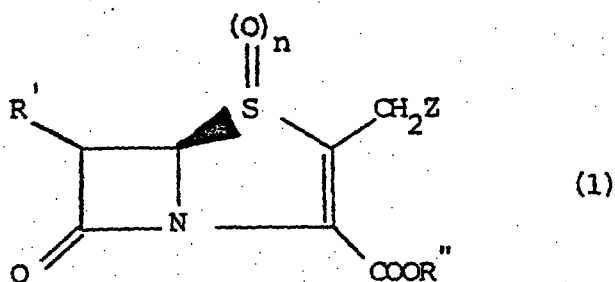


- 1 -

Antibacterial Agents

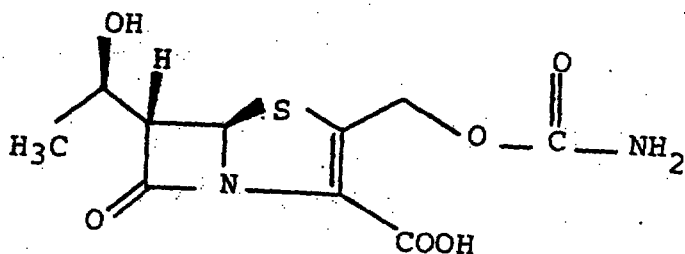
The invention relates to (5R)-2-carbamoyloxymethyl-6 α -
 5 -(1R-hydroxyethyl)-2-penem-3-carboxylic acid and
 its sodium salt.

Our British Patent Specification No. 2043639 describes
 penem-carboxylic acids and esters of the general
 formula (1)



10 wherein n is 0 or 1; R'' represents a hydrogen atom,
 an alkyl group having from 1 to 4 carbon atoms, a
 2,2,2-trichloroethyl, benzyl, acetonyl, p-nitrobenzyl,
 p-methoxybenzyl, phenyl, p-nitrophenyl, benzhydryl,
 acetoxymethyl, pivaloyloxymethyl or phthalidyl group
 15 or a group of the formula $-\text{CH}(\text{CH}_3)-\text{OCOOC}_2\text{H}_5$ or

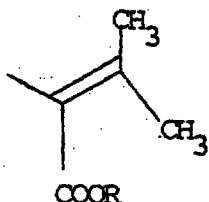
-CH₂NHCOR₂ wherein R₂ represents an alkyl group having from 1 to 4 carbon atoms or a cycloalkyl or aryl group; Z represents a hydroxy, amino, carbamoyloxy, mercapto or pyridimum group, or a group of the formula OR₁,
5 OCOR₁ or SR₃ wherein R₁ represents an alkyl group having from 1 to 4 carbon atoms and R₃ represents a 5-methyl-
-1,3,4-thiadiazol-2-yl, 1-methyl-1H-tetrazol-5-yl,
1,2,3-triazol-5-yl or pyrazinyl group; and R' represents
a hydrogen atom or an alkyl, alkoxy or hydroxyalkyl
10 group, each of which has from 1 to 4 carbon atoms, the alcoholic function of the hydroxyalkyl group being free or protected by a p-nitrobenzyloxycarbonyl or diethyl-
-t-butyl-silyloxy group.
We have now found that one of these compounds, namely the aforesaid (5R)-2-carbamoyloxymethyl-6α-(1R-
15 -hydroxyethyl)-2-penem-3-carboxylic acid, the compound in which n is 0, R'' represents a hydrogen atom, Z represents a carbamoyloxy group and R' represents a
1R-hydroxyethyl group in the α orientation, is,
together with its sodium salt, of particular value as
20 an antibacterial agent, having high and broad spectrum activity against bacterial strains which produce β-lactamases and against anerobic bacteria. This compound has the formula



The preparation of most compounds of the general formula I may be summarised by the following reaction scheme in which X represents a group of the formula $\text{CH}_2\text{Z}'$, Z' has any of the meanings ascribed to Z above except a mercapto or pyridinium group or a group of the formula SR_3 , Y represents a hydrogen atom, a lower alkyl, cyano or alkoxycarbonyl group or a group of the formula $\text{CH}_2\text{Z}'$, Ph represents a phenyl group and the other variables are as above defined.

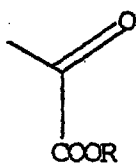
A penicillanic acid S-oxide ester (2) is reacted in an inert solvent at elevated temperature with a compound $\text{XC}\equiv\text{CY}$ and the resultant compound (3) is isomerised in basic conditions to the azetidinone (4). This is converted to the azetidinone (11) by seven steps:

(a) ozonolysis of the 1-substituent of the formula



in a solution at reduced temperature,

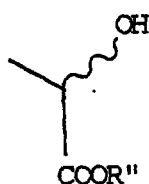
(b) removal of the 1-substituent of the formula



10 resulting from step (a) by mild alkaline hydrolysis or by the action of silica gel,

(c) condensation of the 1-unsubstituted azetidinone resulting from step (b) with a glyoxylate of the formula $\text{CHO.COOR}''$ by refluxing in a solvent,

5 (d) chlorination of the 1-substituent of the formula

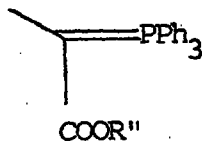


introduced in step (c) by the action of a chlorinating agent,

(e) conversion of the 1-substituent of the formula



10 introduced in step (d) into one of the formula

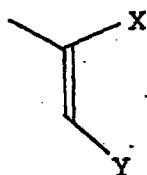


by reaction with triphenylphosphine,

(f) reduction of the 4 β -(substituted vinylsulphinyl) group by the action of a reducing agent, and

(g) ozonolysis of the double bond of the group

5 of the formula



in solution at reduced temperature.

These steps may be carried out in the order (a), (b), (c), (d), (e), (f), (g) or (a), (f), (b), (c),

10 (d), (e), (g) or (f), (a) and (g) simultaneously, (b), (c), (d), (e). The compound (11) is

cyclised by heating in an inert solvent at from 50°C to 140°C. A fuller description of this process may be found in our aforesaid British Patent

15 Specification No. 2043639, to which reference should be made.

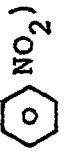
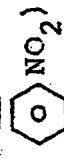
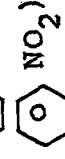
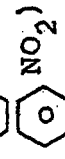
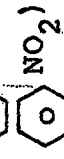
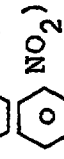
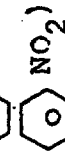
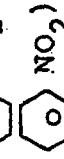
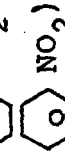
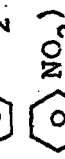
The compound of this invention, (5R)-2-carbamoyloxymethyl-
-6 α -(1R-hydroxyethyl)-2-penem-3-carboxylic acid, may

be prepared according to this general process.

The alcoholic function of the hydroxyethyl group in the 6-position in the final product is preferably protected throughout the reaction sequence, suitably
5 by a p-nitrobenzyloxy-carbonyl or dimethyl-t-butyl-silyloxy group. The carbamoyloxymethyl group in the 2-position in the final product need not be introduced only by the compound $XC \equiv CY$. An alternative
is to introduce a hydroxymethyl group and subsequently
10 react the hydroxy group thereof with trichloroacetyl-isocyanate followed by cleavage of the trichloroacetyl moiety, conveniently effected simultaneously with the reduction of the vinylsulphonyl group.

The preparation of the compound of the invention is
15 illustrated by the following Example. The compounds referred to therein have the formulae as set out in Table 1 hereunder.

TABLE 1

	Formula	R	R' (α -orientation)	R''	X	Y	n
2A	(2)	CH ₃	(R)-CH ₃ CH(O.CO.OCH ₂ )	-	-	-	-
3A	(3)	CH ₃	(R)-CH ₃ CH(O.CO.OCH ₂ )	-	CH ₂ OH	H	-
4A	(4)	CH ₃	(R)-CH ₃ CH(O.CO.OCH ₂ )	-	CH ₂ OH	H	-
4B	(4)	CH ₃	(R)-CH ₃ CH(O.CO.OCH ₂ )	-	CH ₂ OCONHCOCl ₃	H	-
12A	(12)	CH ₃	(R)-CH ₃ CH(O.CO.OCH ₂ )	-	CH ₂ OCONH ₂	H	-
13A	(13)	CH ₃	(R)-CH ₃ CH(O.CO.OCH ₂ )	-	CH ₂ OCONH ₂	-	-
14A	(14)	-	(R)-CH ₃ CH(O.CO.OCH ₂ )	-	CH ₂ OCONH ₂	-	-
15A	(15)	-	(R)-CH ₃ CH(O.CO.OCH ₂ )	CH ₂ COCH ₃	CH ₂ OCONH ₂	-	-
16A	(16)	-	(R)-CH ₃ CH(O.CO.OCH ₂ )	CH ₂ COCH ₃	CH ₂ OCONH ₂	-	-
11A	(11)	-	(R)-CH ₃ CH(O.CO.OCH ₂ )	CH ₂ COCH ₃	CH ₂ OCONH ₂	-	-
1A	(1)	-	(R)-CH ₃ CH(O.CO.OCH ₂ )	CH ₂ COCH ₃	CH ₂ OCONH ₂	-	0
1B	(1)	-	(R)-CH ₃ CH(OH)	CH ₂ COCH ₃	CH ₂ OCONH ₂	-	0
1C	(1)	-	(R)-CH ₃ CH(OH)	H	CH ₂ OCONH ₂	-	0
1D	(1)	-	(R)-CH ₃ CH(OH)	Na	CH ₂ OCONH ₂	-	0

A solution of 2.6g of methyl (1R—p-nitrobenzyloxy
-carbonyloxyethyl)-3-penicillinate-S-oxide (2A),
prepared as described in Example 34 of British Patent
Specification No. 2043639, and 8 ml of propargyl alcohol
5 in 20 ml of toluene was refluxed under nitrogen for
40 hours. The solvent was evaporated off and the
product was purified by silica gel column chromatography,
eluting with dichloromethane: ethyl acetate (9:1 by
volume), to give 2.0g of 4β-(1-hydroxymethylvinylsulphiny)-
10 -3α-(1R-p-nitrobenzyloxycarbonyloxyethyl)-1-(1-methoxy-
carbonyl-2-methyl-2-propenyl)-azetidin-2-one (3A).

2.0g of compound (3A) were dissolved in 50 ml of
dichlormethane and a few drops of triethylamine were
added. The mixture was left at room temperature for
15 12 hours, and the solvent was then evaporated off to
give 4β-(1-hydroxymethyl-vinylsulphiny)-3α-(1R—p-
-nitrobenzyloxycarbonyloxyethyl)-1-(1-methoxycarbonyl-2-
-methyl-1-propenyl)-azetidin-2-one (4A) in
quantitative yield.

20 2.0g of compound (4A) were dissolved in 30 ml of acetone
and cooled to 0°C. 0.8 ml of trichloroacetylisocyanate
were added under nitrogen and the mixture was stirred
for 5 minutes. The solvent was evaporated off, giving
2g of 4β-[I-(N-trichloroacetyl-carbamoyloxymethyl)-
25 -vinylsulphiny]-3α-(1R—p-nitrobenzyloxycarbonyl-

oxyethyl)-1-(1-methoxycarbonyl-2-methyl-1-propenyl)-
-azetidin-2-one (4B).

2.2g of compound (4B) were dissolved in 50 ml of
dimethylformamide. After cooling to -20°C , 0.7 ml
5 of pyridine and 3.2. ml of phosphorus tribromide were
added and the mixture was stirred for 15 minutes. Ethyl
acetate was added and the organic phase was separated
off, shaken with a saturated solution of sodium
bicarbonate, washed with water and dried over anhydrous
10 sodium sulphate. The ethyl acetate was evaporated off
and the product was dissolved in methanol and stirred
over silica gel for a few hours. The silica gel was
filtered off and the methanol evaporated off to give
1.5 g of 4β -(1-carbamoyloxymethylvinylthio)- 3α -(1R-
15 -p-nitrobenzyloxycarbonyloxyethyl)-1-(methoxycarbonyl-2-
-methyl-1-propenyl)-azetidin-2-one (12A).

2.2g of compound (12A) were dissolved in 250 ml of
dichloromethane and cooled to -78°C . Ozonized oxygen
was bubbled through the solution until a blue colour
20 appeared. A saturated solution of sodium bisulphite
was added and the temperature was raised to ambient
temperature. The solvents were evaporated off
to give 1.4g of 4β -carbamoyloxyacetylthio- 3α -(1R-p-
-nitrobenzyloxycarbonyloxyethyl)-1-methoxyoxalyl-
azetidin-2-one (13A).

1.4g of compound (13A) were dissolved in methanol:
ethyl acetate (1:1 by volume), a few grams of
silica gel were added and the mixture was maintained
at ambient temperature under vigorous stirring. After
5 filtering off the silica gel, the filtrate was evaporated
to leave an oil which chromatographed on silica gel
using dichlormethane:ethyl acetate (8:2 by volume)
giving 0.9g of 4 β -carbamoyloxyacetylthio-3 α -(1R -p-
-nitrobenzyloxycarbonyloxyethyl)-azetidin-2-one (14A)
10 in pure form.

0.9g of compound (14A) was dissolved in 40 ml of
benzene. 0.6g of acetonyl glyoxylate was added and
the solution was refluxed for 3 hours. The solvent
was evaporated off to give 4 β -carbamoyloxyacetylthio-
15 -3 α -(1R -p-nitrobenzyloxycarbonyloxyethyl)-1-(1-
-acetonilyloxycarbonyl-1-hydroxymethyl)-azetidin-2-one
(15A) as a crude oil. This was dissolved in 30ml
of anhydrous tetrahydrofuran and cooled to 0°C.
Equimolar amounts of pyridine and thionyl chloride were
20 added to the solution until the starting material had
been consumed. Insoluble material was filtered off.
The filtrate, containing 4 β -carbamoyloxyacetylthio-3 α -
-(1R -p-nitrobenzyloxycarbonyloxyethyl)-1-(1-acetonilyoxy-
carbonyl-1-chloromethyl)-azetidin-2-one (16A),

- was dissolved in 20ml of tetrahydrofuran. 700mg of triphenylphosphine and 0.35ml of pyridine were added and the solution was kept under nitrogen at 70°C for a few hours. Purification by silica gel chromatography, using dichloromethane:ethyl acetate (1:1 by volume) as eluant, gave 0.4g of 4β-carbamoyl-oxyacetylthio-3α-(1R-p-nitrobenzyloxycarbonyloxyethyl)-1-(1-acetonyloxycarbonyl-1-triphenylphosphoranylidenemethyl)-azetidin-2-one (11A).
- 5
- 10 0.4g of compound (11A) was dissolved in 50ml of toluene and refluxed under nitrogen for 3 hours. After purification by short column chromatography on silica gel, eluting with dichloromethane:ethyl acetate (8:2 by volume), there was obtained 0.11 g of acetonyl
- 15 (5R)-2-carbamoyloxymethyl-6α-(1R-p-nitrobenzyloxycarbonyloxyethyl)-2-penam-3-carboxylate (1A).

0.35g of compound (1A) was dissolved in 25 ml of acetonitrile containing a few drops of ethanol and hydrogenated over 400 mg of 10% palladium-on-carbon. The catalyst was filtered off. Silica gel

20 chromatography, using dichloromethane: ethyl acetate (7:3 by volume) as eluant, gave 0.11g of acetonyl

(5R)-2-carbamoyloxymethyl-6α-(1R-hydroxymethyl)-2-

-penem-3-carboxylate (1B).

A solution of 0.11g of compound (1B) in 30 ml of acetonitrile containing a few drops of water was cooled to 0°C. 5ml of 0.1 N sodium hydroxide solution were added under nitrogen and the mixture was stirred for 10 minutes. The mixture was extracted twice with dichloromethane, acidified with 10% aqueous citric acid, and twice further extracted with dichloromethane. The combined extracts were dried over anhydrous sodium sulphate and then evaporated to dryness to give 0.060g of (5R)-2-carbamoyloxymethyl-6 α -(1R-hydroxyethyl)-2-penem-3-carboxylic acid (1C). This was converted into its sodium salt (1D) by exchange with sodium ethylexanoate.

15	UV λ max (H ₂ O), nm	: -259 -308	(ϵ 3600) (ϵ 5400)
	PMR (D ₂ O), δ (ppm):	1.31 3.91 4.25 5.02 5.36 5.66	(d, J=6.5 Hz, 3H, CH ₃ CH) (dd, J=1.5 Hz, 6.0 Hz, 1H, H-6) (m, 1H, CHOH) (two d, 2H, CH ₂ OCO) (d, J=1.5 Hz, 1H, H-5)
20	$[\alpha]_D^{20}$	= +143° (c=0.97, H ₂ O)	

BIOLOGICAL ACTIVITY of sodium (5R)-2-carbamoyloxymethyl-
-6 α -(1R-hydroxyethyl)-2-penem-3-carboxylate (1D).

In vitro :MIC_s determined by the serial dilution
technique in nutrient medium.

Results : high and broad spectrum activity
also against strains producers of β -lactamase.

Relevant antibacterial activity against
anaerobes (Bacteroides, Clostridium).

In comparison with cefotaxime, compound
(1D) shows significantly high inhibiting
action against gram-positive strains and
anaerobes; higher resistance to some
 β lactamases. See Table 2.

In vivo :ED₅₀ determined in mice injected i.p. with
Staphylococcus aureus Smith and treated by
s.c. route 2 hours after injection.
ED₅₀ of compound (1D) was 0.6 mg/Kg
in comparison with cefotaxime 2.5 mg/Kg.

TABLE 2

S t r a i n s	Compound 1D MIC µg / ml	CEFOTAXIME
Escherichia coli B	0.39	0.011
Escherichia coli B cef.R	0.78	0.19
Escherichia coli O26:B6	0.18	0.78
Escherichia coli O26:B6 cef.R °°	0.78	1.56
Escherichia coli K ₁₂	0.39	0.011
Escherichia coli R6K (+)	0.78	0.023
Escherichia coli R P1(+)	0.18	0.045
Escherichia coli R 997	0.39	0.023
Enterobacter cloacae P ₉₉ °°	0.78	> 50
Klebsiella aerogenes 1082E °°	0.78	3.12
K. pneumoniae ATCC 10031	0.39	0.011
Salmonella abortusovae	0.18	0.011
Staphylococcus aureus 209P	0.018	1.56
Staphylococcus aureus PV3	0.018	0.78
Staphylococcus aureus Smith	0.018	0.78
Staphylococcus epidermidis SK 109	0.018	6.25
Listeria monocytogene ATCC 4428	0.18	1.56
Bacteroides fragilis ATCC 23745	0.12	6.25
Clostridium perfringens ATCC 13124	0.06	1.56

Remarks : ° = Producer of β lactamase type I

°° = Producer of β lactamase type IV

(+) = Producer of β lactamase type III



- 17 -

CLAIM

(5R)-2-carbamoyloxymethyl-6 α -(1R-hydroxyethyl)-2-
-penem-3-carboxylic acid or its sodium salt.