueous solution of potassium hydroxide, while stirring, and then decanted and finally lyophilised to give 370 mg. (0.000788 mole; yield: 78.8% of theory) potassium 6-(2"-phenylacetamido)-2', 3', 5', 6'-tetrahydro-spiro[penam-2,4'-[4H]-pyran]-3-carboxylate monohydrate; m.p. 218—219°C.

Analysis for $C_{18}H_{20}KN_2O_5S.H_2O$ (M.W. = 433)

calculated:	C 50.0%;	H 4.86%;	N 6.48%
found:	49.2%;	4.87%;	6.50%

Infra-red spectrum (KBr) in cm^{-1}:

3460	H_2O
3250	NH

2940 2842	CH and CH_2
1750	CO β-lactam
1647	CO amide
1600	CO of COOK group
715 690	monosubstituted phenyl

b) 6 - (2″ - Phenylacetamido) - 2′, 3′, 5′, 6′ - tetrahydro - spiro - [penam - 2,4′ - [4H]-thiopyran] - 3 - carboxylic acid (potassium salt).

This compound is prepared in the same manner as the preceding one. Yield: 50% of theory; m.p. 160—167°C.

Analysis for $C_{18}H_{20}KN_2O_4S_2.4H_2O$ (M.W. = 502)

calculated:	C 43.0%;	H 5.38%;	N 5.58%
found:	42.83%;	4.42%;	5.55%

c) The sodium salts corresponding to the potassium salts prepared hereinabove were prepared in the same way as the latter. The same method was used to prepare the sodium and potassium salts of the (2″-phenylacetamido)-spiro[oxa-(or thia)cycloalkane-penam]-carboxylic acids from the corresponding benzyl esters described in l.c) above.

d) 6 - (2″ - Phenylacetamido) - 2′, 3′, 5′, 6′ - tetrahydro - spiro - [penam - 2, 4′ - [4H]-thiopyran] - 3 - carboxylic acid 1 - oxide (potassium salt).

This compound is prepared in the same manner from the corresponding benzyl ester described in l.d.) above; m.p. 177—203°C. (decomposition).

Infra-red spectrum (KBr) in cm^{-1}:

1750	CO β-lactam
1650	CO amide
1590	CO of COOK group

N.M.R. (D_2O-sodium dimethyl-2,2-sila-2-pentanesulphonate (DSS))

ppm	multiplicity	integration	attribution
1.5 to 3.4	m	8 H	twice: $H_{2'}$, $H_{3'}$, $H_{5'}$ and $H_{6'}$
3.65	s	2 H	CH_2 of phenylacetyl group
4.35	d	1 H	H_3
5 to 5.8	m	2 H	H_5 and H_6
7.35	s	5 H	one phenyl group

Example II.

Preparation of (2" - amino - 2" - phenylacetamido) - spiro [oxa(or thia)cycloalkane-penam] - carboxylic acids and their derivatives.

II.1. Preparation of benzyl (2" - amino - 2" - phenylacetamido) - spiro[oxa(or thia)-cycloalkane-penam] - carboxylates.

a) benzyl 6 - (2" - benzyloxycarbonylamino - 2" - phenylacetamido) - 2', 3', 5', 6' - tetrahydro - spiro[penam - 2,4' - [4H]pyran] - 3 - carboxylate.

1.56 g. (0.003 mole) benzyl 6-amino-2', 3', 5', 6'-tetrahydro-spiro[penam-2,4'-[4H]pyran]-3-carboxylate *p*-toluenesulphonate (prepared as described in Example I.1.a) and 303 mg. (0.003 mole) triethylamine are mixed in 60 ml. dichloromethane. The light yellow solution obtained is cooled to between 0 and −5°C. and 939 mg. (0.0034 mole) N-(benzyloxycarbonyl)-D(-)-2-phenylglycine and 678 mg. dicyclohexylcarbodiimide are then added all at once. After stirring for 1 hour in the cold, the reaction mixture is allowed to return to ambient temperature overnight. After filtering off the N,N'-dicyclohexylurea formed, the organic phase is washed successively with dilute hydrochloric acid, with a 5% aqueous solution of sodium bicarbonate and finally with water. After drying, it is evaporated to dryness to give 2.8 g. of crude product. This product is purified by chromatography on a silica column (eluent: chloroform). In this way, there is obtained 1.6 g. (yield 86.7% of theory) benzyl 6-(2"-benzyloxycarbonylamino-2"-phenylacetamido)-2', 3', 5', 6'-tetrahydro-spiro[penam-2,4'-[4H]pyran]-3-carboxylate; m.p. 58—59°C.

Analysis for $C_{33}H_{33}N_3O_7S$ (M.W. = 615)

calculated:	C 64.39%;	H 5.37%;	N 6.83%
found:	64.42%;	5.45%;	6.71%

Infra-red spectrum (KBr) in cm^{-1}:

3310	NH
2945, 2850	CH_2 and CH
1775	CO β-lactam
1733	CO ester
1675	CO amide
1230	ester
745, 692	monosubstituted phenyl

b) Benzyl 6 - (2" - benzyloxycarbonylamino - 2" - phenylacetamido) - 2', 3', 5', 6' - tetrahydro - spiro[penam - 2,4' - [4H]thiopyran] - 3 - carboxylate.

This compound is obtained, in a yield of 95% of theory, in the same manner as the preceding one from benzyl 6-amino-2', 3', 5', 6'-tetrahydro-spiro[penam-2,4'-[4H]thiopyran]-3-carboxylate *p*-toluenesulphonate (prepared as described in Example I.1.b); m.p. 66—690°C.

c) Benzyl 6 - (2" - benzyloxycarbonylamino - 2" - phenylacetamido) - 2', 3', 5', 6' - tetrahydro-spiro[penam - 2, 4' - [4H]thiopyran] - 3 - carboxylate 1' - oxide.

2.5 g. (0.0039 mole) Benzyl 6-(2"-benzyloxycarbonylamino-2"-phenylacetamido)-2', 3', 5', 6'-tetrahydro-spiro[penam-2,4'-[4H]thiopyran]-3-carboxylate (prepared as described in I.b.) above) are dissolved in 18 ml. chloroform. The solution is cooled in an ice-bath and 757 mg. (0.0037 mole) *m*-

chloro-perbenzoic acid (85%) in 12 ml. chloroform are added thereto within the course of 30 minutes. The solution is stirred for 30 minutes and then washed successively with a 5% aqueous sodium bicarbonate solution and twice with water. After drying the organic phase and evaporating off the solvent, 2.6 g. benzyl 6-(2″-benzyloxycarbonylamino-2″-phenylacetamido)-2′, 3′, 5′, 6′-tetrahydro-spiro[penam-2,4′-[4H]thiopyran]-3-carboxylate 1′-oxide are obtained. This product gives a single spot in a thin layer chromatogram on silica:

eluent	Rf
acetone	0.8
ethyl acetate	0.5 to 0.6
98:2 chloroform/methanol	0.4

Infra-red spectrum (KBr) in cm^{-1}:

3350	NH
3060, 2900	CH_2 and CH
1775	CO β-lactam
1720	CO ester
1675	CO amide
1490	NH amide
1040	SO
695	monosubstituted phenyl

N.M.R. ($CDCl_3$–TMS):

ppm	multiplicity	integration	attribution
1.2 to 3.1	m	8 H	twice: $H_{2'}$, $H_{3'}$, $H_{5'}$ and $H_{6'}$
4.6	s	1 H	H_3
5.1 and 5.2	2 s	4 H	two CH_2 of the benzyl groups
5.2 to 5.7	m	3 H	H_5, H_6 and $H_{2''}$
7.1 to 7.7	m	15 H	three phenyl groups

II.2 Preparation of (2″ - amino - 2″ - phenylacetamido) - spiro - [oxa(or thia)-cycloalkane - penam] - carboxylic acids.

a) 6 - (2″ - Amino - 2″ - phenylacetamido) - 2′, 3′, 5′, 6′ - tetrahydrospiro[penam - 2,4′-[4H]pyran] - 3 carboxylic acid.

1.5 g. (0.0024 mole) benzyl 6-(2″-benzyloxycarbonyl-amino-2″-phenylacetamido)-2′, 3′, 5′, 6′-tetrahydro-spiro-[penam-2,4′-[4H]pyran]-3-carboxylate are dissolved in 3 ml. ethyl acetate, followed by dilution with 300 ml. 96% ethanol. 5 g. Pd/C catalyst containing 10% of palladium are added and

hydrogenolysis is carried out under a pressure of 3 kg. hydrogen for 30 minutes. After filtering off the catalyst and evaporating off the ethanol under reduced pressure at 30 to 35°C., the residue is taken up in water.

This aqueous phase is extracted with ethyl acetate and then lyophilised to give 490 mg. (0.0012 mole; yield: 50% of theory) 6-(2″-amino-2″-phenylacetamido)-2′, 3′, 5′, 6′-tetrahydro-spiro[penam-2,4′-[4H]pyran]-3-carboxylic acid; m.p. 188—200°C.

Infra-red spectrum (KBr) in cm^{-1}:

3390	NH_2
2930, 2880	CH_2
1764	CO β-lactam
1680	CO amide
720, 690	monosubstituted phenyl

N.M.R. (DMSO–TMS):

ppm	multiplicity	integration	attribution
1 to 2.2	m	4 H	two $H_{2'}$ and two $H_{6'}$
2.7 to 4.2	m	4 H	two $H_{3'}$ and two $H_{5'}$
4.5	s	1 H	H_3
5.1 to 5.6	m	2 H	H_5 and H_6
7.5	s	5 H	one phenyl group

b) 6 - (2″-Amino-2″-phenylacetamido)-2′, 3′, 5′, 6′ - tetrahydrospiro[penam - 2, 4′-[4H]thiopyran] - 3 - carboxylic acid.

This compound is obtained in the same manner as the preceding one from benzyl 6-(2″-benzyloxycarbonylamino-2″-phenylacetamido)-2′, 3′, 5′, 6′-tetrahydro-spiro[penam-2,4-[4H]thiopyran]-3-carboxylate; m.p. 188—190°C.

Infra-red spectrum (KBr) in cm^{-1}:

3400	NH_2
2900	CH_2
1770	CO β-lactam
1690	CO amide
695	monosubstituted phenyl

c) 6 - (2″ - Amino - 2″ - phenylacetamido) - 2′, 3′, 5′, 6′ - tetrahydrospiro[penam - 2,4′-[4H]thiopyran] - 3 - carboxylic acid 1 - oxide.

This compound is prepared, in a 44% yield, in the same manner as the two preceding ones from benzyl 6-(2″-benzyloxycarbonylamino-2″-phenylacetamido)-2′, 3′, 5′, 6′-tetrahydrospiro[penam-2,4′-[4H]thiopyran]-3-carboxylate 1′-oxide (described in l.c) above); m.p. 190—194°C.

Infra-red spectrum (KBr) in cm^{-1}:

3390	NH_2
2920, 2820	CH_2
1770	CO β-lactam
1675	CO amide
1500	$COO\ominus$
695	monosubstituted phenyl

d) The following acids are prepared in the same manner:

6' - (2" - amino - 2" - phenylacetamido) - 4,5 - dihydro - spiro[furan - 3(2H), 2' - penam]-3' - carboxylic acid;
6 - (2" - amino - 2" - phenylacetamido) - 4', 5' - dihydro - spiro[penam - 2,3' (2'H)-thiophene] - 3 - carboxylic acid;
6' - (2" - amino - 2" - phenylacetamido) - spiro[oxetane - 3,2' - penam] - 3' - carboxylic acid;
6 - (2' - amino - 2" - phenylacetamido) - spiro[penam - 2,3' - thietane] - 3 - carboxylic acid.

Example III.

Preparation of (5" - methyl - 3" - phenyl - 4" - isoxazolecarboxamido) - spiro[oxa(or thia)cycloalkane - penam] - carboxylic acids and their derivatives.

a) 6 - (5" - Methyl - 3" - phenyl - 4" - isoxazolecarboxamido) - 2', 3', 5', 6' - tetrahydro-spiro[penam - 2,4' - [4H]pyran] - 3 - carboxylic acid (sodium salt).

2 ml. hexamethyldisilazane are added to a suspension of 840 mg. (0.00325 mole) 6-amino-2', 3', 5', 6'-tetrahydrospiro[penam-2,4'-[4H]pyran]-3-carboxylic acid (prepared by the method described in Example 6.1 of co-pending Patent Application No. 1905/77) (Serial No. 1567561) in 23 ml. anhydrous chloroform. The mixture is heated under reflux for 2 hours, dissolution being rapid. The solvent is distilled off *in vacuo* and the residue is taken up in 5 ml. chloroform and 5 ml. anhydrous dioxan. After cooling to 0°C., 325 mg. (0.00325 mole) triethylamine in 1 ml. chloroform are added. To this solution are added 720 mg. (0.00325 mole) 5-methyl-3-phenyl-4-isoxazolecarbonyl chloride in an anhydrous solvent mixture consisting of 3 ml. chloroform and 3 ml. dioxan, followed by stirring for 30 minutes at about °C. The reaction mixture is allowed to return to ambient temperature, a small amount of insoluble material is filtered off and 2 ml. ethanol are added. Stirring is continued for 20 minutes and then 414 mg. (0.003 mole) sodium 2-ethyl-butyrate in 5 ml. isopropanol are added. The reaction mixture is allowed to stand for 2 hours and then 50 ml. anhydrous diethyl ether are added. There immediately appears a white precipitate, which filtered off. There are thus obtained 700 mg. sodi9um 6-(5"-methyl-3"-phenyl-4"-isoxazolecarboxamido)-2', 3', 5', 6'-tetrahydrospiro-[penam-2,4'-[4H]pyran]-3-carboxylate dihydrate (yield 47% of theory); m.p. 198—203°C. (decomposition).

Analysis for $C_{21}H_{20}N_3NaO_6S.2H_2O$ (M.W. = 501)

calculated:	C 50.29%	H 4.79%;	N 8.38%
found:	50.20%;	4.77%;	8.33%

Infra-red spectrum (KBr) in cm^{-1}:

3400	NH and H_2O
2950	CH_2 and CH_3

1760	CO β-lactam
1645	CO amide
1595	CO of COONa
764, 690	monosubstituted phenyl

N.M.R. (D_2O–DSS):

ppm	multiplicity	integration	attrition
1.2 to 2.5	m	4 H	two $H_{2'}$ and two $H_{6'}$
2.65	s	3 H	CH_3 at 5"
3.2 to 4.4	m	4 H	two $H_{3'}$ and two $H_{5'}$
4.33	s	1 H	H_3
5.65	q (J = 4 cps)	2 H	H_5 and H_6
7.7	s	5 H	one phenyl group

b) In the same way, there are prepared the sodium salts of the following acids:

6 - (5" - methyl - 3" - phenyl - 4" - isoxazolecarboxamido) - 2', 3', 5', 6' - tetrahydro-spiro[penam - 2,4' - [4H]thiopyran] - 3 - carboxylic acid;
6' - (5" methyl - 3" - phenyl - 4" - isoxazolecarboxamido) - 4,5 - dihydro - spiro[furan-3 (2H), 2' - penam] - 3 carboxylic acid;
6 - (5" - methyl - 3" - phenyl - 4" - isoxazolecarboxamido) - 4',5' - dihydro - spiro-[penam - 2,3' (2'H) - thiophene] - 3 - carboxylic acid;
6' - (5" - methyl - 3" - phenyl - 4" - isoxazolecarboxamido) - spiro-[oxetane - 3,2'-penam] - 3' - carboxylic acid;
6 - (5" - methyl - 3" - phenyl - 4" -isoxazolecarboxamido) - spiro[penam - 2,3' - thietane] - 3 - carboxylic acid.

Example IV

Preparation of (2", 6" - dimethoxybenzamido) - spiro [oxa(or thia) - cycloalkane-penam] - carboxylic acids and their derivatives.

IV.1. Benzyl 6 - (2", 6" - dimethoxybenzamido) - 2', 3', 5', 6' - tetrahydro-spiro[penam-2,4' - [4H]thiopyran] - 3 - carboxylate.

470 mg. (0.0046 mole) triethylamine in 4 ml. dichloromethane are added to a suspension of 2.5 g. (0.0046 mole) benzyl 6-amino-2', 3', 5', 6'-tetrahydro-spiro[penam-2,4'-[4H]-thiopyran]-3-carboxylate *p*-toluenesulphonate (prepared as described in Example I.1.b)) in 40 ml. dichloromethane. The compound dissolves. A solution of 1.028 g. (0.0051 mole) 2,6-dimethoxybenzoyl chloride in 8 ml. dichloromethane and a solution of 518 mg. (0.0051 mole) triethylamine in 8 ml. dichloromethane are added successively thereto. The resulting solution is then successively washed with 0.05 N hydrochloric acid, with water, with an aqueous 5% sodium bicarbonate solution and again with water. After drying and evaporating off the solvent, a residue of 2 g. benzyl 6-(2",6"-dimethoxybenzamido)-2', 3', 5', 6'-tetrahydro-spiro-[penam-2,4'-[4H]thiopyran]-3-carboxylate is obtained. This compound is recrystallised from diethyl ether; m.p. 187—190°C.

Analysis for $C_{26}H_{28}N_2O_6S_2$ (M.W. = 528)

calculated:	C 59.0%;	H 5.30%;	N 5.30%
found:	58.89%;	5.28%;	5.32%

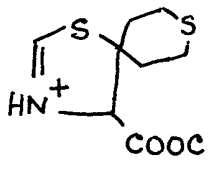
Infra-red spectrum (KBr) in cm^{-1}:

3380	NH
2850	CH_3
1775	CO β-lactam
1740	CO ester
1675	CO amide
1515	NH amide
695	monosubstituted phenyl

N.M.R. ($CDCl_3$–TMS)

ppm	multiplicity	integration	attribution
1.6 to 3.1	m	8 H	twice: $H_{2'}$, $H_{3'}$, $H_{5'}$ and $H_{6'}$
3.82	s	6 H	H of the two methoxy groups
4.5	s	1 H	H_3
5.22	s	2 H	CH_2 of benzyl group
5.5 to 6.1	m	2 H	H_5 and H_6
6.5 to 6.8	m	3 H	NH + $H_{3''}$ and $H_{5''}$
7.1 to 7.6	m	6 H	one phenyl group + $H_{4''}$

Mass spectrum = $M^{\vee}$ at m/e 528



at m/e 308

IV.2.a) 6 - (2", 6" - Dimethoxybenzamido) - 2', 3', 5', 6', - tetrahydrospiro[penam - 2,4' - [4H]thiopyran] - 3 - carboxylic acid (sodium salt).

This compound is obtained using the process described in Example I.2.c); m.p. 180—200°C. (decomposition). Infra-red spectrum (KBr) in cm^{-1}:

2830	CH_3
1765	CO β-lactam
1660	CO amide
1590	$COO^{\ominus}$

N.M.R. (D_2—DSS):

ppm	multiplicity	integration	attribution
1.8 to 3.2	m	8 H	twice: $H_{2'}$ $H_{3'}$ $H_{5'}$ and $H_{6'}$
3.85	s	6 H	H of the two methoxy groups
4.28	s	1 H	H_3
4.7 to 5.62	m	2 H	H_5 and H_6
6.5 to 7.7	m	3 H	$H_{3''}$ $H_{4''}$ and $H_{5''}$

b) The sodium salts of the following acids are prepared in the same manner:
6 - (2″, 6″ - dimethoxybenzamido) - 2′, 3′, 5′, 6′, - tetrahydro - spiro - [penam - 2,4′-[4H]pyran] - 3 - carboxylic acid;
6′ - (2″, 6″ - dimethoxybenzamido) - 4,5 - dihydro - spiro[fuuran - 3 (2H),2′ - penam]-3′ - carboxylic acid;
6 - (2″, 6″ - dimethoxybenzamido) - 4′, 5′ - dihydro - spiro [penam - 2,3′ (2′H)-thiophene] - 3 - carboxylic acid;
6′ - (2″, 6″ - dimethoxybenzamido) - spiro[oxetane - 3,2′ - penam] - 3′ - carboxylic acid;
6 - (2″, 6″ - dimethoxybenzamido) - spiro[penam - 2,3′ - thietane] - 3 - carboxylic acid.

Pharmacological properties.

Numerous comparative tests have been carried out concerning the intrinsic biological activity of the compounds of general formula (I) according to the present invention towards various bacterial strains of the Gram-positive and Gram-negative type. The reference compounds for the comparative tests were penicillin G, oxacillin, ampicillin and methicillin. Information is given below regarding the origin and characteristics of the bacterial strains employed:

A. Gram-positive bacterial strains.

Staphylococcus aureus 6538.

This is a Gram-positive coccus which is particularly sensitive to penicillins and which has a very low resistance mechanism. This strain of *Staphylococcus* is, therefore representative of a maximum sensitivity of the species.

Staphylococcus aureus 52149.

This is a Gram-positive coccus, the intrinsic sensitivity of the receptor of which is equivalent to that of the preceding strain but which produces a β-lactamase which is typical of the species and which renders it resistant to all the penicillins which are sensitive to hydrolysis.

B. Gram-negative bacterial strains.

Escherichia coli B.

This is a well known collection strain of *Escherichia coli* which produces very little β-lactamase (of type I) and is, therefore, very sensitive to penicillins. As regards the classification of the β-lactamases, use is here made of that proposed by M. H. Richmond and R. B. Sykes (see Advances in Microbial Physiology, 9, (1973), 43 and 45).

Escherichia coli B Ampi-R.

This is a mutant of the preceding strain, which we have produced. This strain is, however, a hyperproducer of β-lactamase of type I, already produced by the parent strain *Escherichia coli B*. It has an increased resistance to penicillins, which would appear to be directly connected with the production of β-lactamase.

Escherichia coli K 12—44.

This is a mutant of *Escherichia coli* K 12, the reference parent strain typical of the species. This mutant is not a producer of β-lactamase.

Escherichia coli K 12—44 S.

This is a pleiotropic mutant of *Escherichia coli* K 12—44, which does not produce β-lactamase and which we have produced. It is very sensitive to penicillins due to hyperpermeability.

Escherichia coli K 12—44 R.

This strain is a pleiotropic mutant of *Escherichia coli* K 12—44, which we have produced. It is not a producer of β-lactamase but it has a resistance to penicillins, probably as a result of the modifications of the permeability of the cell membranes.

C. Results of the comparative tests of activity.

For a certain number of compounds of general formula (I) according to the present invention, a determination was carried out of the minimum inhibitory concentration (abbreviated MIC) according to the procedure described below.

The products to be tested are introduced in increasing concentrations into a gelose culture medium in Petri dishes. A multiple inoculator is used simultaneously to deposit drops (10 microlitres) of inocolum (suspension of about 10^5 bacteria per ml.) on to the surface of the medium. After incubation at 37°C. for 24 hours, the growth of the bacteria is observed. By definition, the MIC is expressed as the minimum concentration of the tested compounds which inhibits multiplication of the bacteria. However, in the results which follow, the MIC is given as being equal to 1 for the reference compounds and the activity figures given for the tested compounds of general formula (I) are, therefore, relative values. This presentation of the result is more correct and the most reproducible because, for one and the same bacterial strain, different MIC values can be observed if they are measured at different times. This is bound up with the "seasonal" variations in the strains and their nutrient medium.

The compounds which were tested are as follows:—

Compound A:
6 - (2″ - phenylacetamido) - 2′, 3′, 5′, 6′ - tetrahydro - spiro[penam-2,4′ - [4H]pyran] - 3 - carboxylic acid (potassium salt) (I: $n = m = 2$, X = O, R_1 = H, R_2 = 2 - phenylacetyl):

Compound B:
6 - (2″ - amino - 2″ - phenylacetamido) - 2′, 3′, 5′, 6′ - tetrahydro - spiro[penam - 2,4′-[4H]pyran] - 3 - carboxylic acid (I: $n = m = 2$, X = O, R_1 = H, R_2 = 2 - amino - 2-phenylacetyl);

Compound C:
6 - (5″ - methyl - 3″ - phenyl - 4″ - isoxazolecarboxamido) - 2′, 3′, 5′, 6′ - tetrahydro - spiro[penam - 2,4′ - [4H]pyran] - 3 - carboxylic acid (sodium salt) (I: $n = m = 2$, X = O, R_1 = H, R_2 = 5 - methyl - 3 - phenyl - 4 - isoxazolecarbonyl);

Compound E:
6 - (2″ - phenylacetamido) - 2′, 3′, 5′, 6′ - tetrahydro - spiro [penam - 2,4′ - [4H]thiopyran] - 3 - carboxylic acid (potassium salt) (I: $n = m = 2$, X = S, R_1 = H, R_2 = 2-phenylacetyl);

Compound F:
6 - (2″ - amino - 2″ - phenylacetamido) - 2′, 3′, 5′, 6′ - tetrahydrospiro[penam - 1, 4′-[4H]thiopyran] - 3 - carboxylic acid (I: $n = m = 2$), X = S, R_1 = H, R_2 = 2 - amino - 2-phenylacetyl);

Compound G:
6 - (2″, 6″ - dimethoxybenzamido) - 2′, 3′, 5′, 6′ - tetrahydro - spiro - [penam - 2,4′-[4H]thiopyran] - 3 - carboxylic acid (sodium salt) (I: $n = m = 2$, X = S, R_1 = H, R_2 = 2,6 - dimethoxybenzoyl);

Compound H:
6 - (2″ - phenylacetamido) - 2′, 3′, 5′, 6′ - tetrahydro - spiro[penam - 2,4′ - [4H]thiopyran] - 3 - carboxylic acid 1′ - oxide (potassium salt) (I: $n = m = 2$, X = SO, R_1 = H, R_2 = 2 - phenylacetyl);

Compound J:
6 - (2″ - amino - 2″ - phenylacetamido) - 2′, 3′, 5′, 6′-tetrahydrospiro[penam - 2,4′-

[4H]thiopyran] - 3 - carboxylic acid 1' - oxide (I: $n = m = 2$, $X = SO$, $R_1 = H$, $R_2 = 2$-amino - 2 - phenylacetyl).

a) Comparative tests with penicillin G.

Strain used	Penicillin G	Compound A	Compound E	Compound H
S. aureus 6538	1	2	10	1
S. aureus 52149	1	2	1	1
E. coli B	1	4	5	1
E. coli B Ampi–R	1	2	>2	–

b) Comparative tests with oxacillin.

Strain used	Oxacillin	Compound C
S. aureus 6538	1	2
S. aureus 52149	1	1

c) Comparative tests with ampicillin.

Strain used	Ampicillin	Compound B	Compound F	Compound J
S. aureus 6538	1	1	2	2
E. coli B	1	3.5	7	1
E. coli B Ampi–R	1	1	3	2
E. coli K 12–44	1	3.5	10	3
E. coli K 12–44S	1	1.9	15	3
E. coli K 12–44R	1	1.5	>1.5	2

d) Comparative test with methicillin.

Strain used	Methicillin	Compound G
S. aureus 52149	1	0.5

It can be seen from these results that the compounds of general formula (I) according to the present invention have activities whch are comparable with those of the corresponding known penicillins. However, the essential interest of these new compounds comes forth from the following: the compounds of general formula (I) differ from the known penicillins by an increased resistance to β-lactamases, which are the deactivating enzymes hydrolysing the lactam function of the penicillanic ring.

This property of the compounds of the present invention is demonstrated by the results of the comparison of the hydrolysis kinetics of compound B with that of ampicillin, in the presence of two β-lactamases of Gram-negative bacteria. These results are set out in the following Table:

Residual activities (in micromoles) of ampicillin and Compound B in the presence of β-lactamases, as a function of time

β-lactamase TEM			β-lactamase P 99		
Incubation time (minutes)	Ampicillin	Compound B	Incubation time (minutes)	Ampicillin	Compound B
0	500	500	0	500	500
2		469	3	262	458
4		453	6	138	422
6	<50	438	9	<50	334
12	<50	334	12	<50	312
18	<50	225	20	<50	300

These results show that, after incubation for 6 minutes in β-lactamase TEM, the activity of ampicillin is no longer detectable, whilst there still remains an activity of compound B corresponding to 438 micromoles, i.e. 87%. The same observation was made in the case of β-lactamase P 99: after incubation for 9 minutes, the activity of ampicillin is no longer detectable, whereas there still remains an activity of compound B corresponding to 334 micromoles, i.e. 67%.

These results are also confirmed by the activity tests which have been described above. Indeed, it can be seen that between the activity of compound B on *E. coli* B and on its mutant, which is a hyperproducer of β-lactamase, *E. coli* B Ampi-R, there is a difference of a factor of 3.5.

An increased resistance to these β-lactamase also has been found for compound F. This is, however, less pronounced than in the case of compound B. No change in behaviour towards these β-lactamases has been detected for compound J, as compared with ampicillin.

A very pronounced increase in resistance fo β-lactamase TEM has also been found for compounds E and H, which are homologues of penicillin G. This property can be shown by measuring the rate of hydrolysis expressed in terms of the number of microliters of sodium hydroxide of a given concentration which are consumed per minute. This method allows the dosage of the penicilloic acid resulting from the hydrolysis of the β-lactam function by the lytic enzyme. The results of these kinetics, as given in the following Table, clearly show the superiority of compounds E and H as compared with penicillin G.

Compound	Microlitres 0.005 N NaOH consumed per minute
Penicillin G	3.83
Compound E	0.64
Compound H	0.50

This confirms the importance of the compounds of general formula (I), as compared with the known penicillins, particularly for combating the Gram-negative strains which produce β-lactamases.

D. *Posology and use.*

The compounds of general formula (I), like all penicillins, canbe administered orally or parenterally.

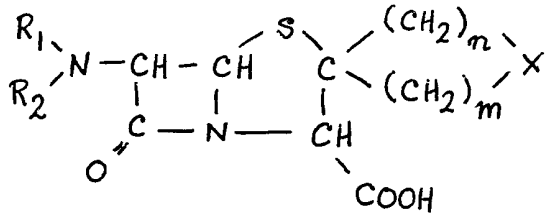
For example, the posology of compound A is between 0.4 and 6 g. per day, that of compound C between 4 and 8 g. per day, that of compound E between 2 and 10 g. per day and that of compound H between 0.2 and 3 g. per day. For the homologues of ampicillin (compounds B, F and J), an identical posology of 0.7 to 7 g. per day can be adopted; these doses can of course be adapted depending upon the patient and the disease to be treated.

Thus, the present invention also provides pharmaceutical compositions comprising at least one of the new compounds according to the present invention in admixture with a solid or liquid pharmaceutical diluent or carrier.

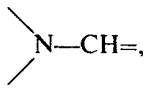
Furthermore, the present invention provides a "method of treating infectious diseases caused by Gram-positive and Gram-negative bacteria in non-human warm-blooded animals, which comprises administering to said animals a therapeutically effective amount of at least one of the new compounds according to the present invention.

WHAT WE CLAIM IS:—

1. Amino-spiro[oxa(or thia) cycloalkane-penam]-carboxylic acid derivatives of the general formula:—



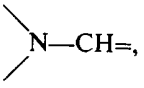
wherein X is a sulphur or oxygen atom or a sulphinyl group, *n* is 1 or 2, *m* is 1 or 2, R_1 is a hydrogen atom and R_2 is a monocarboxylic acyl radical selected from those known in the chemistry of the penicillins, or R_1 and R_2 together represent a bivalent aminoethylene radical



and the pharmaceutically-acceptable non-toxic salts thereof.

2. A compound according to claim 1, wherein R_2 is a 2-phenylacetyl, 2-amino-2-phenylacetyl, 5-methyl-3-phenyl-4-isoxazolecarbonyl or 2,6-dimethoxybenzoyl radical.

3. A compound according to claim 1, wherein the aminoethylene radical



is a (hexahydro-1H-azepin-1-yl)-methylene radical.

4. Potassium 6-(2″-phenylacetamido)-2′,3′,5′,6′-tetrahydro-spiro[penam-2,4′-[4H]pyran]-3-carboxylate.

5. Potassium 6-(2″-phenylacetamido)-2′,3′,5′,6′-tetrahydro-spiro[penam-2,4′-[4H]thiopyran]-3-carboxylate.

6. Potassium 6-(2″-phenylacetamido)-2′,3′,5′,6′-tetrahydro-spiro[penam-2,4′-[4H]thiopyran]-3-carboxylate 1′-oxide.

7. 6-(2″-Amino-2″-phenylacetamido)-2′,3′,5′,6′-tetrahydro-spiro[penam-2,4′-[4H]pyran]-3-carboxylic acid.

8. 6-(2″-Amino-2″-phenylacetamido)-2′,3′,5′,6′-tetrahydro-spiro[penam-2,4′-[4H]thiopyran-3-carboxylic acid.

9. 6-(2″-Amino-2″-phenylacetamido)-2′,3′,5′,6′-tetrahydro-spiro[penam-2,4′-[4H]thiopyran]-3-carboxylic acid 1′-oxide.

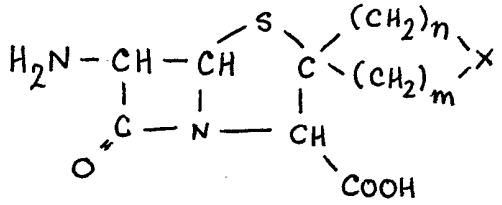
10. Sodium 6-(5″-methyl-3″ phenyl-4″-isoxazole carboxamido)-2′,3′,5′,6-tetrahydro-spiro[penam-2,4′-[4H]thiopyran]-3-carboxylate.

11. sodium 6-(2″,6″-dimethoxy benzoxamido)-2′,3′,5′,6′-tetrahydro-spiro-[penam-2,4′-[4H]-pyran]-3-carboxylate.

12. Sodium 6-(5″-methyl-3″-phenyl-4″-isoxazolecarboxamido)-2′,3′,5′,6′-tetrahydro-spiro[penam-2,4′-[4H]pyran]-3-carboxylate.

13. Sodium 6-(2″,6″-dimethoxybenzamido)-2′,3′,5′,6′-tetrahydro-spiro-[penam-2,4′-[4H]thiopyran]-3-carboxylate.

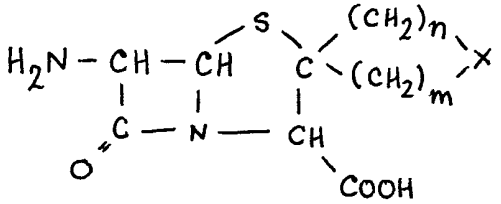
14. A process of preparing compound according to claim 1, in which X is a sulphur or oxygen atom, n is 1 or 2, m is 1 or 2, R_1 is hydrogen and R_2 is a monocarboxylic acyl radical selected from those known in the chemistry of penicillins, which comprises reacting an amino-spiro[oxa(or thia)cycloalkane-penam]-carboxylic acid of the general formula:—



wherein X, n and m have the same meanings as above, with a halide of a monocarboxylic acid of the general formula R_2OH or a functional equivalent thereof, R_2 having the same meaning as above.

15. A process according to claim 14, wherein R_2 is a 2-phenylacetyl, 2-amino-2-phenylacetyl, 5-methyl-3-phenyl-4-isoxazolecarbonyl or 2,6-dimethoxybenzoyl radical.

16. A process of preparing compounds according to claim 1, in which X is a sulphur or oxygen atom, n is 1 or 2, m is 1 or 2, R_1 is hydrogen and R_2 is a monocarboxylic acyl radical selected from those known in the chemistry of penicillins, which comprises (a) reacting an ester of an amino-spiro[oxa(or thia)cycloalkane-penam]-carboxylic acid of the general formula:—



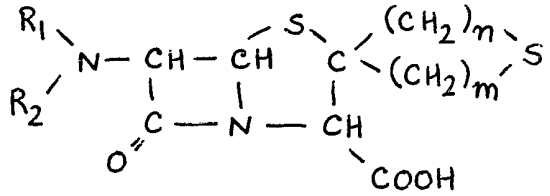
wherein X, n and m have the meanings given above, with a halide of a monocarboxylic acid of the general formula R_2OH or a functional equivalent thereof, R_2 having the same meaning as above, and

(b) converting the resulting ester into the corresponding acid by hydrogenolysis.

17. A process according to claim 16, wherein the ester of the amino-spiro[oxa(or thia)cycloalkane-penam]carboxylic acid is a benzyl ester.

18. A process according to claim 16 or 17, wherein R_2 is a 2-phenylacetyl, 2-amino-2-phenylacetyl, 5-methyl-3-phenyl-4-isoxazolecarbonyl or 2,6-dimethoxybenzoyl radical.

19. A process of preparing compounds according to claim 1, in which X is a sulphinyl group and *n*, *m*, R_1 and R_2 have the meanings as in claim 1, which comprises subjecting an amino-spiro[thiacycloalkane-penam]-carboxylic acid derivative of the general formula:—

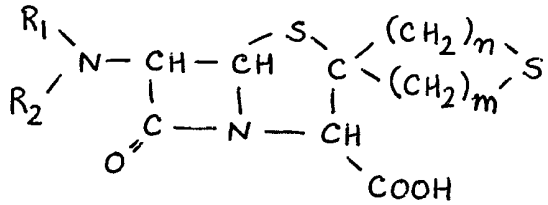


wherein *n*, *m* R_1 and R_2 have the same meanings as in claim 1, to an oxidation by means of an organic peracid.

20. A process according to claim 19, wherein R_1 is a hydrogen atom and R_2 is a 2-phenylacetyl, 2-amino-2-phenylacetyl, 5-methyl-3-phenyl-4-isoxazolecarbonyl or 2,6-dimethoxybenzoyl radical.

21. A process of preparing compounds according to claim 1, in which X is a sulphinyl group and *n*, *m*, R_1 and R_2 have the same meanings as in claim 1, which comprises

(a) subjecting an ester of an amino-spiro[thiacycloalkane-penam]-carboxylic acid derivative of the general formula:—



wherein *n*, *m*, R_1 and R_2 have the same meanings as in claim 1, to an oxidation by means of an organic peracid, and

(b) converting the resulting ester into corresponding acid by hydrogenolysis.

22. A process according to claim 21, wherein the ester of the amino-spiro[thiacycloalkane-penam]-carboxylic acid derivative is a benzyl ester.

23. A process according to claim 21 or 22, wherein R_1 is a hydrogen atom and R_2 is a 2-phenylacetyl, 2-amino-2-phenylacetyl, 5-methyl-3-phenyl-4-isoxazolecarbonyl or 2,6-dimethoxybenzoyl radical.

24. A process of preparing compounds according to claim 1, substantially as hereinbefore described and exemplified.

25. Compounds according to claim 1 whenever prepared by the process according to any of claims 14 to 24.

26. Pharmaceutical compositions, comprising at least one compound according to claim 1, in admixture with a solid or liquid pharmaceutical diluent or carrier.

27. A method of treating infectious diseases caused by Gram-positive and Gram-negative bacteria in non-human warm-blooded animals, which comprises administering to said animals a therapeutically effective amount of at least one compound according to claim 1.

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