PATENT SPECIFICATION (11) 1 561 236

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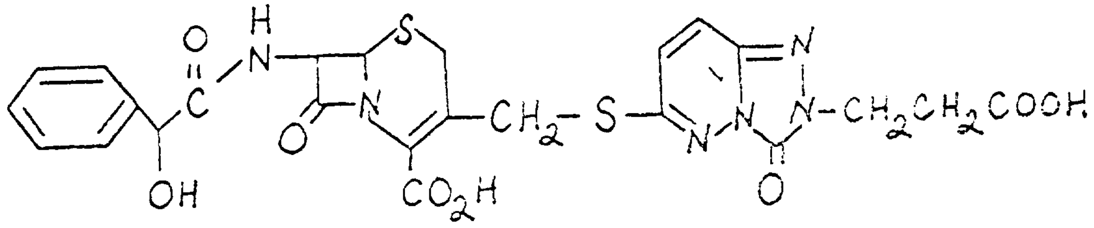
(21) Application No. 29212/77 (22) Filed 12 Jul. 1977 (19)
(31) Convention Application No's. 705226 (32) Filed 13 Jul. 1976
771859 24 Feb. 1977
777986 16 Mar. 1977
788056 15 Apr. 1977 in
(33) United States of America (US)
(44) Complete Specification Published 13 Feb. 1980
(51) INT. CL.^{3} C07D 501/48
A61K 31/545
(52) Index at Acceptance
C2C 1314 145X 1470 1510 214 215 220
226 22Y 246 247 250 252 253 254
256 25Y 28X 292 29Y 30Y 311 313
314 31Y 321 32Y 332 338 33Y 342
346 34Y 351 352 360 362 364 365
366 367 368 36Y 371 373 37Y 553
614 620 625 628 638 652 658 660

PATENTS ACT 1949

SPECIFICATION NO 1561236

The following corrections were allowed under Section 76 on 26 March 1980:

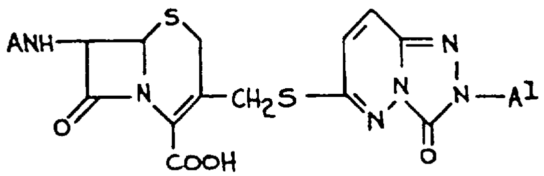
Page 40, lines 20 to 30, *delete* existing formula *insert*



THE PATENT OFFICE
30 April 1980

Bas 76194/5

sometimes hereinafter also written as



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(21) Application No. 29212/77 (22) Filed 12 Jul. 1977

(19) THE PATENT OFFICE LONDON

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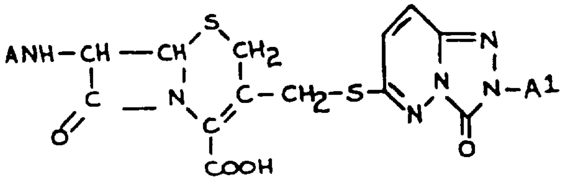
366 367 368 36Y 371 373 37Y 553
614 620 625 628 638 652 658 660
662 670 694 697 761 768 801 802
80Y AA QS RL RP

(72) Inventors: TAKAYUKI NAITO
JUN OKUMURA
HAJIME KAMACHI
SEIJI IIMURA

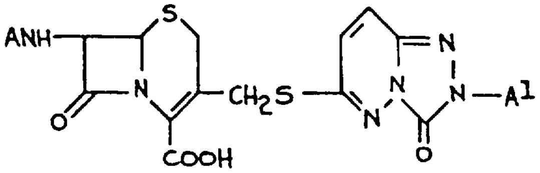
(54) CEPHALOSPORINS

(71) We, BRISTOL-MYERS COMPANY, a Corporation organised and existing under the laws of the State of Delaware, United States of America, having offices located at 345 Park Avenue, New York, New York 10022, United States of America, do hereby declare the invention for which we pray that a patent may be granted to us and the method by which it is to be performed to be particularly described in and by the following statement:-

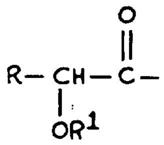
The present invention relates to novel acids having the structure


I

sometimes hereinafter also written as



wherein A is



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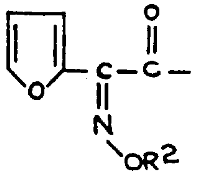
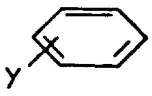


wherein R^1 is hydrogen or formyl,
R is



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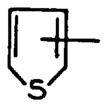
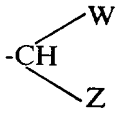


and Y is hydrogen, chlorine, bromine, fluorine, triflurormethyl, amino, nitro, hydroxy, (lower)alkyl of 1-4 carbon atoms or (lower)alkoxy of 1-4 carbon atoms,

R^2 is (lower)alkyl of 1-4 carbon atoms; and wherein A^1 is methyl or -$(CH_2)_nCOOH$ and n is one or two; the easily hydrolyzed esters and the non-toxic, pharmaceutically acceptable salts of those acids.

Said easily hydrolyzed esters of the acids of formula I include those having the group of the formula

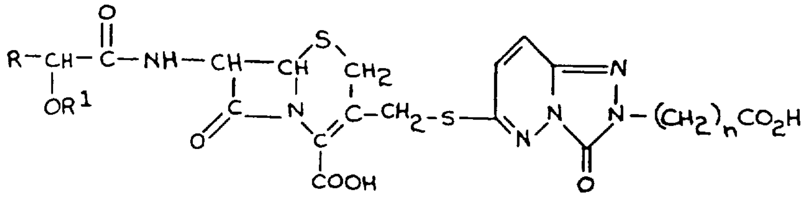


$$\text{-CH}\begin{smallmatrix}W\\ Z\end{smallmatrix}$$

wherein when W represents hydrogen, Z represents (lower)-alkanoyl, benzoyl, naphthoyl, furoyl, thenoyl, nitrobenzoyl, methylbenzoyl, halobenzoyl, phenyl-benzoyl, N-phthalimido, N-succinimido, N-saccharino, N-(lower)alkylcarbamoyl, (lower)alkoxy, (lower)-alkylthio, phenoxy, carbalkoxy, carbobenzoxy, carbamoyl, benzyloxy, chlorobenzyloxy, carbophenoxy, carbo-tert.-butoxy or (lower)alkylsulfonyl, and when W represents carbalkoxy, Z represents carbalkoxy and, when W represents phenyl, Z represents benzoyl or cyano or wherein W and Z taken together represent 2-oxocycloalkyl containing 4 to 8 carbon atoms inclusive. By "(lower)-alkanoyl", "(lower)alkylcarbamoyl", "(lower)alkoxy", "(lower)alkylthio" and "(lower)alkylsulfonyl" we mean alkanoyl, alkylcarbamoyl, alkoxy, alkylthio and alkylsulfonyl groups having up to 4 carbon atoms in the alkyl groups.

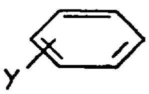
As set forth below in more detail the present invention also provides salts of these acids. The stereochemistry of the bicyclic nucleus is that found in Cephalosporin C.

A preferred embodiment of the present invention consists of the acids having the D configuration in the 7-side chain and the formula II

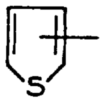


II

wherein n is one or two and R^1 is hydrogen or formyl and R is



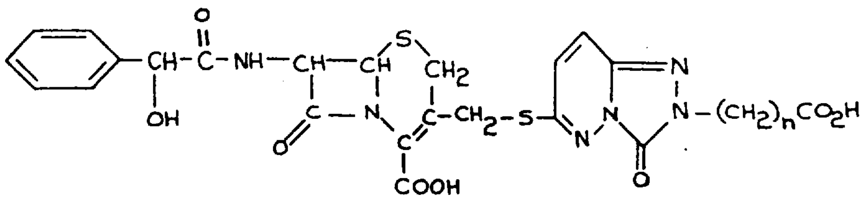
or



and Y is hydrogen, chlorine, bromine, fluorine, trifluoromethyl, amino, nitro, hydroxy, lower-alkyl or 1-4 carbon atoms or lower alkoxy of 1-4 carbon atoms and the nontoxic, pharmaceutically acceptable salts of those acids and the easily hydrolyzed esters of those acids including especially the pivaloyloxymethyl, acetoxymethyl, acetonyl, phenacyl and methoxymethyl esters and the silyl esters such as the trimethylsilyl ester. The invention also includes the benzaldehyde and salicylaldehyde Schiff bases or acetaldehyde or acetone derivatives of such compounds when they contain an amino group.

A further preferred embodiment of this invention consists of the compounds of formula II, wherein R is 2-thienyl, 3-thienyl, phenyl, chlorophenyl, bromophenyl, trifluoromethylphenyl, tolyl or methoxyphenyl.

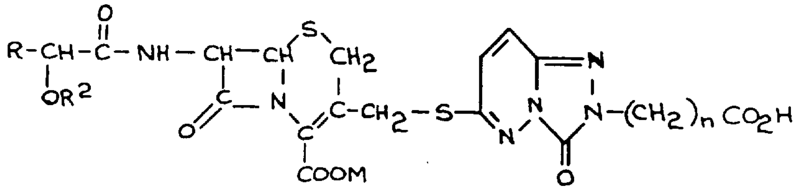
Particularly preferred embodiments of this invention comprises the acids having the D configuration in the 7-sidechain and the formula



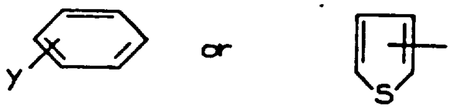
wherein n is one or two and their nontoxic, pharmaceutically acceptable salts and easily hydrolyzed esters.

Also included in this invention are the compounds (used as either intermediates or metabolic precursors) in which the α-hydroxy group is "blocked" by substituents such as dichloroacetyl (U.K. 962,024 and U.K. 1,328,340), formyl (U.S. 3,641,021), trimethylsilyl or tetrahydropyranyl (U.K. 1,328,340) and the term ester as used herein includes compounds having such blocked α-hydroxy groups.

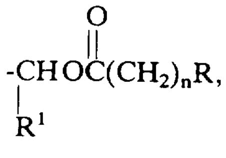
There is also provided by the present invention a compound having the formula



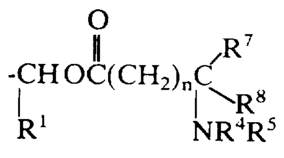
wherein n is one or two, R^2 is hydrogen or formyl and R is



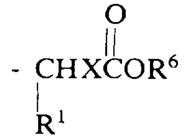
and Y is hydrogen, chlorine, bromine, fluorine, trifluoromethyl, amino, nitro, hydroxy, lower alkyl of 1-4 carbon atoms or lower alkoxy of 1-4 carbon atoms and M is



$$-\underset{R^1}{\underset{|}{CH}}O\overset{O}{\overset{\|}{C}}(CH_2)_nR,$$

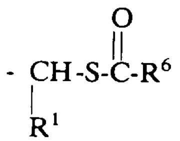


$$-\underset{R^1}{\underset{|}{CH}}O\overset{O}{\overset{\|}{C}}(CH_2)_n\underset{NR^4R^5}{\underset{|}{C}}(R^7)(R^8)$$



$$-\underset{R^1}{\underset{|}{CH}}X\overset{O}{\overset{\|}{C}}OR^6$$

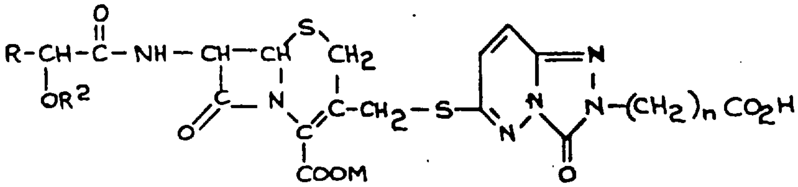
or



$$-\underset{R^1}{\underset{|}{CH}}\text{-}S\text{-}\overset{O}{\overset{\|}{C}}\text{-}R^6$$

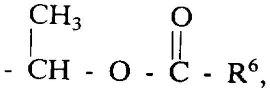
n is 0 to 4; R is hydrogen, alkyl having 1 to 8 carbon atoms, cycloalkyl of 3 to 6 carbon atoms, phenyl, C_1-C_4 phenalkyl, pyridyl, thienyl, or pyrrolyl; R^1 is hydrogen, methyl or ethyl; R^7 and R^8 are each hydrogen, alkyl having 1 to 6 carbon atoms, phenyl, pyridyl, or thienyl; R^4 and R^5 are each hydrogen or alkyl of 1 to 4 carbon atoms; R^6 is alkyl having 1 to 4 carbon atoms, phenyl, phenalkyl having 1 to 4 carbon atoms, pyridyl, thiadiazolyl, amino or C_1-C_4 alkylamino; X is NH or oxygen; and each phenyl group is unsubstituted or substituted with one or two substituents selected from alkyl having 1 to 6 carbon atoms, alkoxy having 1 to 4 carbon atoms, hydroxy, amino, NHR^1, $N(R^1)_2$, nitro, fluoro, chloro, bromo or carboxy, or a nontoxic, pharmaceutically acceptable salt thereof.

There is also provided by the present invention a compound having the formula

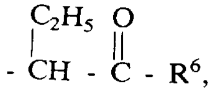


wherein n is one or two, R^2 is hydrogen or formyl and R is

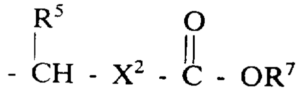
and Y is hydrogen, chlorine, bromine, fluorine, trifluoro-methyl, amino, nitro, hydroxy, lower alkyl of 1-4 carbon atoms or lower alkoxy of 1-4 carbon atoms and M is selected from



$$-\underset{}{\overset{CH_3}{\underset{|}{CH}}}-O-\overset{O}{\overset{||}{C}}-R^6,$$

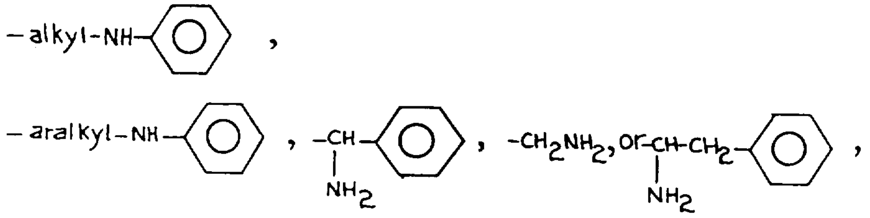


$$-\overset{C_2H_5}{CH}-\overset{O}{\overset{||}{C}}-R^6,$$



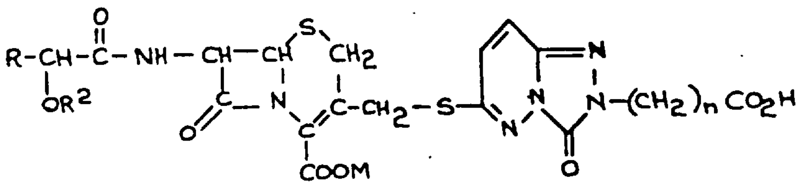
$$-\overset{R^5}{CH}-X^2-\overset{O}{\overset{||}{C}}-OR^7$$

wherein R^5 is a hydrogen atom, a methyl or an ethyl group; X^2 is -O-, -NH-; R^6 is a basic group such as alkyl or aralkyl substituted with substituted or unsubstituted NH_2, such as alkyl-$NHCH_3$, aralkyl-$NHCH_3$,



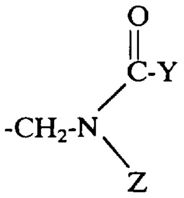
R^7 is an alkyl group such as a methyl, ethyl, propyl, isopropyl, butyl, isobutyl, pentyl or 2-ethyl-hexyl group; a cycloalkyl group such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl or cycloheptyl; an aryl group such as phenyl or naphthyl; an aralkyl group such as benzyl or naphthylmethyl; a heterocyclic group and wherein the alkyl, cycloalkyl, aryl, aralkyl and heterocyclic groups may be substituted with one or more groups selected from amino groups, substituted amino groups such as methylamino, diethylamino or acetamido groups, the halogen atoms such as fluorine, chlorine or bromine, the nitro group, alkoxy groups such as methoxy, ethoxy, propyloxy, isopropyloxy, butoxy or isobutoxy; or a nontoxic, pharmaceutically acceptable salt thereof.

There is also provided by the present invention a compound having the formula



wherein n is one or two, R^2 is hydrogen or formyl and R is

and Y is hydrogen, chlorine, bromine, fluorine, trifluoromethyl, amino, nitro, hydroxy, lower alkyl of 1-4 carbon atoms or lower alkoxy of 1-4 carbon atoms and M is



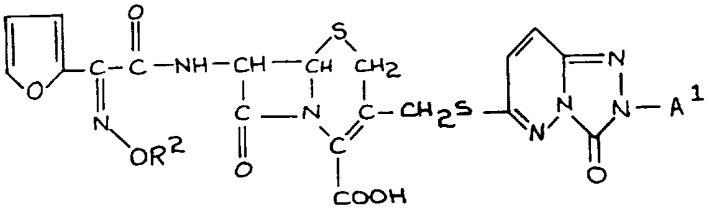
$$-CH_2-N\begin{matrix}\nearrow C(=O)-Y\\ \searrow Z\end{matrix}$$

wherein Y is alkyl of one to six carbon atoms, phenyl, benzyl, alkoxy of one to six carbon atoms, or benzyloxy; Z is alkyl of one to six carbon atoms, phenylbenzyl, alkoxy of one to six carbon atoms, cyclopentyl, cyclohexyl and phenyl, or Y+Z taken together are a 3-benzoxa-zolidine ring; or a nontoxic, pharmaceutically acceptable salt thereof.

Also included within the present invention are pharmaceutical compositions comprising a mixture of an antibacterially effective amount of a compound of the present invention and a semisynthetic penicillin or another cephalosporin or a cephamycin or a β-lactamase inhibitor or an aminoglycoside antibiotic.

In the treatment of bacterial infections in man, the compounds of this invention of formula II are administered parenterally, in accordance with conventional procedures for antibiotic administration, in an amount of from about 5 to 200 mg./kg./day and preferably about 5 to 20 mg./kg/day in divided dosage, e.g. three to four times a day. They are administered in dosage units containing, for example, 125, 250 or 500 mg. of active ingredient with suitable physiologically acceptable carriers or excipients. The dosage units are preferably in the form of liquid preparations such as solutions or suspensions.

Another preferred embodiment of this invention comprises the acids having the formula III



III

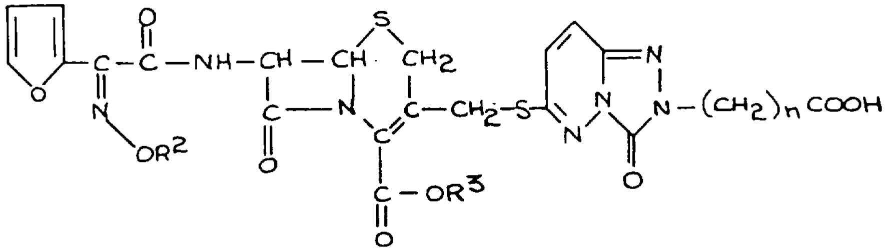
wherein A^1 is methyl or $-(CH_2)_nCOOH$ and n is one or two and R^2 is alkyl containing 1-4 carbon atoms, the easily hydrolyzed esters and the non-toxic pharmaceutically acceptable salts of those acids as hereinbefore set forth.

The compounds of formula III of the present invention are *syn* isomers or else are mixtures of *syn* and *anti* isomers containing at least 75% of the *syn* isomer. Preferably such mixtures of isomers contain at least 90% of the *syn* isomer and not more than 10% of the *anti* isomer. Most preferably the compounds of formula III are *syn* isomers essentially free of the corresponding *anti* isomer.

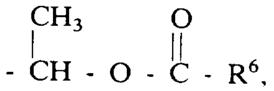
The preferred embodiments of the present invention are the *syn* isomers of the compounds of Formula III wherein R^2 is methyl or ethyl, n is one or two in its acid or pivaloyloxymethyl, acetoxymethyl, methoxymethyl, acetonyl, phenacyl, p-nitrobenzyl, β,β,β-trichloroethyl, 3-phthalidyl or 5-indanyl ester form.

Reference to the *syn* (*cis*) isomeric form refers to the configuration of the group OR^2 with respect to the carboxamido group.

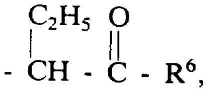
There is also provided by the present invention a compound having the formula



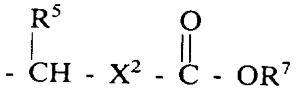
wherein R^2 is alkyl containing 1-4 carbon atoms, n is one or two and R^3 is selected from



$$-\overset{\text{CH}_3}{\overset{|}{\text{CH}}}-\text{O}-\overset{\text{O}}{\overset{\|}{\text{C}}}-R^6,$$

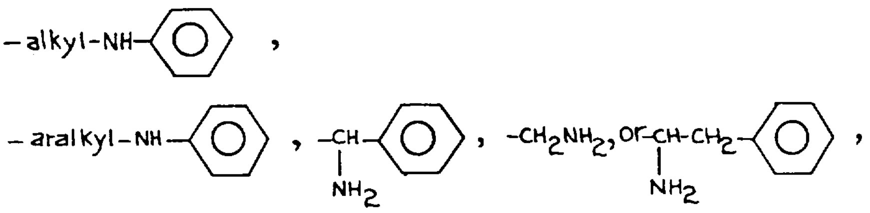


$$-\overset{\text{C}_2\text{H}_5}{\overset{|}{\text{CH}}}-\overset{\text{O}}{\overset{\|}{\text{C}}}-R^6,$$



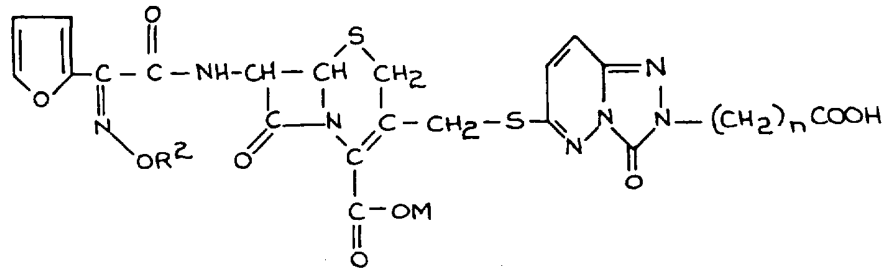
$$-\overset{R^5}{\overset{|}{\text{CH}}}-X^2-\overset{\text{O}}{\overset{\|}{\text{C}}}-\text{OR}^7$$

wherein R^5 is a hydrogen atom, a methyl or an ethyl group; X^2 is -O-, -NH-; R^6 is a basic group such as alkyl or aralkyl substituted with substituted or unsubstituted NH_2, such as alkyl-$NHCH_3$, aralkyl-$NHCH_3$,

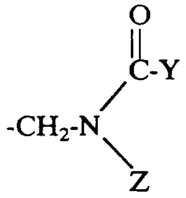


R^7 is an alkyl group such as a methyl, ethyl, propyl, isopropyl, butyl, isobutyl, pentyl or 2-ethyl-hexyl group; a cycloalkyl group such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl or cycloheptyl; an aryl group such as phenyl or naphthyl; an aralkyl group such as benzyl or naphthylmethyl; a heterocyclic group and wherein the alkyl, cycloalkyl, aryl, aralkyl and heterocyclic groups may be substituted with one or more groups selected from amino groups, substituted amino groups such as methylamino, diethylamino or acetamido groups, the halogen atoms such as fluorine, chlorine or bromine, the nitro group, alkoxy groups such as methoxy, ethoxy, propyloxy, isopropyloxy, butoxy or isobutoxy; or a nontoxic, pharmaceutically acceptable salt thereof, said compound being at least 75% by weight in the form of its *syn* isomer and preferably in the form of its *syn* isomer essentially free of the corresponding *anti* isomer.

There is also provided by the present invention a compound having the formula



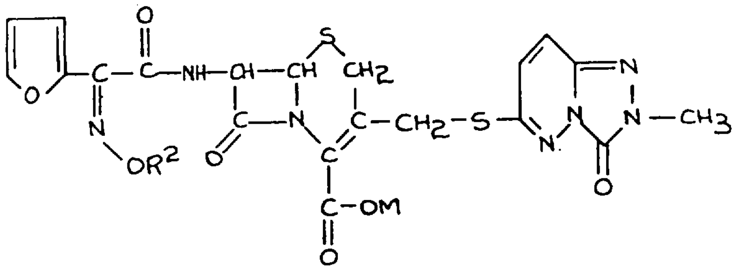
wherein R^2 is alkyl containing 1-4 carbon atoms, n is one or two and M is



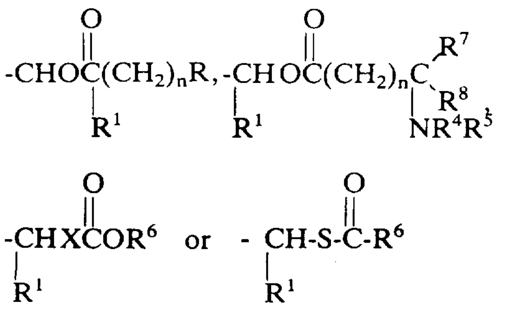
$$-CH_2-N\begin{matrix}\overset{O}{\overset{\|}{C}}-Y\\ Z\end{matrix}$$

wherein Y is alkyl of one to six carbon atoms, phenyl, benzyl, alkoxy of one to six carbon atoms, or benzyloxy; Z is alkyl of one to six carbon atoms, phenylbenzyl, alkoxy of one to six carbon atoms, cyclopentyl, cyclohexyl and phenyl, or Y+Z taken together are a 3-benzoxazolidine ring; or a nontoxic, pharmaceutically acceptable salt thereof, said compound being at least 75% by weight in the form of its *syn* isomer and preferably in the form of its *syn* isomer essentially free of the corresponding *anti* isomer.

There is also provided by the present invention a compound having the formula



wherein R^2 is alkyl containing 1-4 carbon atoms and M is



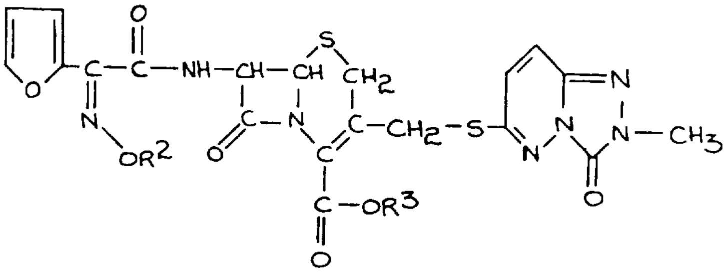
$$\underset{R^1}{-\underset{|}{CH}}O\overset{O}{\overset{\|}{C}}(CH_2)_nR,\ \underset{R^1}{-\underset{|}{CH}}O\overset{O}{\overset{\|}{C}}(CH_2)_n\underset{NR^4R^5}{\underset{|}{C}}\begin{matrix}R^7\\ R^8\end{matrix}$$

$$\underset{R^1}{-\underset{|}{CH}}X\overset{O}{\overset{\|}{C}}OR^6 \text{ or } -\underset{R^1}{\underset{|}{CH}}\text{-S-}\overset{O}{\overset{\|}{C}}\text{-}R^6$$

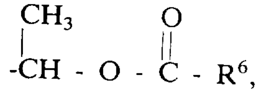
n is 0 to 4; R is hydrogen, alkyl having 1 to 8 carbon atoms, cycloalkyl of 3 to 6 carbon atoms, phenyl, C_1-C_4 phenalkyl, pyridyl, thienyl, or pyrrolyl:

R^1 is hydrogen, methyl or ethyl; R^7 and R^8 are each hydrogen, alkyl having 1 to 6 carbon atoms, phenyl, pyridyl, or thienyl; R^4 and R^5 are each hydrogen or alkyl of 1 to 4 carbon atoms; R^6 is alkyl having 1 to 4 carbon atoms, phenyl, phenalkyl having 1 to 4 carbon atoms, pyridyl, thiadiazolyl, amino or C_1-C_4 alkylamino; X is NH or oxygen; and each phenyl group is unsubstituted or substituted with one or two substituents selected from alkyl having 1 to 6 carbon atoms, alkoxy having 1 to 4 carbon atoms, hydroxy, amino, NHR^1, $N(R^1)_2$, nitro, fluoro, chloro, bromo or carboxy, or a nontoxic, pharmaceutically acceptable salt thereof, said compound being at least 75% by weight in the form of its *syn* isomer and preferably in the form of its *syn* isomer essentially free of the corresponding *anti* isomer.

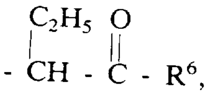
There is also provided by the present invention a compound having the formula



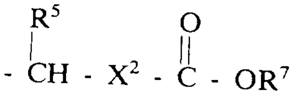
wherein R^2 is alkyl containing 1-4 carbon atoms and R^3 is selected from the group consisting of



$$\begin{array}{l} CH_3 \quad\quad O \\ | \quad\quad\quad\quad \| \\ -CH - O - C - R^6, \end{array}$$

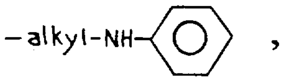


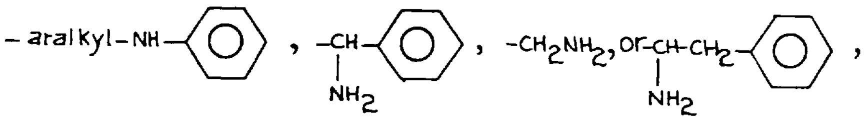
$$\begin{array}{l} C_2H_5 \;\; O \\ | \quad\quad\quad \| \\ -CH - C - R^6, \end{array}$$



$$\begin{array}{l} R^5 \quad\quad\quad O \\ | \quad\quad\quad\quad \| \\ -CH - X^2 - C - OR^7 \end{array}$$

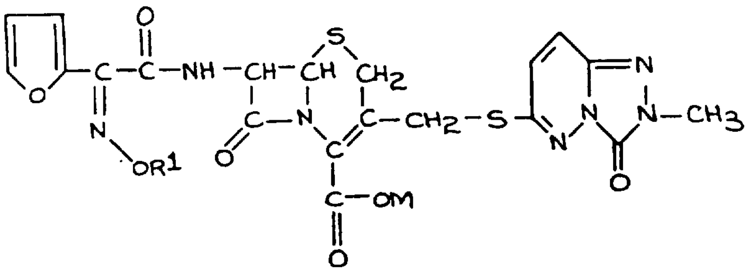
wherein R^5 is a hydrogen atom, a methyl or an ethyl group; X^2 is -O-, -NH-; R^6 is a basic group such as alkyl or aralkyl substituted with substituted or unsubstituted NH_2, such as alkyl-$NHCH_3$, aralkyl-$NHCH_3$,



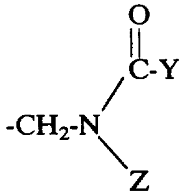


R^7 is an alkyl group such as a methyl, ethyl, propyl, isopropyl, butyl, isobutyl, pentyl or 2-ethyl-hexyl group; a cycloalkyl group such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl or cycloheptyl; an aryl group such as phenyl or naphthyl; an aralkyl group such as benzyl or naphthylmethyl; a heterocyclic group and wherein the alkyl, cycloalkyl, aryl, aralkyl and heterocyclic groups may be substituted with one or more groups selected from of amino groups, substituted amino groups such as methylamino, diethylamino or acetamido groups, the halogen atoms such as fluorine, chlorine or bromine, the nitro group, alkoxy groups such as methoxy, ethoxy, propyloxy, isopropyloxy, butoxy or isobutoxy; or a nontoxic, pharmaceutically acceptable salt thereof, said compound being at least 75% by weight in the form of its *syn* isomer and preferably in the form of its *syn* isomer essentially free of the corresponding *anti* isomer.

There is also provided by the present invention a compound having the formula



wherein R^2 is alkyl containing 1-4 carbon atoms and M is

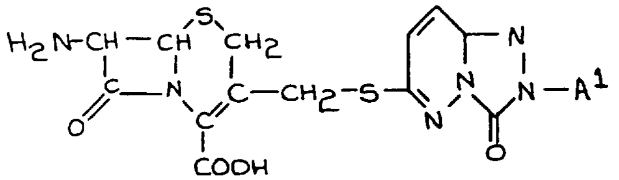


wherein Y is alkyl of one to six carbon atoms, phenyl, benzyl, alkoxy of one to six carbon atoms, or benzyloxy; Z is alkyl of one to six carbon atoms, phenylbenzyl, alkoxy of one to six carbon atoms, cyclopentyl, cyclohexyl and phenyl, or Y+Z taken together are a 3-benzoxa-zolidine ring; or a nontoxic, pharmaceutically acceptable salt thereof, said compound being at least 75% by weight in the form of its *syn* isomer and preferably in the form of its *syn* isomer essentially free of the corresponding *anti* isomer.

In the treatment of bacterial infections in man, the compounds of this invention are administered parenterally in an amount of from about 10 to 90 mg./kg./day and preferably about 14 to 50 mg./kg./day in divided dosage, e.g. two to four times a day. They are administered in dosage units containing, for example, 125, 250 or 500 mg. of active ingredient with suitable physiologically acceptable carriers or excepients. The dosage units are in the form of liquid preparations such as solutions or suspensions and preferably are aqueous solutions of a sodium or potassium salt which are injected intravenously or intramuscularly or by continuous or intermittent infusion in concentrations of about 125-500 mgm./ml., and preferably, 250 mgm./ml. as is customary in therapy with cephalosporin antibiotics.

It was an unexpected finding that the leading compound of the present invention (BB-S510; see below) having a 2-methyl substituent on the triazolopyridazinone showed *in vitro* antibacterial potency considerably superior to that of the corresponding compound lacking such methyl group (BB-S515; see below).

The present invention also provides a process for the production of the antibacterial agents of formula I which comprises reacting a compound of the formula



IV

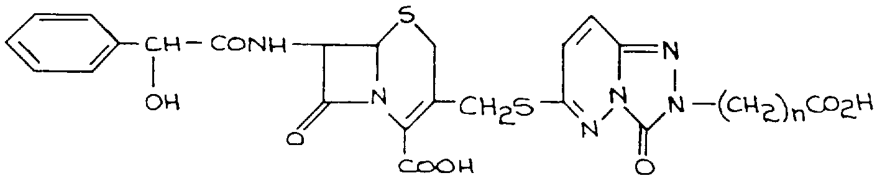
where A^1 is as hereinbefore defined or a salt or easily hydrolyzed ester of Schiff base as with benzaldehyde or salicylaldehyde thereof (including, but not limited to, those of U.S. 3,284,451 and U.K. 1,229,453 and any of the silyl esters described in U.S. patent 3,249,622 for use with 7-aminopenicillanic acid and used in Great Britain 1,073,530 and particularly the pivaloyloxymethyl, acetoxymethyl, methoxymethyl, acetonyl, phenacyl, p-nitrobenzyl, β,β,β-trichloroethyl, 3-phthalidyl and 5-indanyl esters) thereof with an organic monocarboxylic acid chloride or a functional equivalent thereof as an acylating agent corresponding to the acid AOH.

Such functional equivalents include the corresponding acid anhydrides, including mixed anhydrides and particularly the mixed anhydrides prepared from stronger acids such as the lower aliphatic monoesters of carbonic acid, or alkyl and aryl sulfonic acids and of more hindered acids such as diphenylacetic acid. In addition, an acid azide or an active ester or thioester (e.g. with p-nitrophenyl, 2,4-dinitrophenol, thiophenol, thioacetic acid) may be used or the free acid itself may be coupled with compound IV after first reacting said free acid with N,N'-dimethylchloroformiminium chloride [cf. Great Britain 1,008,170 and Novak and Weichet, *Experientia XXI*, 6, 360 (1965)] or by the use of enzymes or of an N,N'-carbonyl-diimidazole or an N,N'-carbonylditriazole [cf. South African patent specification 63/2684] or a carbodiimide reagent [especially N,N'-dicyclohexylcarbodiimide, N,N'-diisopropylcarbodiimide or N-cyclo-hexyl-N'-(2-morpholinoethyl)carbodiimide; cf. Sheehan and Hess, *J. Amer. Chem. Soc.*, *77*, 1967 (1955)], or of alkylylamine reagent [cf. R. Buijle and H.G. Viehe, *Angew, Chem. International Edition 3*, 582, (1964)] or of an isoxazolium salt reagent [cf. R. B. Woodward, R. A. Olofson and H. Mayer, *J. Amer. Chem. Soc.*, *83*, 1010 (1961)], or of a ketenimine reagent [cf. C. L. Stevens and M. F. Munk, *J. Amer. Chem. Soc.*, *80*, 4065 (1958)] or of hexachlorocyclotriphosphatriazine or hexabromocyclotriphosphatriazine (U.S. 3,651,050) or of diphenylphosphoryl azide [DPPA; *J. Amer. Chem. Soc.*, *94*, 6203-6205 (1972)] or of diethylphosphoryl cyanide [DEPC; Tetrahedron Letters No. 18, pp. 1595-1598 (1973)] or of diphenyl phosphite [Tetrahedron Letters No. 49, pp. 5047-5050 (1972)]. Another equivalent of the acid chloride is a corresponding azolide, i.e., an amide of the corresponding acid whose amide nitrogen is a member of a quasiaromatic five membered ring containing at least two nitrogen atoms, i.e., imidazole, pyrazole, the triazoles, benzimidazole, benzotriazole and their substituted derivatives. As an example of the general method for the preparation of an azolide, N,N'-carbonyl-diimidazole is reacted with a carboxylic acid in equimolar proportions at room temperature in tetra-hydrofuran, chloroform, dimethylformamide or a similar inert solvent to form the carboxylic acid imidazolide in practically quantitative yield with liberation of carbon dioxide and one mole of imidazole. Dicarboxylic acids yield dimidazolide. The by-product, imidazole, precipitates and may be separated and the imidazolide isolated, but this is not essential. The methods for carrying out these reactions to produce a cephalosporin and the methods used to isolate the cephalosporin so produced are well known in the art.

Mention was made above of the use of enzymes to couple the free acid with compound IV. Included in the scope of such processes are the use of an ester, e.g. the methyl ester, of that free acid with enzymes provided by various micro-organisms, e.g. those described by T. Takahashi et al., J. Amer. Chem. Soc., *94(11)*, 4035-4037 (1972) and by T. Nara et al., J. Antibiotics (Japan) *24(5)*, 321-323 (1971) and in U.S. 3,682,777.

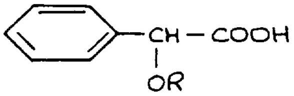
For the coupling of the organic carboxylic acid as described above with compound IV (or a salt or preferably an easily hydrolyzed ester of Schiff base, as with benzaldehyde, thereof) it is also convenient and efficient to utilize as the coupling agent phosphonitrilic chloride trimer (J. Org. Chem., *33(7)*, 2979-81, 1968) or N-ethoxy-1,2-dihydroquinoline (EEDQ) as described in J. Amer. Chem. Soc., *90*, 823-824 and 1652-1653 (1968) and U.S. Patent 3,455,929. The reaction is preferably carried out at 30-35°C. in benzene, ethanol or tetrahydrofuran using about equimolar quantities of all three reagents followed by conventional isolation and removal by conventional methods of any blocking groups present.

One process of the present invention stated more specifically in the process for the preparation of a product having the D-configuration in the sidechain and the formula



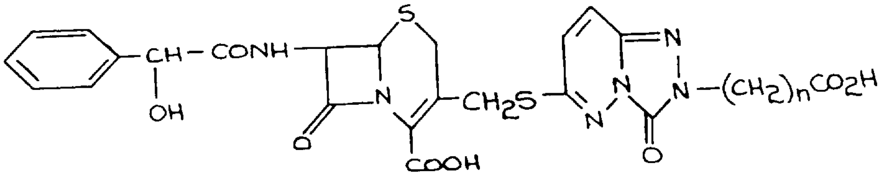
wherein n is one or two or a salt thereof which comprises the consecutive steps of

a. preparing an acylating derivative of D-mandelic acid having the formula



wherein the hydroxyl blocking group R represents dichloroacetyl, silyl and preferably trimethylsilyl, tetra-hydropyranyl or, preferably, formyl in an anhydrous organic solvent such as benzene, ethanol or preferably tetrahydrofuran, at room temperature or below and preferably at about 5° C;

b. mixing therewith, preferably slowly, a solution at about the same temperature in a solvent, preferably aqueous tetrahydrofuran, containing substantially the same number of moles of a tertiary amine, preferably a tertiary alkylamine such as triethylamine and substantially the same number of moles of 7-amino-3-(2-carboxymethyl or 2-carboxyethyl-2,3-dihydro-s-triazolo[4,3-b]-pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid or a salt or an easily hydrolyzed Schiff base, as with benzaldehyde, thereof to produce the intermediate acid having the formula



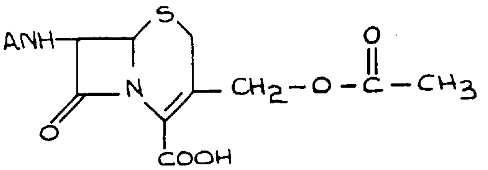
wherein n is one or two or a salt thereof wherein R has the meaning set out above; and

c. removing said hydroxyl blocking group R by conventional chemical methods to produce said product or salt thereof.

In preferred embodiments of the present invention R represents formyl which is removed in step C by treatment with aqueous alkali such as aqueous sodium bicarbonate or R represents dechloroacetyl which is removed in step C by alkaline hydrolysis, preferably at about pH 9-10, or R represents trimethylsilyl which is removed in step C by exposure to aqueous acid.

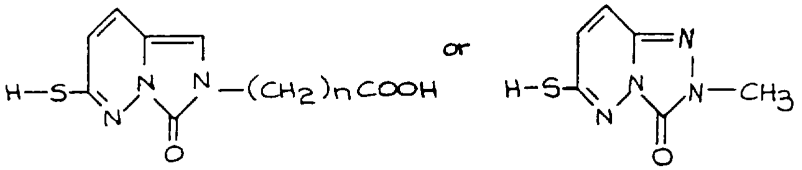
Other compounds of formula II are made in like manner.

An additional process of the present invention comprises the preparation of the compounds of the present invention by the displacement of the 3-acetoxy group from a cephalosporanic acid of the formula



V

where A is as defined hereinbefore above, prepared by substituting 7-amino cephalosporanic acid for the 3-thiolated-7-amino cephalosporanic acids in the acylation procedures described herein and elsewhere reported with the appropriate thiol HSR^3 having the formula



where n is one or two and then removing the protecting group if any is present as on the carboxyl group.

The displacement of such a 3-acetoxy group with such a thiol may be accomplished in solution as in water or aqueous acetone at a temperature of at least room temperature and preferably within the range of about 50° to 100°C. in the presence of a mild base such as sodium bicarbonate, e.g. preferably near neutrality such as at about pH 6. An excess of the thiol is preferably employed. The reaction product is isolated by careful acidification of the reaction mixture followed by extraction with a water-immiscible organic solvent. As noted above, the preparation of many other 7-acylamidocephalosporanic acids is described in the patent and scientific literature, e.g. in U.S. Class 260-243C.

The salts of the compounds of this invention include the nontoxic carboxylic acid salts thereof, including non-toxic metallic salts such as sodium, potassium, calcium and aluminum, the ammonium salt and substituted ammonium salts, e.g. salts of such nontoxic amines as trialkyl-amines including triethylamine, procaine, dibenzylamine, N-benzyl-beta-phenethylamine, 1-ephenamine, N,N'-dibenzylethylenediamine, dehydroabietylamine, N,N'-bis-dehydroabietylethylenediamine, and other amines which have been used to form salts with benzylpenicillin, L-lysine, arginine and histidine.

The preferred esters of the cephalosporins of the present invention are the pivaloyloxymethyl, acetoxymethyl, methoxymethyl, acetonyl and phenacyl esters. All are useful intermediates in the production of the cephalosporin having a free carboxyl group.

As indicated above, these five esters of 7-amino-cephalosporanic acid are each prepared by known methods. One excellent procedure is that of U.S. patent 3,284,451 in which sodium cephalothin is esterified by reaction with the corresponding active chloro or bromo compound (e.g. phenacyl bromide, chloroacetone, chloromethyl ether, pivaloyloxymethyl chloride [also called chloromethyl pivalate], acetoxymethyl chloride) and then the thienylacetic acid sidechain is removed enzymatically as in the same patent or chemically as in U.S. patent 3,575,970 and in Journal of Antibiotics, XXIV (11), 767-773 (1971). In another good method the triethylamine salt of 7-aminocephalosporanic acid is reacted directly with the active halogen compound, as in United Kingdom 1,229,453.

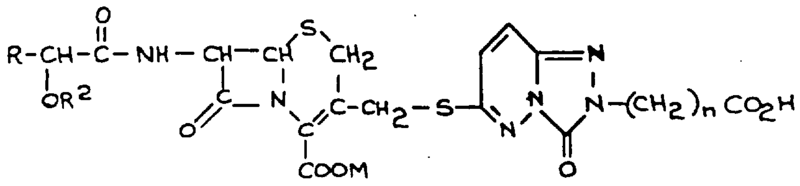
These esters of 7-aminocephalosporanic acid are then reacted with the nucleophile HSR^3 in the same manner as is illustrated herein for 7-aminocephalosporanic acid itself. The 3-thiolated ester of 7-aminocephalosporanic acid is then coupled with the organic carboxylic acid as before.

The ester of the cephalosporin so obtained is, if not used *per se*, converted to its free acid and, if desired, any salt by removal of the esterifying group, as by aqueous or enzymatic hydrolysis (as with human or animal serum) or acidic or alkaline hydrolysis or by treatment with sodium thiophenoxide as taught in U.S. 3,284,451 and, in the penicillin series, by Sheehan et al., J. Org. Chem. *29(7)*, 2006-2008 (1964).

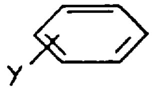
The cephalosporins obtained by the above method, when they contain an amino group, can be converted to the corresponding benzaldehyde or salicylaldehyde Schiff base, or, with acetaldehyde or acetone to the corresponding derivatives.

In another alternative synthesis, the 3-thiolated 7-aminocephalosporanic acid is prepared as described herein and then acylated at the 7-amino group and finally esterified, as by reaction of the appropriate alcohol with the acid chloride prepared, for example, by reaction of the final cephalosporin with thionyl chloride or by other essentially acidic esterification procedures.

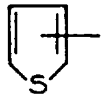
There is further provided by the present invention a pharmaceutical composition comprising an antibacterially effective amount of a compound having the formula



wherein n is one or two, R^2 is hydrogen or formyl and R is

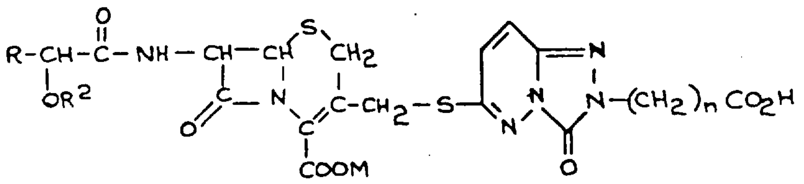


or



and Y is hydrogen, chlorine, bromine, fluorine, trifluoromethyl, amino, nitro, hydroxy, lower alkyl of 1-4 carbon atoms or lower alkoxy of 1-4 carbon atoms and M is hydrogen, pivaloyloxymethyl, acetoxymethyl, methoxymethyl, acetonyl, phenacyl, p-nitrogenzyl, β,β,β-trichloroethyl, 3-phthalidyl or 5-indanyl and preferably is hydrogen or a nontoxic, pharmaceutically acceptable salt thereof.

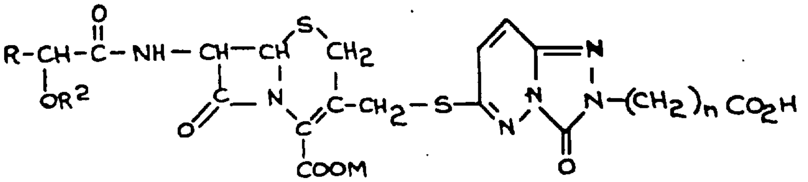
There is further provided by the present invention a method of treating bacterial infections comprising administering by injection to an infected warm-blooded non-human animal, an effective but nontoxic dose for example of 250-1000 mgm., of a compound having the formula



wherein n is one or two, R^2 is hydrogen or formyl and R is

and Y is hydrogen, chlorine, bromine, fluorine, trifluoromethyl, amino, nitro, hydroxy, lower alkyl of 1-4 carbon atoms or lower alkoxy of 1-4 carbon atoms and M is hydrogen, pivaloyloxymethyl, acetoxymethyl, methoxymethyl, acetonyl, phenacyl, p-nitrobenzyl, β,β,β-trichloroethyl, 3-phthalidyl or 5-indanyl or a nontoxic, pharmaceutically acceptable salt thereof.

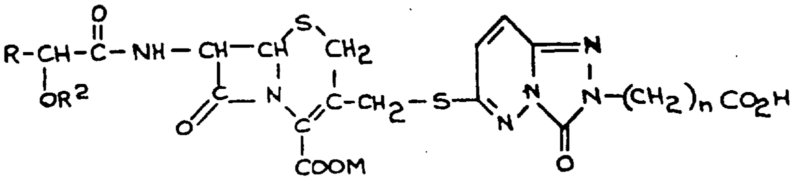
There is also provided by the present invention a method for combatting *Shig. dysenteriae* infections which comprises administering to a warm-blooded non-human mammal infected with a *Shig. dysenteriae* infection an amount effective for treating said *Shig. dysenteriae* infection of a composition comprising a compound having the formula



wherein n is one or two, R^2 is hydrogen or formyl and R is

and Y is hydrogen, chlorine, bromine, fluorine, trifluoromethyl, amino, nitro, hydroxy, lower alkyl of 1-4 carbon atoms or lower alkoxy of 1-4 carbon atoms and M is hydrogen, pivaloyloxymethyl, acetoxymethyl, methoxymethyl, acetonyl, phenacyl, p-nitrobenzyl, β,β,β-trichloroethyl, 3-phthalidyl or 5-indanyl and preferably is hydrogen or a nontoxic, pharmaceutically acceptable salt thereof.

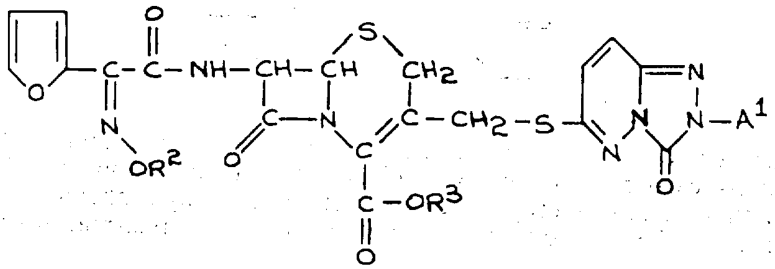
There is also provided by the present invention a method for combatting *Sal. enteritidis* infections which comprises administering to a warm-blooded non-human mammal infected with a *Sal. enteritidis* infection an amount effective for treating said *Sal. enteritidis* infection of a composition comprising a compound having the formula



wherein n is one or two, R^2 is hydrogen or formyl and R is

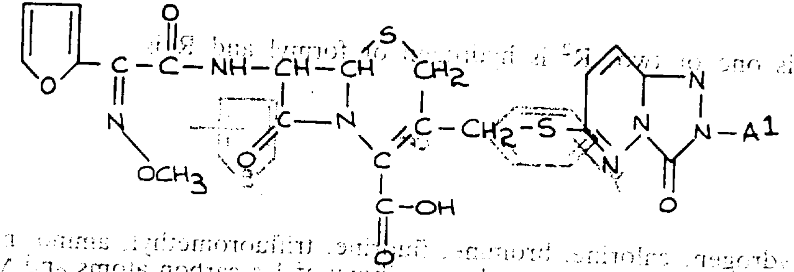
and Y is hydrogen, chlorine, bromine, fluorine, trifluormethyl, amino, nitro, hydroxy, lower alkyl of 1-4 carbon atoms or lower alkoxy of 1-4 carbon atoms and M is hydrogen, pivaloyloxymethyl, acetoxymethyl, methoxymethyl, acetonyl, phenacyl, p-nitrobenzyl, β,β,β-trichloroethyl, 3-phthalidyl or 5-indanyl and preferably is hydrogen or a nontoxic pharmaceutically acceptable salt thereof.

There is further provided by the present invention a pharmaceutical composition comprising an antibacterially effective amount of a compound having the formula



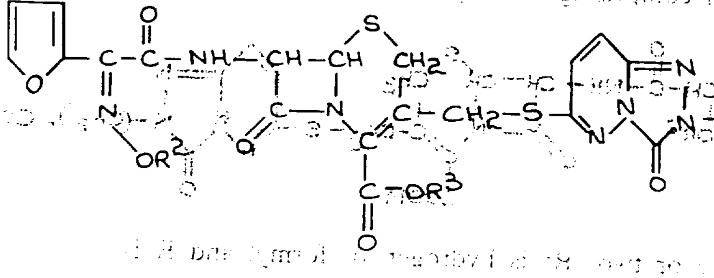
wherein R^2 and A^1 are as hereinbefore defined and R^3 is hydrogen, pivaloyloxymethyl, acetoxymethyl, methoxymethyl, acetonyl, phenacyl, p-nitrobenzyl, β,β,β-trichloroethyl, 3-phthalidyl or 5-indanyl and preferably is hydrogen or a nontoxic, pharmaceutically acceptable salt thereof, said compound being at least 75% by weight in the form of its *syn* isomer and preferably in the form of its *syn* isomer essentially free of the corresponding *anti* isomer, and a pharmaceutically acceptable carrier therefor.

There is further provided by the present invention a pharmaceutical composition comprising an antibacterially effective amount of the *syn* isomer of a compound having the formula



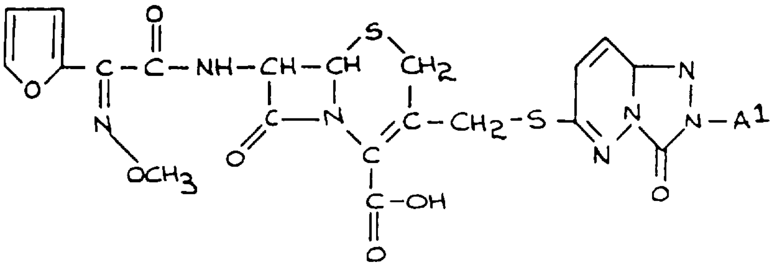
wherein A^1 is as hereinbefore defined or a nontoxic, pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier therefore.

There is further provided by the present invention a method of treating bacterial infections comprising administering by injection to an infected warm-blooded non-human animal, an effective but nontoxic dose, for example 250-1000 mgm., of a compound having the formula



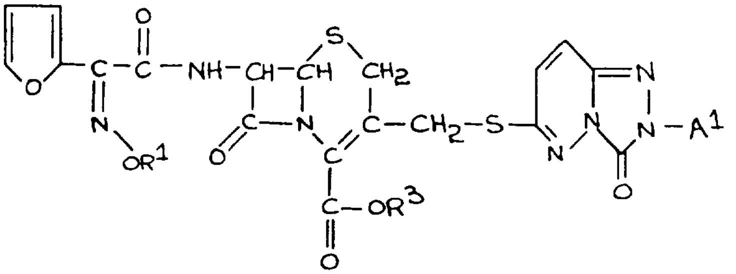
wherein R^2 and A^1 are as hereinbefore defined and R^3 is hydrogen, pivaloloxymethyl, acetoxymethyl, methoxymethyl, acetonyl, phenacyl, p-nitrobenzyl, β,β,β-trichloroethyl, 3-phthalidyl or 5-indanyl or a nontoxic, pharmaceutically acceptable salt thereof, said compound being at least 75% by weight in the form of its *syn* isomer and preferably in the form of its *syn* isomer essentially free of the corresponding *anti* isomer.

There is further provided by the present invention a method of treating bacterial infections comprising administering by injection to an infected warm-blooded non-human animal, an effective but nontoxic dose, for example of 250-1000 mgm., of the *syn* isomer of a compound having the formula



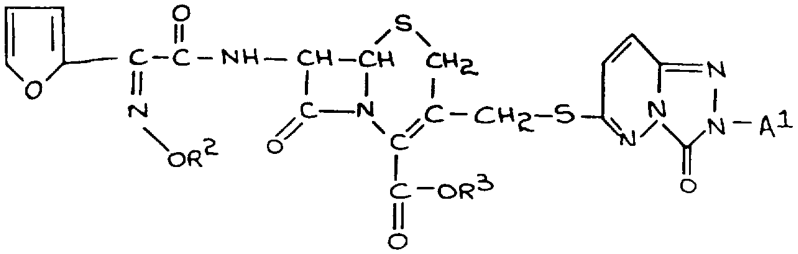
wherein A^1 is as hereinbefore defined or a nontoxic, pharmaceutically acceptable salt thereof, and wherein n is preferably 1.

There is also provided by the present invention a method for combatting *Haemophilus* infections which comprises administering to a warm-blooded non-human mammal infected with an *Haemophilus* infection an amount effective for treating said *Haemophilus* infection of a composition comprising a compound having the formula



wherein A^1 and R^2 are as hereinbefore defined and R^3 is hydrogen, pivaloyloxymethyl, acetoxymethyl, methoxymethyl, acetonyl, phenacyl, p-nitrobenzyl, β,β,β-trichloroethyl, 3-phthalidyl or 5-indanyl and preferably is hydrogen or a nontoxic, pharmaceutically acceptable salt thereof, said compound being at least 75% by weight in the form of its *syn* isomer and preferably in the form of its *syn* isomer essentially free of the corresponding *anti* isomer, and a pharmaceutically acceptable carrier therefor.

There is also provided by the present invention a method for combatting *Neisseria* infections which comprises administering to a warm-blooded non-human mammal infected with a *Neisseria* infection an amount effective for treating said *Neisseria* infection of a composition comprising a compound having the formula



wherein R^2 and A^1 are as hereinbefore defined and R^3 is hydrogen, pivaloyloxymethyl, acetoxymethyl, methoxymethyl, acetonyl, phenacyl, p-nitrobenzyl, β,β,β-trichloroethyl, 3-phthalidyl or 5-indanyl and preferably is hydrogen or a nontoxic, pharmaceutically acceptable salt thereof, said compound being at least 75% by weight in the form of its *syn* isomer and preferably in the form of its *syn* isomer essentially free of the corresponding *anti* isomer, and a pharmaceutically acceptable carrier therefor.

Starting materials

2-Furoylcyanide

To a suspension of 26-1 g. (0.4 mole) of ground potassium cyanide in 300 ml. of acetonitrile at 5° C. was added 26.1 g. (0.2 mole) of α-furoyl chloride while keeping the temperature below 8° C. The mixture was stirred in the cold for 15 minutes then heated at reflux for 30 minutes. The reaction was cooled, filtered and the acetonitrile was removed at 15 mm. (steam-bath) leaving 24.5 g. of a dark oil which was used without further purification. An infrared spectrum showed a nitrile band at 2265 cm^{-1}.

2-Furaneglyoxylic Acid

The 24.5 g of crude 2-furoylcyanide was mixed with 160 ml. concentrated hydrochloric acid at 25° C. with intermittent stirring. The reaction was stored for 24 hours at 25° C. and diluted with 80 ml. of water. The reaction was stirred for 5 minutes and filtered. The filtrate was saturated with sodium chloride and extracted with 5 × 120 ml. of 1:1 ether-ethyl acetate solution. The extracts were combined, dried over anhydrous magnesium sulfate and evaporated at 30° C. (15 mm.) to give a brownish-orange solid. The solid was dissolved in methanol, treated with charcoal and evaporated under reduced pressure (15 mm.) to dryness to yield 17 g. of the acid.

The product was recrystallized from toluene to give 11.5 g. (m.p. 76° C.). The ir and nmr spectra were consistent for the structure.

2-Methoxyimino-2-furylacetic Acid

To a solution of 4.5 g. (0.032 mole) of 2-furaneglyoxylic acid in 40 ml. of 50% alcohol and 3.1 g. (0.037 mole) of methoxyamine hydrochloride in 6 ml. water at 20° C. was added dilute sodium hydroxide solution to pH 4-5. The solution was stirred at pH 4-5 at 25° C. for 24 hours. The alcohol was removed under reduced pressure (15 mm.) and the solution was adjusted to pH 7-8 with 50% sodium hydroxide solution. The reaction was extracted with 3 × 50 ml. of ether and the aqueous layer was adjusted to pH 1.9 using concentrated hydrochloric acid. The mixture was extracted with 5 × 50 ml. of ethyl acetate. The organic fractions were combined, washed with brine, dried over anhydrous magnesium sulfate and evaporated under reduced pressure (15 mm.) to an oil which was cooled for one hour in an ice bath. The product was slurried with Skellysolve B and collected to yield 3.1 g. of yellow crystals, m.p. 78° C. An analytical sample was recrystallized from toluene, dried for 16 hours *in vacuo* over P_2O_5 at 25° C. The ir and nmr spectra were consistent for the structure.

Anal. Calc'd for C_7H_7NO: C, 49.65; H, 4.17; N, 8.28. Found: C, 49.30; H, 4.21; N, 8.37.

2-Ethoxyiminofurylacetic Acid

The 7.85 g. (0.056 mole) of furyl-2-glyoxylic acid was dissolved in 100 ml. of water and adjusted to pH 7 with 50% sodium hydroxide. The 6.83 g. (0.070 mole) of ethoxyamine hydrochloride in 10 ml. of water was added, while keeping the pH at 4-5. The reaction was diluted with 25 ml. of alcohol, stirred 3 hours at room temperature and then filtered. The alcohol was removed at 35° C. (15 mm.) and the aqueous portion was adjusted with dilute sodium hydroxide solution to pH 7-8 and then was washed with ether and the washes were discarded. The aqueous fraction was adjusted with 6N hydrochloric acid to pH 1.5 and extracted into 3 × 80 ml. of ethyl acetate. The acetate fractions were combined, washed with brine and reduced in volume at 35°C. (15 mm.) to an oil. The oil was cooled in an ice bath, triturated with Skellysolve B, collected and dried over P_2O_5 *in vacuo* at 25° C.

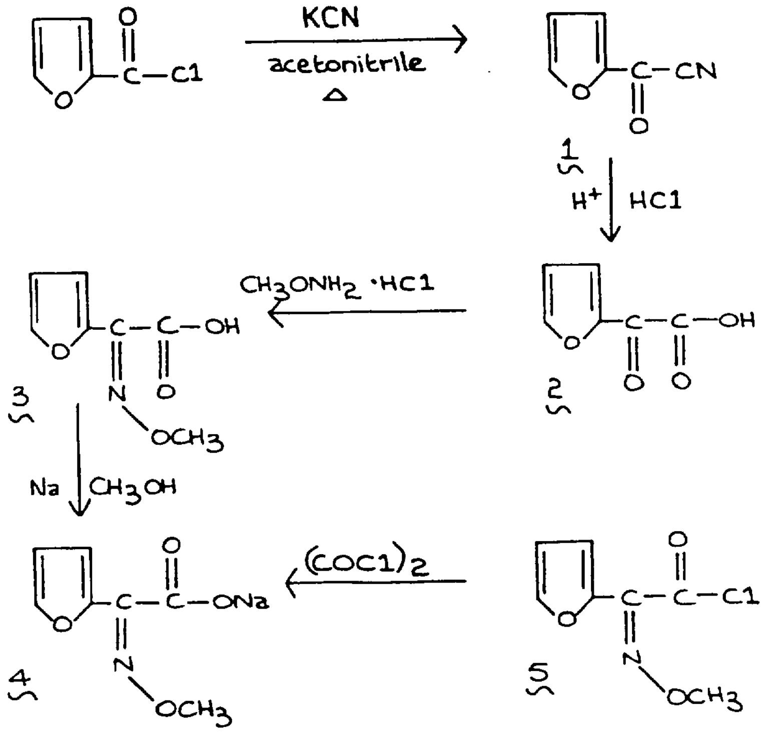
Yield: 4.8 g., m.p. 83-85° C. The ir and nmr were consistent for the structure.

Anal. Calc'd for $C_8H_9NO_4$: C, 52.46; H, 4.95; N, 7.65. Found: C, 52.22; H, 4.94; N, 7.60.

Sodium α-Ethoxyimino-α-(2-furyl)acetate

To 50 ml. of methanol was added 250 mg. (0.0109 mole) of metallic sodium and stirred until all the sodium had dissolved. This sodium methoxide solution was treated with 2.0 g. (0.0109 mole) of α-ethoxyimino-α-(2-furyl)acetic acid dissolved in 10 ml. of methanol and stirred at room temperature for one hour. The methanol was removed at 40° C. (15 mm.) and the product was dried *in vacuo* over P_2O_5 at 25° C. to yield 2.22 g. white solid, m.p. decomp. >240° C. The ir and nmr were consistent for the structure.

"Skellysolve B" is a petroleum ether fraction of b.p. 60°-68° C. consisting essentially of n-hexane.



2-Furoylcyanide 1

To a suspension of 78.3 g. of powdered potassium cyanide in 900 ml. acetonitrile at 5° C. was added 59.25 ml. (68.5 g.) of α-furoyl chloride with vigorous stirring while keeping the temperature at 4-8° C. The mixture was stirred at 4-8° C. for 15 minutes and then heated at reflux for 30 minutes. The mixture was cooled to 23-25° C., filtered, washed with 50 ml. of acetonitrile which was added to the filtrate, and the acetonitrile was removed at 60° C. (15 mm.) leaving 51 g. of 1 as a dark oil. An IR spectrum showed a nitrile band at 2265 cm^{-1} and an NMR spectrum showed a ratio of approximately 70/30 of product 1/furoic acid. The crude product 1 was used without further purification (49% yield of product).

Furyl-2-glyoxylic Acid 2

The 51 g. of crude 2-furoyl cyanide 1p was mixed with 500 ml. concentrated hydrochloric acid at 25° C. The reaction was stirred for 24 hours at 25° C. and then diluted with 240 ml. of water. The mixture was stirred for 5 minutes and filtered. The black filtrate was saturated with sodium chloride and extracted with 6 × 500 ml. of 1:1 ether-ethyl acetate solution. (*Note*: Initially the extractions were difficult due to the inability to see the separation of two black phases. As additional ether-ethyl acetate extractions were run the task was simplified.) The extracts were combined and evaporated to dryness at 60° C. (15 mm.). The resultant solid was dissolved in 600 ml. ether, (*Note*: Use of alcohol should be avoided at this point as esters may form), treated with 10 g. of charcoal ("Darko-KB"), filtered after stirring for 0.5 hour and evaporated to dryness at 50° C. (15 mm.) to yield 46.6 g. of 2 as a light tan colored acid. This product 2 was found to contain a ratio of approximately 56/44 of product 2/furoic acid. This represented a 63% yield of product 2

Purification was accomplished by dissolving the above crude product 2 in H_2O (50 mg./ml.), titrating to pH 2.8 with HCl and extracting with 2 × 200 ml. of ethyl acetate. Evaporation of the ethyl acetate extracts gave 35% furoic acid and 15% product 2. The pH 2.8 aqueous phase was adjusted to pH 0.8 (HCl) and extracted with 2 × 200 ml. ethyl acetate. The organic extracts were combined and washed with 50 ml. H_2O. The organic phase was evaporated at 50° C. (15 mm.) yielding a solid with a ratio of approximately 86/14 of product 2/furoic acid. This solid was then recrystallized by dissolving the product 2 in toluene at 50 mg./ml. at 80° C., decanting, and leaving to crystallize at room temperature for 18 hours, yielding 13.3 g. of pure acid 2 by NMR. This represented a 51% yield in the purification and recrystallization step and an overall yield from the 2-furoyl chloride to the pure furyl-2-glyoxylic acid 2 of 16%.

Syn-α-methoxyiminofurylacetic Acid 3

A solution of 4.5 g. of furyl-2-glyoxylic acid 2 in 40 ml. of 50% ethanol was titrated to pH

6 with IN sodium hydroxide and then 3.1 g. of methoxyamine·HCl in 6 ml. of H_2O at 20° C. was added. The solution was titrated to a constant pH 4.9 and stirred at pH 4.9 for 24 hours at 20-23° C. The ethanol was then removed at 50° C. (15 mm.) and the residual aqueous solution was titrated to pH 8 with 50% sodium hydroxide and washed with 3 × 50 ml. ether (pH adjusted to 8 after each wash). The aqueous layer was titrated to pH 1.9 with concentrated HCl and extracted with 5 × 50 ml. ethyl acetate with the pH readjusted to 1.9 after each extraction. The ethyl acetate extracts were combined and evaporated to a solid 3 at 50° C. (15 mm.). This solid was then slurried with 75 ml. of "Skellysolve B". The suspension was filtered and the solids were redissolved in 16 ml. of toluene at 80° C. The hot solution was decanted and left to crystallize at 20-23° C. for 18 hours to yield 1.17 g. 3 (22% yield of product). The NMR was clean and consistent for the structure 3 with a trace of *anti* isomer present.

Sodium Syn-α-methoxyiminofurylacetate 4

To 40 ml. of methanol was added 0.16 g. of sodium. The mixture was stirred until all of the sodium dissolved and then decanted. The resulting sodium methoxide solution was cooled to 3° C. and 1.12 g. of *syn*-α-methoxyiminofurylacetic acid 3 in 7.8 ml. of methanol was added. The solution was stirred for 10 minutes at room temperature. The solvent was evaporated at 40° C. (15 mm.). The residue 4 was dried by azeotropic distillation with 3 × 20 ml. of benzene at 40° C. (15 mm.). The product 4 was dried for 18 hours at 23° C. under high vacuum (0.7 mm.) over P_2O_5 yielding 1.25 g. (99% yield of product). The NMR showed this product 4 to be clean and consistent for the structure with 0.15 mole methanol and a trace of *anti* isomer.

To 0.63 g. of sodium *syn*-α-methoxyiminofurylacetate 4 suspended in 25 ml. of benzene was added four drops of dry dimethylformamide and 0.31 ml. (1.1 eq.) of oxalyl chloride. This mixture was stirred for 40 minutes at 20-23° C. The benzene was removed at 35° C. (15 mm.) to provide the acid chloride 5 as the gummy residue.

6-Chloro-2,3-dihydro-2-ethoxycarbonylmethyl-s-triazolo-[4,3-b]pyridazin-3-one

To a solution of 6-chloro-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-one [P. Francavilla and F. Lauria, J. Het. Chem., *8*, 415 (1971)] (*1*, 1.00 g., 5.9 m.mole) in dry DMF (30 ml.) was added sodium hydride (50% in paraffin, 0.3 g., 6.3 m.mole) under stirring with formation of yellow crystals. To the mixture was added ethyl chloroacetate (1.4 ml., 13 m.mole) and the mixture was heated at 90° C. for 8 hours with stirring. After cooling, the reaction mixture was poured into water (50 ml.) and extracted with toluene (5 × 40 ml.). The organic extracts were combined, dried over anhydrous sodium sulfate and evaporated at reduced pressure. The residue was crystallized with benzene-n-hexane to give yellow needles (1.16 g., 77%), m.p. 114-115° C. (lit. 110° C.).

ir: ν_{max}^{KBr} 1735, 1710 cm^{-1}.

uv: λ_{max}^{EtOH} 231 nm (ε, 26000)

nmr: $\delta_{ppm}^{CDCl_3}$ 7.58 (1H, d, J=10 Hz, pyridazine-H),

6.98 (1H, d, J=10 Hz, pyridazine-H), 4.80 (2H, s, -$C\underline{H}_2CO$), 4.27 (2H, q, J=7.5 Hz, $C\underline{H}_2CH_3$), 1.29 (3H, t, J=7.5 Hz, $CH_2C\underline{H}_3$).

Anal. Calc'd. for $C_9H_9N_4O_3Cl$: C, 42.12; H, 3.53; N, 21.83; Cl, 13.81. Found: C, 41.54, 41.46; H, 3.22, 3.49; N, 21.51, 21.53; Cl, 13.88, 13.99.

2-Carboxymethyl-2,3-dihydro-6-mercapto-s-triazolo[4,3-b]-pyridazin-3-one

To a solution of 6-chloro-2,3-dihydro-2-ethoxy-carbonylmethyl-s-triazolo[4,3-b]pyridazin-3-one (30 g., 0.12 mole) in ethanol (900) ml.) was added $NaSH \cdot 2H_2O$ (70% pure, 45.9 g., 0.36 mole) and the mixture was refluxed for 0.5 hour. The reaction mixture was evaporated at reduced pressure. The residue was dissolved in water (200 ml.) and concentrated HCl was added to the solution to adjust to pH 2. The precipitate of 2-carboxymethyl-2,3-dihydro-6-mercapto-s-triazolo[4,3-b]pyridazin-3-one was collected by filtration and washed with water. Yield 18.3 g. (69%).

ir: ν_{max}^{KBr} 2900, 2450, 1750, 1660 cm^{-1}.

uv: $\lambda_{max}^{1\%NaHCO_3aq.}$ 260 nm (ε,19500), 313 nm (ε, 7000)

nmr: $\delta_{ppm}^{DMSO\text{-}d_6}$ 7.88 (1H, d, J=10 Hz, pyridazine-H), 7.45 (1H, d, J=10 Hz, pyridazine-H), 4.72 (2H, s, $C\underline{H}_2CO$).

Anal. Calc'd. for $C_7H_6N_4O_3S$: C, 37.17; H, 2.67; N, 24.77; S, 14.17. Found: C, 37.35, 37.23; H, 2.26, 2.28; N, 23.58, 23.69; S, 14.32.

7-Amino-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]-pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic Acid

To a suspension of 7-aminocephalosporanic acid (8.79 g., 32.2 m.mole) in 0.1 M phosphate buffer (pH 7, 149ml.) were added $NaHCO_3$ (8.14 g., 97.0 m.mole) and the thiol 2-carboxymethyl-2,3-dihydro-6-mercapto-s-triazolo-[4,3-b]pyridazin-3-one (7.30 g., 32.2 m.mole) with stirring. The mixture was heated at 80° C. for 0.5 hour under N_2 stream. The mixture was treated with active carbon and adjusted to pH 3 with concentrated HCl. The resulting precipitate was collected by filtration and washed with water to give 7.59 g. (54%) of 7-amino-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid.

ir: ν_{max}^{KBr} 1800, 1720, 1600, 1540, 1470 cm^{-1}.

uv: $\lambda_{max}^{Buffer\ (pH\ 7)}$ 252 nm (ε,19500), 298 nm (ε, 8400).

nmr: $\delta_{ppm}^{D_2O+K_2CO_3}$ 7.56 (1H, d, J=9 Hz, pyridazine-H), 7.05 (1H, d, J=9 Hz, pyridazine-H), 5.45 (1H, d, J=5 Hz, 6-H), 5.05 (1H, d, 5 Hz, 7-H), 4.43 (1H, d, J=14 Hz, 3-CH_2), 4.04)1H, d, J=14Hz, 3-CH_2), 3.88 (1H, d, J=18 Hz, 2-H), 3.45 (1H, d, J=18 Hz, 2-H).

6-Chloro-2-(2-cyanoethyl)-2,3-dihydro-s-triazolo[4,3-b]-pyridazin-3-one

To a solution of 6-chloro-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on [P. Francabilla and F. Lauria, J. Het. Chem. 8, 415 (1971)] (17 g., 0.1 mole) in dry DMF (300 ml.) was added potassium tert.-butoxide (0.5 g., 4.5 m.moles) with stirring. Acrylonitrile (6.6 g., 0.12 mole) in dry DMF (10 ml.) was added to the mixture. The mixture was stirred at 100-110° C. for 24 hours, then poured into water (700 ml.) and extracted with ethyl acetate (5 × 400 ml.). The organic extracts were combined, dried over Na_2SO_4 and evaporated. The residue was crystallized from ethyl acetate to give light yellow needles of 6-chloro-2-(2-cyanoethyl)-2,3-dihydro-s-triazolo [4,3-b]- pyridazin-3-one (2.5 g., 11%). M.p. 166-168° C.

ir: ν_{max}^{KBr} 2230, 1720, 1550, 1500 cm^{-1}.

uv: $\lambda_{max}^{dioxane}$ 373 nm (ε 2000).

nmr: $\delta_{ppm}^{DMSO\text{-}d_6}$ 3.03 (2H, t, J=6.0 Hz, $C\underline{H}_2$), 4.21 (2H, t, J=6.0 Hz, $C\underline{H}_2$), 7.23 (1H, d, J=10.0 Hz, pyridazine-H), 7.93 (1H, d, J=10.0 Hz, pyridazine-H).

Anal. Calc'd. for $C_8H_6N_5OCl$: C, 42.97; H, 2.70; N, 31.32; Cl, 15.86. Found: C, 42.73, 42.56; H, 2.57, 2.50; N, 31.36, 31.68; Cl, 15.96, 15.81.

2-(2-Carboxyethyl)-6-chloro-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-one

A solution of 6-chloro-2-(2-cyanoethyl)-2-3-dihydro-s-triazolo[4,3-b]pyridazin-3-on (724 mg.) in 6*N*-HCl (15 ml.) was refluxed for 6 hours. The reaction mixture was extracted with ethyl acetate (10 × 20 ml.). The combined extracts were washed with saturated aqueous sodium chloride (50 ml.), dried over Na_2SO_4 and evaporated to give light yellow, solid 2-(2-carboxyethyl)-6-chloro-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-one (567 mg., 72%). M.p. >170° C. (sublimation).

ir: ν_{max}^{KBr} 3400-2400, 1730, 1710, 1540 cm^{-1}.

uv: $\lambda_{max}^{dioxane}$ 377 nm (ϵ 1500).

nmr: $\delta_{ppm}^{D_2O+NaHCO_3}$ 2.70 (2H, t, J=7.0 Hz, $C\underline{H}_2$),

4.24 (2H, t, J=7.0 Hz, $C\underline{H}_2$), 7.17 (1H, d, J=10.0 Hz, pyridazine-H), 7.70 (1H, d, J=10.0 Hz, pyridazine-H).

Anal. Calc'd. for $C_8H_7N_4O_3Cl$: C, 39.60; H, 2.91; N, 23.09; Cl, 14.61. Found: C, 39.62, 39.48; H, 2.97, 2.67; N, 23.05, 22.70; Cl. 13.93, 14.12.

2-(2-Carboxyethyl)-2,3-dihydro-6-mercapto-s-triazolo[4,3-b]pyridazin-3-one

A mixture of 2-(2-carboxyethyl)-6-chloro-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on (567 mg., 2.34 m.moles) and 70% sodium hydrosulfide dihydrate (924 mg., 7.02 m.mole) in water (10 ml.) was stirred at room temperature for two hours. The reaction mixture was adjusted successively to pH 1 with c. HCl, to pH 10 with NaOH and then to pH 1 with c. HCl. The resulting precipitate of 2-(-carboxyethyl)-2,3-dihydro-6-mercapto-s-triazolo[4,3-b]-pyridazin-3-one was collected by filtration and washed with water. Yield: 418 mg. (74%). M.p. 174-176° C.

ir: ν_{max}^{KBr} 3600-2600, 2440, 1730, 1720 (sh) cm^{-1}.

uv: $\lambda_{max}^{pH\ 7\ buffer}$ 262 nm (ϵ 17000), 318 nm (ϵ 6600).

nmr: $\delta_{ppm}^{DMSO-d_6}$ 2.73 (2H, t, J=7.0 Hz, $C\underline{H}_2$),

4.07 (2H, t, J=7.0 Hz, $C\underline{H}_2$), 7.30 (1H, d, J=10.0 Hz, pyridazine-H), 7.74 (1H, d, J=10.0 Hz, pyridazine-H).

Anal. Calc'd. for $C_8H_8N_4O_3S$: C, 40.00; H, 3.36; N, 23.32; S, 13.35. Found: C, 39.08, 39.06; H, 3.12, 3.20; N, 22.65, 22.70; S, 14.23, 14.29.

7-Amino-3-[2-(2-carboxyethyl)-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl]-3-cephem-4-carboxylic Acid.

A mixture of 7-*ACA* (405 mg., 1.49 m.moles), the thiol 2-(2-carboxyethyl)-2,3-dihydro-6-mercapto-s-triazolo[4,3-b]pyridazin-3-on (357 mg., 1.49 m.moles) and $NaHCO_3$ (375 mg., 4.47 m.moles) in 0.1 *M* phosphate buffer (pH 7, 8 ml.) was stirred at 80° C. for 30 minutes. The reaction mixture was cooled and filtered to remove insolubles. The filtrate was adjusted to pH 1-2 with c. HCl. The resulting precipitate, 7-amino-3-[2-(2-carboxyethyl)-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl]-3-cephem-4-carboxylic acid, was collected by filtration and washed with water. Yield: 519 mg. (77%).

ir: ν_{max}^{KBr} 3600-2200, 1800, 1725, 1620, 1550, 1480 cm^{-1}.

uv: $\lambda_{max}^{pH\ 7\ buffer}$ 253 nm (ϵ 20000), 298 nm (ϵ 10000).

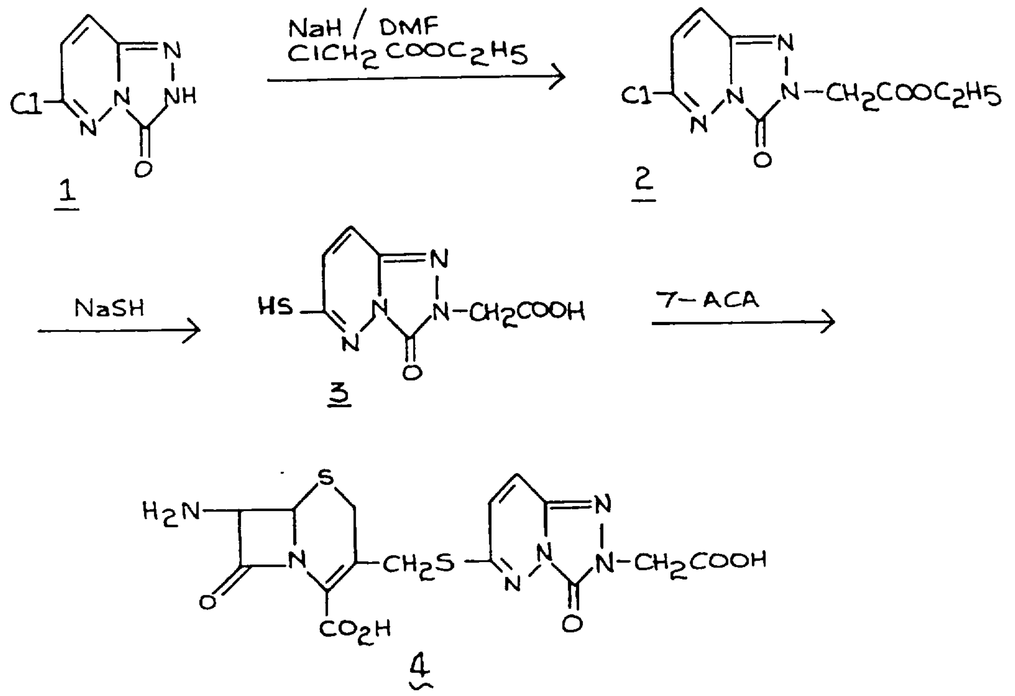
nmr: $\delta_{ppm}^{D_2O+K_2CO_3}$ 2.20 (2H, t, J=7.0 Hz, $C\underline{H}_2$),

3.40 (1H, d, J=17.5 Hz, 2-H), 3.85 (1H, d, J=17.5 Hz, 2-H), 4.00-4.50 (4H, m, 3-$C\underline{H}_2$ and N-$C\underline{H}_2$-), 5.01 (1H, d, J=4.0 Hz, 6-H), 5.40 (1H, d, J=4.0 Hz, 7-H), 6.94 (1H, d, J=10.0 Hz, pyridazine-H), 7.44 (1H, d, J=10.0 Hz, pyridazine-H).

Anal. Calc'd. for $C_{16}H_{16}N_6O_6S_2 \cdot 3/2H_2O$: C, 40.09; H, 3.99; N, 17.52; S, 13.37. Found: C, 40.06, 40.12; H, 3.33, 3.34; N, 16.96, 16.98; S, 13.87, 13.98.

7-ACA refers to 7-aminocephalosporanic acid and DMF to dimethylformamide.

Scheme 1. Preparation of 7-amino-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic Acid.



6-Chloro-2,3-dihydro-2-ethoxycarbonylmethyl-s-triazolo[4,3-b]pyridazin-3-one (2)

To a solution of 6-chloro-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-one [P. Francavilla and F. Lauria, J. Het. Chem., *8*, 415 (1971)] (*1*, 1.00 g., 5.9 m.mole) in dry DMF (30 ml.) was added sodium hydride (50% in paraffin, 0.3 g., 6.3 m.mole) under stirring with formation of yellow crystals. To the mixture was added ethyl chloroacetate (1.4 ml., 13 m.mole) and the mixture was heated at 90° C. for 8 hours with stirring. After cooling, the reaction mixture was poured into water (50 ml.) and extracted with toluene (5 × 40 ml.). The organic extracts were combined, dried over anhydrous sodium sulfate and evaporated at reduced pressure. The residue was crystallized with benzene-n-hexane to give yellow needles of *2* (1.16 g., 77%) m.p. 114-115° C. (lit. 110° C.).

ir: ν_{max}^{KBr} 1735, 1710 cm^{-1}.

uv: λ_{max}^{EtOH} 231 nm (ϵ, 26000).

nmr: $\delta_{ppm}^{CDCl_3}$ 7.58 (1H, d, J=10 Hz, pyridazine-H), 6.98 (1H, d, J=10 Hz, pyridazine-H), 4.80 (2H, s, -C$\underline{H}_2$CO), 4.27 (2H, q, J=7.5 Hz, C$\underline{H}_2$CH$_3$), 1.29 (3H, t, J=7.5 Hz, CH$_2$C$\underline{H}_3$).

Anal. Calc'd. for $C_9H_9N_4O_3Cl$: C, 42.12; H, 3.53; N, 21.83; Cl, 13.81. Found: C, 41.54, 41.46; H, 3.22, 3.49; N, 21.51, 21.53; Cl, 13.88, 13.99.

2-Carboxymethyl-2,3-dihydro-6-mercapto-s-triazolo[4,3-b]pyridazin-3-one (3)

To a solution of 6-chloro-2,3-dihydro-2-ethoxy-carbonylmethyl-s-triazolo[4,3-b]pyridazin-3-one (*2*, 30 g., 0.12 mole) in ethanol (900 ml.) was added NaSH·2H$_2$O (70% pure, 45.9 g., 0.36 mole) and the mixture was refluxed for 0.5 hour. The reaction mixture was evaporated at reduced pressure. The residue was dissolved in water (200 ml.) and concentrated HCl was added to the solution to adjust to pH 2. The precipitate (*3*) was collected by filtration and washed with water. Yield 18.3 g. (69%).

ir: ν_{max}^{KBr} 2900, 2450, 1750, 1660 cm^{-1}.

uv: $\lambda_{max}^{1\%NaHCO_3aq.}$ 260 nm (ϵ, 19500), 313 nm (ϵ, 7000).

nmr: $\delta_{ppm}^{DMSO\text{-}d_6}$ 7.88 (1H, d, J=10 Hz, pyridazine-H),

7.45 (1H, d, J=10 Hz, pyridazine-H), 4.72 (2H, s, $C\underline{H}_2CO$).

Anal. Calc'd. for $C_7H_6N_4O_3S$: C, 37.17; H, 2.67; N, 24.77; S, 14.17. Found: C, 37.35, 37.23; H, 2.26, 2.28; N, 23.58, 23.69; S, 14.32.

7-Amino-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic Acid (4)

To a suspension of 7-aminocephalosporanic acid (8.79 g., 32.2 m.mole) in 0.1 M phosphate buffer (pH 7, 149 ml.) were added $NaHCO_3$ (8.14 g., 97.0 m.mole) and the thiol *3* (7.30 g., 32.2 m.mole) with stirring. The mixture was heated at 80° C. for 0.5 hour under N_2 stream. The mixture was treated with active carbon and adjusted to pH 3 with concentrated HCl. The resulting precipitate was collected by filtration and washed with water to give 7.59 g. (54%) of *4*.

ir: ν_{max}^{KBr} 1800, 1720, 1600, 1540, 1470 cm^{-1}.

uv: $\lambda_{max}^{Buffer\ (pH\ 7)}$ 252 nm (ϵ, 19500), 298 nm (ϵ, 8400).

nmr: $\delta_{ppm}^{D_2O+K_2CO_3}$ 7.56 (1H, d, J=9 Hz, pyridazine-H),

7.05 (1H, d, J=9 Hz, pyridazine-H), 5.45 (1H, d, J=5 Hz, 6-H), 5.05 (1H, d, 5 Hz, 7-H), 4.43 (1H, d, J=14 Hz, $3\text{-}CH_2$), 4.04 (1H, d, J=14 Hz, $3\text{-}CH_2$), 3.88 (1H, d, J=18 Hz, 2-H), 3.45 (1H, d, J=18 Hz, 2-H).

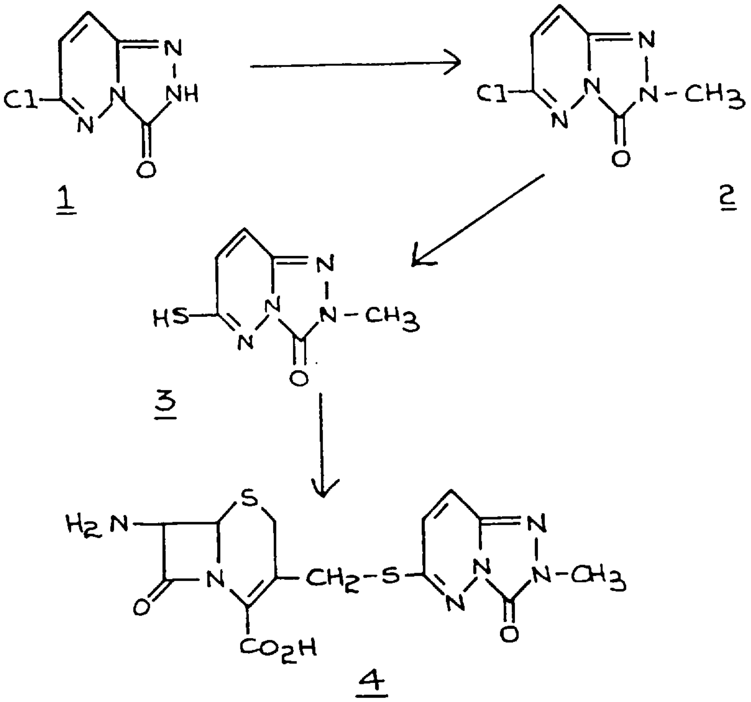
Pivaloyloxymethyl-7-amino-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylate.

Method A. - The title compound is produced by substituting for the 7-aminocephalosporanic acid used immediately above an equimolar weight of pivaloyloxymethyl 7-aminocephalosporanate hydrochloride prepared according to Example 2 of U.K. 1,229,453 from 7-aminocephalosporanic acid. German 1,904,585 (Farmdoc 39,445) is equivalent to U.K. 1,229,453.

Method B. - The title compound is produced by substituting for the 0.025 mole (6.8 g.) 7-amino-cephalosporanic acid used in the procedure of Example 2 of U.K. 1,229,453 an equimolar weight of 7-amino-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid (*4*).

The respective acetoxymethyl, methoxymethyl, acetonyl and phenacyl esters of 7-amino-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid are prepared by substituting in Method B above chloromethyl pivalate used therein an equimolar weight of chloromethyl acetate, chloromethyl methyl ether, chloroacetone and phenacyl bromide, respectively.

Preparation of 7-Amino-3-(2-methyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl-3-cephem-4-carboxylic Acid



6-Chloro-2,3-dihydro-2-methyl-s-triazolo[4,3-b]pyridazin-3-one (*2*)

To a solution of 6-chloro-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-one [P. Francavilla and F. Lauria, J. Het. Chem. *8*, 415 (1971)] (*1*, 8.5 g., 50 m.mol.) in dry DMF (12 ml.) was added NaH (50% dispersion in paraffin, 2.64 g., 55 m.mol) and the mixture was stirred for 1 hour at room temperature. After methyl iodide (21.3 g., 150 m.mole) was added, the mixture was stirred for 40 hours at room temperature, diluted with water (200 ml.) and extracted with $CHCl_3$ (4 × 100 ml.). The combined extracts were washed with water (3 × 50 ml.), treated with a small amount of carbon and dried with anhydrous Na_2SO_4. Evaporation of the solvent under reduced pressure afforded pale yellow residue which was crystallized from chloroform-n-hexane. Yield: 7.23 g. (79%). M.p. 180-181° C.

ir: $\nu^{KBr}_{C=O}$ 1720 cm^{-1}.

uv: λ^{EtOH}_{max} 233 nm (ϵ 25200), 363 nm (ϵ 1600).

nmr: $\delta^{CDCl_3}_{ppm}$ 3.72 (3H, s, N-CH_3),
6.88 (1H, d, J=10 Hz, pyridazine-H), 7.48 (1H, d, J=10 Hz, pyridazine-H).

Anal. Calc'd. for $C_6H_5ClN_4O$: C, 39.04; H, 2.73; N, 30.35; Cl, 19.21. Found: C, 39.24, 39.28; H, 2.54, 261; N, 30.63, 30.80; Cl, 19.59, 19.26.

6-Mercapto-2,3-dihydro-2-methyl-s-triazolo[4,3-b]pyridazin-3-one (*3*)

A mixture of *2* (6.50 g., 37.7 m.mol.) and $NaSH \cdot 2H_2O$ (70% pure, 9.4 g.) in water (100 ml.) was heated under reflux for 15 minutes. The mixture was cooled and acidified to pH 1 with concentrated HCl to precipitate the thiol *3* which was collected by filtration and dissolved in aqueous saturated $NaHCO_3$ (100 ml.). The solution was treated with a small amount of carbon and acidified with dilute HCl to precipitate *3* as pale yellow prisms. Yield: 5.72 g. (89%). M.p. >280° C.

ir: ν_{max}^{KBr} 2450 (-SH), 1710 (C=0) cm^{-1}.

uv: $\lambda_{max}^{1\%NaHCO_3}$ 261 nm (ϵ 16300), 315 nm (ϵ 5800).

nmr: $\delta_{ppm}^{D_2O+KOH}$ 3.60 (3H, s, N-C$\underline{H}_3$),

7.08 (2H, s, pyridazine-H).

Anal. calc'd. for $C_6H_6N_4OS$: C, 39.55; H, 3.32; N, 30.75; S, 17.60. Found: C, 39.57, 39.66; H, 3.14, 3.22; N, 30.32, 30.61; S, 17.80, 17.89.

7-Amino-3-(2-methyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic Acid (*4*)

A mixture of 7-aminocephalosporanic acid (7-ACA, 5.44 g., 20 m.mol), *3*, (3.64 g., 20 m.mol.) and $NaHCO_3$ (3.36 g., 40 m.mol.) in 0.1 M phosphate buffer (pH 7, 100 ml.) was heated with stirring at 80° C. for 30 minutes. The hot mixture was treated with a small amount of carbon and the filtrate was acidified to pH 4 with dilute HCl to precipitate *4* which was collected by filtration, washed with water (50 ml.) and dried. Yield: 5.73 g. (73%). M.p. 240-245° C. (dec.).

ir: ν_{max}^{KBr} 1800 (β-lactam C=0), 1725 (C=0),

1610 and 1410 (COO^-) cm^{-1}.

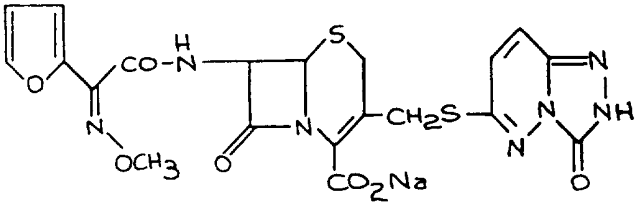
uv: $\lambda_{max}^{1\%NaHCO_3}$ 253 nm (ϵ 20000), 305 (ϵ 8400).

nmr: $\delta_{ppm}^{D_2O+NaHCO_3}$ 3.69 (3H, s, N-C$\underline{H}_3$),

5.08 (1H, d, J=4.5 Hz, 6-H), 5.48 (1H, d, J=4.5Hz, 7-H), 7.00 (1H, d, J=10 Hz, pyridazine-H), 7.52 (1H, d, J=10 Hz, pyridazine-H).

Anal. Calc'd. for $C_{14}H_{14}N_6O_4S_2 \cdot H_2O$: C, 40.76; H, 3.91; N, 20.38; S, 15.55. Found: C, 40.84, 40.63; H, 3.44, 3.31; N, 20.50, 20.36; S, 15.19, 15.57.

Preparation of BB-S515



BB-S515

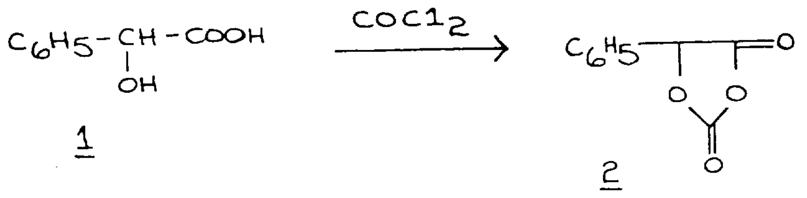
BB-S515; 7-[(2Z)-2-Methoxyimino(fur-2-yl)acetamido]-3-(2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic Acid Sodium Salt.

To a solution of (2Z)-2-methoxyimino(fur-2-yl)-acetic acid (169 mg., 1 m.mole) and triethylamine (0.14 ml., 1 m.mole) in dichloromethane (2 ml.) was added oxalyl chloride (0.09 ml., 1 m.mole) at 0-5° C. and the mixture was stirred for 30 minutes. The solvent was evaporated under reduced pressure to afford an oily residue. A solution of that oily residue in dry acetone (5 ml.), after filtration, was added to a mixture of 7-amino-3-(2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid (380 mg., 1 m.mole) (U.S. 3,907,786) and sodium bicarbonate (336 mg., 4 m.mol.) in water (10 ml.) at 0-5° C. The reaction mixture was stirred at 0-5° C. for 2 hours. Most of the acetone was evaporated at reduced pressure, the aqueous concentrate being washed with ether (2 × 30 ml.) and adjusted to pH 1-2 with concentrated HCl. The resulting precipitate (338 mg.) was collected by filtration and dried *in vacuo*. A suspension of the free acid (303 mg.) in water (10 ml.) was adjusted to pH 6.5 with aqueous NaOH (1 *N*, 0.6 ml.) and filtered to make a clear solution which was lyophilized to give 7-[(2Z)-2-methoxyimino(fur-2-yl)acetamido]-3-(2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid sodium salt as a light brown powder (222 mg., 46%). M.p. >230° C. (dec.).

ir: ν_{max}^{KBr} 3410, 1760, 1720, 1600 cm^{-1}.

uv: $\lambda_{max}^{pH7Buffer}$ 256 nm (ϵ 20600), 274 (ϵ 18800).

Preparation of D-mandelic acid carboxyanhydride



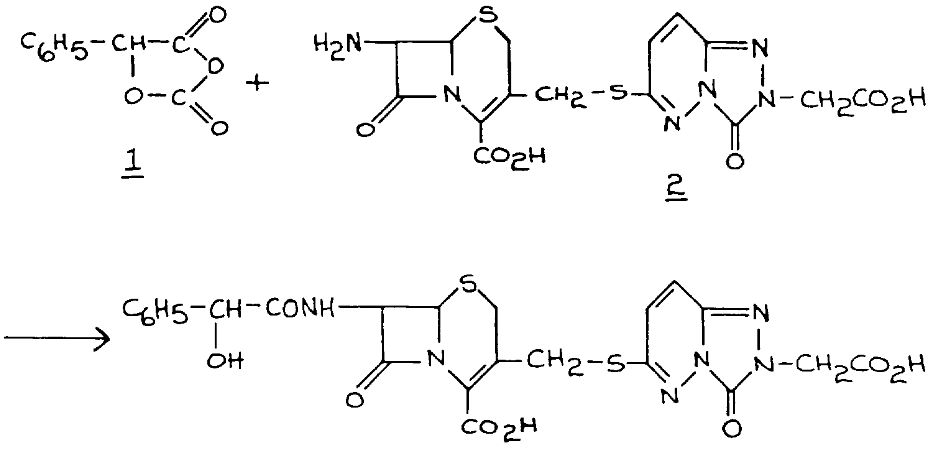
D-Mandelic acid carboxyanhydride (*2*)

Phosgene was bubbled through a solution of 2.0 g. (0.013 mole) and D(-)-mandelic acid (*1*) in dry tetrahydrofuran for 30 minutes. The solution was allowed to stand overnight after which time it was heated under reflux for 10 minutes. Evaporation of the solvent under reduced pressure afforded an oily residue which was solidified by trituration with n-hexane (20 ml.). The product was collected by filtration and dried *in vacuo* on KOH. Yield 2.3 g. of D-mandelic acid carboxyanhydride.

IR: γ_{max}^{nuj} 1895, 1875, 1780 cm^{-1}.

The preferred and most active compounds of the present invention are those having the D configuration at the α-carbon atom in the 7-side-chain, that is, those made from D-mandelic acid or a monosubstituted D-mandelic acid as illustrated herein. In addition, the configuration at the two optically active, asymmetric centers in the β-lactam nucleus is that found in cephalosporin C produced by fermentation and in the 7-aminocephalosporanic acid derived therefrom.

Example 1



3, BB-S488

BB-S488; 7-(D-Mandelamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic Acid (3)

D-(-)-Mandelic acid O-carboxyanhydride (U.S. Patents 3,167,549, 3,840,531 and 3,910,900), (*1*, 400 mg., 2.3 m.mole) was added portionwise to a solution of 7-amino-3-(2-carboxy-methyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid (*2*, 657 mg., 1.5 m. mole) and sodium bicarbonate (445 mg., 5.3 m.mole) in 50% aqueous acetone (30 ml.) at ca 0° C. with vigorous stirring. The mixture was stirred for 1 hour at room temperature and evaporated under reduced pressure below 40° C. to remove acetone. The resulting aqueous solution was washed with ether and acidified to pH 1 with dilute HC1 to afford a gummy precipitate, which was collected by filtration, washed with water and dissolved in tetrahydrofuran (THF) (100 ml.). The THF solution was treated with a small amount of active carbon, dried over anhydrous sodium sulfate and filtered. The filtrate was evaporated under reduced pressure and the residue was triturated with ether. The pale yellow precipitate was collected by filtration and chromatographed on a silica gel column (Wako-gel C-200, 10 g.) eluted with a solution of chloroform-methanol (20:1). The fractions containing the desired product were combined and concentrated under reduced pressure. The concentrate was diluted with ether (100 ml.) to precipitate the product (*3*), which was collected by filtration, washed with ether (30 ml.) and dried. Yield 279 mg. (34%). M.p. 173-176° C. (dec.).

ir: ν_{max}^{KBr} 3600-2400, 1770, 1720, 1520, 1495, 1365, 1245 cm^{-1}.

uv: λ_{max}^{EtOH} 254 nm (ε 18000), 297 nm (ε 9000, sh).

nmr: $\delta_{ppm}^{DMSO\text{-}d_6}$ 3.68 (2H, m, 2-$\underline{H}$),

4.03 (1H, d, J=13 Hz, 3-$\underline{H}$), 4.34 (1H, d, J=13 Hz, 3-$\underline{H}$), 4.64 (2H, s, NC$\underline{H}_2$CO), 5.00 (1H, d, J=4 Hz, 6-$\underline{H}$), 5.02 (1H, s, PhC$\underline{H}$), 5.63 (1H, d-d, J=4 & 9 Hz, a doublet with addition of D_2O, J=4 Hz, 7-$\underline{H}$), 6.97 (1H, d, J=10 Hz, pyridazine-$\underline{H}$), 7.1-7.4 (5H, m, phenyl-$\underline{H}$), 7.60 (1H, d, J=10 Hz, pyridazine-$\underline{H}$), 8.60 (1H, d, J=9 Hz, disappeared with addition of D_2O, CONH).

Anal. Calc'd. for $C_{23}H_{20}N_6O_8S_2\cdot1/2H_2O$: C, 47.50; H, 3.64; N, 14.45; S, 11.03. Found: C, 47.34; H, 3.48; N, 13.90; S, 11.01.

In vitro activity (Table 1)

The MIC's were determined by the Steers' agar dilution method using Mueller-Hinton agar against 4 gram-positive and 28 gram-negative bacteria and the results are shown in Table 1.

In vitro activity (Tables 2 and 3)

MIC determinations were performed by serial two-fold agar dilution method using Steers' apparatus on Mueller-Hinton agar plate against 51-gram-positive and 95 gram-negative bacteria. The results are shown in Tables 2 and 3.

Media effect on MIC

The MIC's were determined by using three kinds of agar media [Nutrient (NA), Mueller-Hinton (MHA) and Heart-Infusion (HIA)]. The results obtained with BB-S488 and cefamandole are shown in Table 4, which indicates little media effect in these cephalosporins.

Blood levels in mice

Groups of mice were administered subcutaneously graded doses of 40, 20 and 10 mg./kg.. The blood samples collected from orbital sinuses were assayed by the paper disc-agar diffusion method on *Sarcina lutea* PCI 1001 plates. The results are shown in Table 5.

In vivo activity

Comparative *in vivo* evaluation was made by the standard experimental infection in mice against the following pathogenic bacteria:

S. aureus Smith
E. coli Juhl
K. pneumoniae A9977

The results are shown in Table 6.

TABLE 1. *The in vitro Antibacterial Activity of BB-S488 by Agar Dilution Method (Mueller-Hinton Agar).*

Test Organism		*MIC (mcg./ml.)* *BB-S488*	*Cefamandole*
S. aureus Smith	A9537	0.2	0.05
S. aureus	A9497	0.1	0.05
S. aureus BX-1633	A9606	0.4	0.1
St. faecalis	A9536	100	50
E. coli NIHJ		0.025	0.025
E. coli ATCC 8739		0.1	0.05
E. coli Juhl	A15119	0.2	0.4
E. coli BX-1373		0.2	0.8
E. coli	A15810	0.1	0.4
E. coli	A9660	0.05	0.1
E. coli	A15147	3.1	0.4
Kl. pneumoniae	A9678	3.1	3.1
Kl. pneumoniae	A9977	0.05	0.2
Kl. pneumoniae	A15130	0.2	0.8
Kl. pneumoniae	A9867	0.2	0.8
Pr. vulgaris	A9436	0.1	0.4
Pr. vulgaris	A9699	0.2	6.3
Pr. mirabilis	A9554	0.05	0.4
Pr. mirabilis	A9900	0.1	0.8
Pr. morganii	A9553	>100	>100
Pr. morganii	A20031	0.1	0.8
Pr. rettgeri	A15167	0.05	0.2
Ps. aeruginosa	A9930	>100	>100
Ps. aeruginosa	A9843	>100	>100
Shig. dysenteriae		0.025	0.1
Shig. flexneri	A9684	12.5	6.3
Shig. sonnei	A9516	0.025	0.05
Serr. marcescens	A20019	100	100
Enterob. cloacae	A9656	3.1	3.1
Sal. enteritidis	A9531	0.05	0.1
Sal. typhosa	A9498	0.05	0.1
B. anthracis	A9504	0.1	0.1

TABLE 2. *In vitro Antibacterial Activity in Mueller-Hinton Agar (Gram-positive)*

Code No.	*Test Organism*	*MIC (mcg./ml.)* *BB-S488*	 *Cefamandole*
Sa-2	S. aureus Smith A9537	0.4	0.2
Sa-3	S. aureus No. 193	0.8	0.2
Sa-8	S. aureus	0.4	0.2
Sa-9	S. aureus No. 193	0.8	0.2
Sa-10	S. aureus A20239	1.6	0.4
Sa-11	S. aureus BX-1633 A9606	0.4	0.2
Sa-12	S. aureus A9497	0.2	0.1
Sa-29	S. aureus No. 193	1.6	0.8
Sa-33	S. aureus Terajima	0.0125	0.0125
Sa-34	S. aureus A15092	0.8	0.2
Sa-35	S. aureus A15094	0.8	0.4
Sa-36	S. aureus Russell	0.8	0.4
Sa-37	S. aureus A9524	1.6	0.8
Sa-38	S. aureus A9534	0.4	0.2
Sa-39	S. aureus A9578	0.8	0.4
Sa-40	S. aureus A9601	0.8	0.4
Sa-41	S. aureus A9602	0.8	0.2
Sa-44	S. aureus A15097	25	25
Sa-56	S. aureus A9630	3.1	0.8
Sa-57	S. aureus A9748	25	3.1
Sa-58	S. aureus A15033	12.5	1.6
Sa-59	S. aureus A15096	100	6.3
Sa-60	S. aureus A20604	50	3.1
Sa-61	S. aureus A20605	100	6.3
Sa-62	S. aureus A20606	3.1	0.8
Sa-63	S. aureus A20607	>100	12.5
Sa-64	S. aureus A20608	100	6.3
Sa-65	S. aureus A20609	100	6.3
Sa-66	S. aureus A20610	100	6.3
Sa-67	S. aureus A20611	100	6.3
Sa-68	S. aureus A20612	1.6	0.4
Sa-69	S. aureus A20613	100	6.3
Sp-1	S. pyogenes S-23	0.4	0.1
Sp-2	S. pyogenes Dick	0.4	0.1
Sp-3	S. pyogenes A9604	0.4	0.1
Sp-4	S. pyogenes A20065	0.2	0.1
Sp-5	S. pyogenes A15040	0.4	0.1
Sp-6	S. pyogenes A20066	0.4	0.1
Sp-7	S. pyogenes Dig 7	0.4	0.1
Sp-8	S. pyogenes A15041	0.4	0.1
Sp-9	S. pyogenes A20201	0.4	0.1
Sp-10	S. pyogenes A20202	0.4	0.1
Dp-1	D. pneumoniae Type II	0.2	0.2
Dp-2	D. pneumoniae Type I Neufeld	0.2	0.2
Dp-3	D. pneumoniae Type III	0.2	0.2
Dp-4	D. pneumoniae A9585	0.2	0.2
Dp-5	D. pneumoniae A15069	0.2	0.2
Dp-6	D. pneumoniae A20167	0.2	0.2
Dp-7	D. pneumoniae A20759	0.2	0.2
Dp-8	D. pneumoniae A20769	0.2	0.2
Dp-9	D. pneumoniae A20770	0.2	0.2

TABLE 3. *In vitro Antibacterial Activity in Mueller-Hinton Agar (Gram-negative)*

Code No.	*Test Organism*	*MIC (mcg./ml.)* *BB-S488*	*Cefamandole*
Ec-1	E. coli NIHJ	0.2	0.1
Ec-3	E. coli Juhl A15119	0.2	0.8
Ec-4	E. coli A15169	12.5	6.3
Ec-5	E. coli K-12, ML-1630 A20363	0.2	0.8
Ec-11	E. coli A20366	50	25
Ec-15	E. coli ATCC 8739	0.2	0.1
Ec-34	E. coli A9660	0.1	0.1
Ec-35	E. coli A9435	0.4	0.8
Ec-36	E. coli A15147	3.1	1.6
Ec-40	E. coli A20361	0.2	0.8
Ec-44	E. coli A9535	0.1	0.1
Ec-45	E. coli A15148	3.1	1.6
Ec-46	E. coli A15164	25	12.5
Ec-47	E. coli A15170	100	50
Ec-49	E. coli A20107	0.4	0.2
Ec-50	E. coli A20109	0.2	0.8
Ec-51	E. coli A20343	50	12.5
Ec-56	E. coli A20365	25	12.5
Ec-58	E. coli A9675	0.4	1.6
Ec-59	E. coli A20766	0.2	0.8
Ec-62	E. coli A20895	0.4	0.8
El-1	E. cloacae A9656	3.1	3.1
El-2	E. cloacae A20364	3.1	3.1
El-4	E. cloacae A20650	1.6	1.6
El-6	E. cloacae A9657	0.8	0.8
El-7	E. cloacae A9659	1.6	0.8
El-8	E. cloacae A9655	1.6	1.6
El-9	E. cloacae A20021	>100	100
El-11	E. cloacae A20344	>100	>100
El-12	E. cloacae A21006	1.6	3.1
El-14	E. cloacae A20953	0.8	3.1
Pm-1	P. mirabilis A9554	0.1	0.8
Pm-2	P. mirabilis A9900	0.2	1.6
Pm-3	P. mirabilis A20119	0.4	3.1
Pm-4	P. mirabilis A20454	0.2	1.6
Pm-5	P. mirabilis A9702	0.1	0.8
Pm-6	P. mirabilis A21222	1.6	1.6
Pg-1	P. morganii A9553	>100	>100
Pg-2	P. morganii A20031	0.2	1.6
Pg-3	P. morganii A9636	0.8	1.6
Pg-5	P. morganii A15166	0.1	0.2
Pg-6	P. morganii A20455	0.4	1.6
Pg-7	P. morganii A20457	0.2	0.8
Pg-8	P. morganii A15153	0.1	0.8
Pg-9	P. morganii A15149	0.8	3.1
Pv-1	P. vulgaris A9436	0.2	0.8
Pv-2	P. vulgaris A9526	6.3	1.6
Pv-3	P. vulgaris A9699	6.3	50
Pv-4	P. vulgaris ATCC 9920	0.1	0.2
Pv-5	P. vulgaris A9539	25	>100
Pv-6	P. vulgaris A9716	0.1	0.8
Pv-7	P. vulgaris A21240	25	>100

TABLE 3 (Continued)

Code No.	*Test Organism*	*BB-S488*	*Cefamandole*
Pr-1	P. rettgeri A15167	0.1	0.2
Pr-2	P. rettgeri A9637	0.1	0.1
Pr-4	P. rettgeri A20645	0.1	0.1
Pr-5	P. rettgeri A20915	0.2	0.8
Pr-6	P. rettgeri A20920	0.1	0.2
Pn-1	P. inconstans A20615	0.1	0.8
Ps-1	P. stuartii A20745	0.4	0.8
Ps-2	P. stuartii A20894	0.2	0.8
Ps-3	P. stuartii A20911	0.8	0.8
Ps-4	P. stuartii A21051	50	25
Ps-5	P. stuartii A21057	0.2	0.8
Kp-1	K. pneumoniae D11	0.1	0.8
Kp-2	K. pneumoniae A9678	3.1	1.6
Kp-3	K. pneumoniae A9977	0.1	0.8
Kp-4	K. pneumoniae A15130	0.2	0.8
Kp-7	K. pneumoniae A9867	0.4	0.8
Kp-8	K. pneumoniae A20680	25	12.5
Kp-9	K. pneumoniae A20636	12.5	12.5
Kp-10	K. pneumoniae A20328	6.3	3.1
Kp-11	K. pneumoniae A20330	1.6	12.5
Kp-12	K. pneumoniae A21228	6.3	6.3
Kx-2	Klebsiella sp. A9662	0.4	1.6
Kx-3	Klebsiella sp. A20346	0.2	0.8
Sm-1	S. marcescens A20019	25	25
Sm-2	S. marcescens A20335	3.1	12.5
Sm-3	S. marcescens A20336	6.3	12.5
Sm-4	S. marcescens A20442	6.3	12.5
Sm-5	S. marcescens A20222	3.1	12.5
Sm-6	S. marcescens A20460	6.3	12.5
Sm-9	S. marcescens A20333	6.3	50
Sm-10	S. marcescens A20334	6.3	50
Sm-11	S. marcescens A20459	6.3	25
Sm-12	S. marcescens A20461	6.3	50
Se-1	S. enteritidis A9531	0.1	0.2
St.-1	S. typhosa	0.1	0.2
Sh-1	S. paratyphi	0.1	0.2
St-101	S. typhimurium	0.1	0.2
Sd-1	S. dysenteriae	0.1	0.2
Sr-1	S. flexneri A9684	12.5	3.1
Ss-1	S. sonnei Yale	0.1	0.1
Cx-1	Citrobacter sp. A20673	1.6	1.6
Cx-2	Citrobacter sp. A20694	1.6	1.6
Cx-3	Citrobacter sp. A20695	1.6	1.6

TABLE 4. *Media effect on MIC of BB-S488 and Reference Compounds*

Code No.	*Test Organism*	*BB-S488*			*Cefamandole*		
		NA	*HIA*	*MHA*	*NA*	*HIA*	*MHA*
Sa-2	S. aureus Smith A9537	0.8	0.8	0.8	0.2	0.2	0.2
Sa-11	S. aureus BX-1633 A9606*	3.1	1.6	0.8	0.4	0.4	0.4
Sa-44	S. aureus A15097	>100	>100	100	6.3	3.1	3.1
Sf-3	S. faecalis A9536	>100	>100	>100	>100	100	100
Ec-1	E. coli NIHJ	0.1	0.05	0.05	0.1	0.1	0.05
Ec-3	E. coli Juhl A15119	0.2	0.4	0.2	0.2	0.4	0.4
Ec-11	E. coli A20366*	>100	>100	>100	50	25	50
Ec-15	E. coli ATCC 8739	0.1	0.2	0.2	0.1	0.1	0.1
Ec-36	E. coli A15147*	6.3	6.3	6.3	1.6	3.1	3.1
Ec-46	E. coli A15164*	50	>100	100	25	12.5	12.5
Ec-51	E. coli A20343*	50	>100	>100	12.5	25	25
El-1	E. cloacae A9656	12.5	6.3	3.1	6.3	3.1	3.1
El-2	E. cloacae A20364*	12.5	6.3	3.1	6.3	100	50
El-11	E. cloacae A20344*	>100	>100	>100	>100	>100	>100
Kp-3	K. pneumoniae A9977	0.4	0.2	0.2	0.4	0.8	0.8
Kp-4	K. pneumoniae A15130	0.8	0.8	0.4	0.8	0.8	0.8
Kx-3	Klebsiella sp. A20346*	0.2	0.2	0.2	0.4	0.4	0.4
Pv-1	P. vulgaris A9436	0.2	0.2	0.1	0.2	0.4	0.4
Pv-3	P. vulgaris A9699*	0.8	100	0.8	6.3	100	50
Pm-1	P. mirabilis A9554	0.1	0.1	0.1	0.2	0.4	0.8
Pm-2	P. mirabilis A9900	0.1	0.2	0.2	0.4	0.4	0.8
Pg-1	P. morganii A9553*	>100	>100	>100	>100	>100	>100
Pg-2	P. morganii A20031	0.2	0.2	0.1	0.4	0.8	0.8
Pg-6	P. morganii A20455*	0.4	0.8	0.4	1.6	3.1	1.6
Pr-1	P. rettgeri A15167	0.4	0.1	0.05	0.2	0.1	0.2
Ps-1	P. stuartii A20745	50	50	12.5	6.3	6.3	1.6
Ps-2	P. stuartii A20894	0.8	0.4	0.2	0.4	0.4	0.2
Ps-3	P. stuartii A20911*	50	25	12.5	6.3	6.3	3.1
Sm-1	S. marcescens A20019*	>100	>100	>100	50	50	100
Sm-3	S. marcescens A20336	100	>100	0.8	50	50	100
Pa-3	P. aeruginosa A9930	100	>100	>100	>100	>100	>100
Ba-3	B. anthracis A9504	0.4	0.2	0.2	0.4	0.2	0.4

*β-lactamase +

TABLE 5. *Subcutaneous mice blood levels* Mcg./Ml.

Dose	*Time*	*BB-S488*	*Cefamandole*
40 mg./kg.	15'	19	18
	30'	17	11
	60'	12	3.9
	120'	1.6	0.3
20 mg./kg.	15'	7.4	9.5
	30'	6	4.1
	60'	4.3	1
	120'	0.7	<0.1
10mg./kg.	15'	5	3.8
	30'	3	1.5
	60'	0.8	0.3
	120'	--	0.1

TABLE 6. *In vivo activity*

Test Organism	*Dose*	*BB-S488*	*Cefamandole*
S. aureus Smith	25 mg./kg.	5/5*	5/5
	6.3	5/5	5/5
	1.6	5/5	5/5
	0.4	5/5	1/5
	0.1	2/5	
	PD_{50}	*0.12 mg./kg.*	*0.6 mg./kg.*
E. coli Juhl	25 mg./kg	5/5	5/5
	6.3	5/5	5/5
	1.6	5/5	2/5
	0.4	5/5	0/5
	0.1	2/5	
	PD_{50}	*0.12 mg./kg.*	*1.8 mg./kg.*
K. pneumoniae A9977	25 mg./kg.	5/5	5/5
	6.3	5/5	1/5
	1.6	5/5	0/5
	0.4	4/5	0/5
	0.1	0/5	
	PD_{50}	*0.26 mg./kg.*	*6.25 mg./kg.*

*No. of survivors/No. tested

Test Organism	*Run No.*	*No. of LD_{50}*	*Dose (mg./kg.)*	*BB-S 488*	*Cefam-andole*
E. cloacae	C-811	1 × 10	100	5/5	5/5
A20464 (El.19)			25	5/5	5/5
			6.3	5/5	5/5
			1.6	4/5	4/5
			0.4	2/5	1/5
		PD_{50}(mg.kg.)		*0.54*	*0.8*

Urinary Recovery in Rats

Dose (sc)	*% Recovery (0-24 Hrs.)* *BB-S488*	*Cefamandole*
10 mg./kg.	38.8	58.3

Nephrotoxicity Test in Rabbits

No nephrotoxic sign was seen in the rabbits treated with 100 mg./kg. (iv) of BB-S488 while cephaloridine showed severe nephrotoxicity in the comparative test.

Additional PD_{50} Data (Single sc Treatment)

Test Organism	*Run No.*	*No. of LD_{50}*	*Dose (mg./kg.)*	*BB-S 488*	*Cefam-andole*
K. pneumoniae	C-805	3×10^3	25	5/5	5/5
A9977 (Kp-3)			6.3	5/5	1/5
			1.6	5/5	0/5
			0.4	4/5	0/5
			0.1	0/5	---
		PD_{50}(mg./kg.)		*0.26*	*9.4*
P. vulgaris	C-808	1×10	50	---	3/5
A9436 (Pv-1)			25	5/5	---
			12.5	---	1/5
			6.3	5/5	---
			3.1	---	0/5
			1.6	3/5	---
			0.8	---	0/5
			0.4	1/5	---
		PD_{50}(mg./kg.)		*1.1*	*36*
P. mirabilis	C-810	1×10^3	50	---	2/5
A9900 (Pm-2)			25	5/5	---
			12.5	---	1/5
			6.3	5/5	---
			3.1	---	0/5
			1.6	4/5	---
			0.8	---	0/5
			0.4	0/5	---
			0.2	---	0/5
			0.1	0/5	---
		PD_{50}(mg./kg.)		*1.1*	*50*

Stability of BB-S488

Stability of BB-S488 was determined in both a 10% and an 0.02% solution. The stability is indicated as the relative activity remaining in the test solution at given periods to the initial solution. The activity was assayed using paper discs on *B. subtilis* PCI 219 plate (pH 6).

(1) *Stability in a 10% Aqueous Solution at Room Temperature*

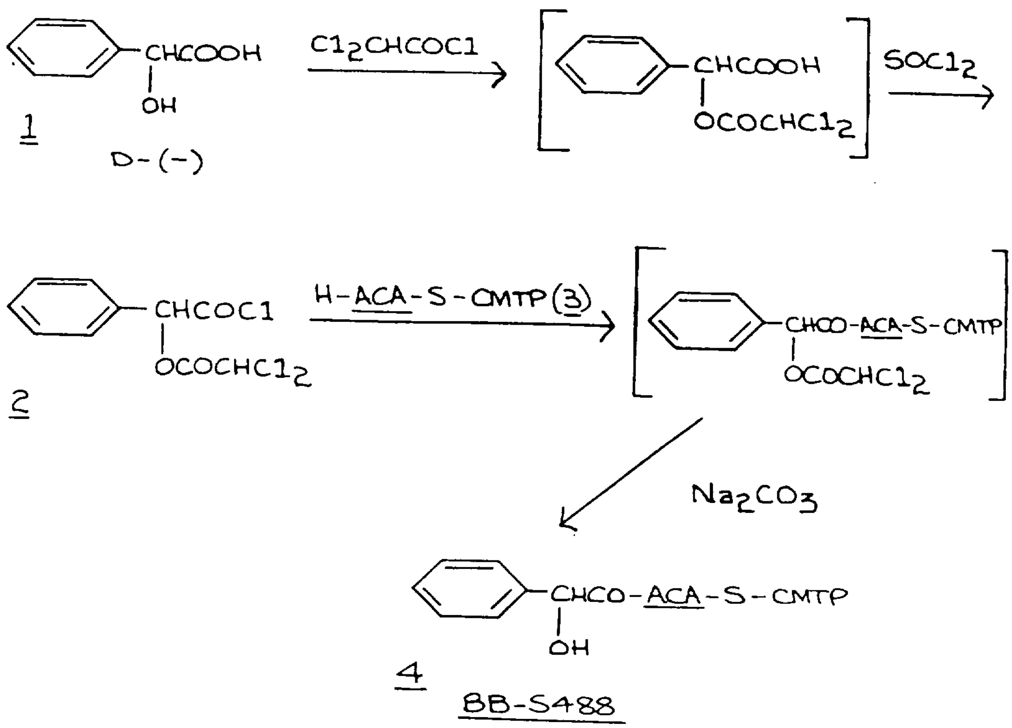
Compound	*pH*[(1)]	*Remaining Activity (%)* 0	2	3	7 *Days*
BB-S488	6.1	100	128	90	116

[(1)] Unadjusted pH of the 10% solution.

Compound	*pH*[(2)]	*Remaining Activity (%)* 0	1	2	3	7 *Days*
BB-S488	4	100		93	78	102
	7	100		87	64	56
	9	100	20	14	13	0

[(2)] pH 4: 0.1 M AcOH - NaOAc buffer.
pH 7: 0.1 M phosphate buffer.
pH 9: 0.1 M NH_4OH-NH_4Cl buffer.

Example 2



Dichloroacetylmandeloyl chloride(2)

A mixture of D(-)-mandelic acid (*1*, 1.52 g., 10 m.mole) and dichloroacetyl chloride (4.41 g., 30 m.mole) was heated at 80-85° C. for 1.5 hrs. and the excess dichloroacetyl chloride was removed under diminished pressure. To the residue was added thionyl chloride (2.5 ml.) and the mixture was heated under reflux for 1.5 hrs. Excess thionyl chloride was removed by distillation and dry benzene was added. Evaporation was repeated. The residual oil was kept over KOH at 1 mm Hg overnight at room temperature to remove dichloroacetyl chloride. Yield, 2.8 g. (100%). This product was used in the next step without further purification.

ir: $\nu_{\text{max}}^{\text{liq.}}$ 1780, 1160 cm^{-1}.

nmr: $\delta_{\text{ppm}}^{\text{CCl}_4}$ 5.91 (1H, s, PhCH or COCHCl$_2$), 6.00 (1H, s, PhCH or COCHCl$_2$), 7.32 (5H, s, phenyl-$\underline{H}$).

BB-S488; 7-(D-Mandelamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic Acid. (*4*)

A solution of the above-obtained dichloroacetyl-mandeloyl chloride (2, 2.8 g., 10 m.mole) in dry acetone (30 ml.) was added dropwise to a stirred solution of 7-amino-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]-pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid (H-*ACA*-S-CMTP)(*3*, 3.94 g., 9 m.mole) and triethylamine (3.54 g., 35 m.mole) in 50% aqueous acetone (120 ml.) at 0°-5° C. The mixture was allowed to rise to room temperature during 1 hour with stirring and was adjusted to pH 11 with 5% aqueous sodium carbonate (ca 12 ml. was required). The mixture was allowed to stand at room temperature for 30 minutes, acidified to pH 1 with dilute HCl and evaporated under reduced pressure to remove acetone below 40° c. The precipitate was collected by filtration, washed with water (20 ml.) and air-dried. The dried material was dissolved in THF (150 ml.), stirred for 5 minutes at 40-50° C. and filtered to remove insoluble unreacted *3* (0.54 g., 14% recovery). The filtrate was chromatographed on a silica gel column (Wako-gel, C-200, 30 g.) and eluted with chloroform-methanol (100:5). The eluates were collected in 50 ml. fractions monitoring by tlc (silica gel, solvent, CH$_3$CN-water = 4:1, detected with I$_2$). The fractions containing the desired product were combined, treated with a small amount of carbon and evaporated under reduced pressure. The residue was triturated with chloroform (50 ml.) to yield, 2.36 g. (46%) of 7-(D-mandelamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid (*4*). M.P., 165-170° C. (dec.).

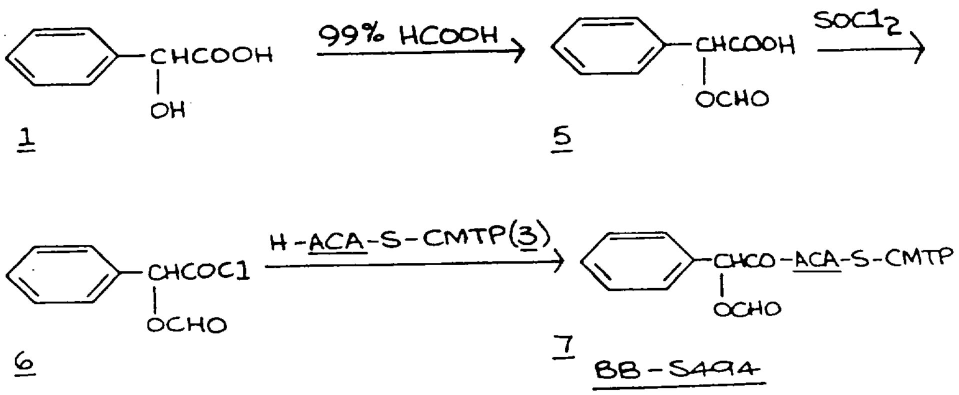
ir: $\nu_{\text{max}}^{\text{KBr}}$ 3600-2500, 1780, 1720, 1500, 1410, 1355, 1220, 1195 cm^{-1}.

uv: $\lambda_{\text{max}}^{\text{EtOH}}$ 254 nm (ϵ, 18300), 297 nm (sh, ϵ, 9300).

nmr: $\delta_{\text{ppm}}^{\text{DMSO-d}_6}$ 3.84 (2H, m, 2-$\underline{H}$), 4.17 (2H, d, 13 Hz, 3-$\underline{H}$), 4.50 (1H, d, 13 Hz, 3-$\underline{H}$), 4.82 (2H, s, NCH$_2$COO), 5.20 (1H, d, 4.5 Hz, 6-$\underline{H}$), 5.25 (1H, s, PhCH), 5.87 (1H, d-d, 4.5 & 9 Hz, 7-$\underline{H}$, a doublet (J=4.5 Hz) by addition of D$_2$O), 7.25 (1H, d, 11 Hz, pyridazine-$\underline{H}$), 7.4-7.7 (5H, m, phenyl-$\underline{H}$), 7.90 (1H, d, 11 Hz, pyridazine-$\underline{H}$), 9.0 (1H, d, 9 Hz, 7-CONH, disappear by addition of D$_2$O).

Anal. Calc'd for C$_{23}$H$_{20}$N$_6$O$_8$S$_2$3/4CHCl$_3$: C, 43.08; H, 3.16; N, 12.69; S, 9.69. Found: C, 43.11, 43.22; H, 2.97, 3.06; N, 12.80, 12.77; S, 9.64.

Example 3



O-Formyl-D(-)-mandelic Acid (5)

A mixture of D(-)-mandelic acid (*1*, 5.0 g., 33 m.mole) and 99% formic acid (80 ml.) was heated at 80-90° C. for 12 hours. The mixture was evaporated and toluene (100 ml.) was added to the residue and evaporated under reduced pressure to remove formic acid azeotropically. The residue was dissolved in benzene (200 ml.) and the solution was washed with water (2 × 50 ml.). The organic layer was separated, dried with anhydrous sodium sulfate and evaporated under reduced pressure. The residual oil was triturated with cyclohexane (50 ml.) to crystallize. Yield, 3.70 g. (63%) of O-formyl-D(-)-mandelic acid (*5*) as colorless prisms. M.P., 56-59° C. (lit. M.P., 55-58° C.).

ir: ν_{max}^{KBr} 3400-2800, 1755, 1720, 1160, 990 cm^{-1}.

nmr: $\delta_{ppm}^{CDCl_3}$ 5.98 (1H, s, PhCH),

7.31 (5H, m, phenyl-$\underline{H}$), 8.05 (1H, s, OCHO), 10.05 (1H, s, COOH, disappeared by addition of D_2O).

O-Formyl-D(-)-mandeloyl Chloride (6)

A mixture of *5* (2.0 g., 11 m.mole) and thionyl chloride (10 ml.) was heated under reflux for 2 hours. Evaporation of the excess thionyl chloride and distillation of the residue under reduced pressure afforded the acid chloride O-formyl-D(-)-mandeloyl chloride (*6*). Yield, 1,53 g. (70%). B.P., 120-122° C./15 mmHg.

ir: $\nu_{max}^{liq.}$ 1805, 1740, 1160, 1140 cm^{-1}.

BB-S494; 7-(D-O-Formylmandelamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic Acid (7)

A solution of O-formyl-D(-)-mandeloyl chloride (*6*) (1.0 g., 5.1 m.mole) in dry acetone (10 ml.) was added dropwise to a cold (0 to 5° C.) solution of 7-amino-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid (*3*, 1.75 g., 4 m.mole) in 50% aqueous acetone (70 ml.) containing sodium bicarbonate (1.34 g., 16 m.mole). The mixture was stirred for 30 minutes at room temperature and washed with ether. The aqueous layer was acidified to pH 1 with dilute HCl. The separated oily gum was collected and dissolved in THF (100 ml.). The solution was treated with a small amount of carbon and dried with anhydrous sodium sulfate. Evaporation of the solvent under reduced pressure to 10 ml. and dilution with ether afforded the title compound (*7*) as a pale yellow amorphous powder, 0.91 g. (38%). M.P., 172-176° C.(dec.).

ir: ν_{max}^{KBr} 3600-2400, 1775, 1720, 1550, 1355, 1230, 1160 cm^{-1}.

uv: λ_{max}^{EtOH} 254 nm (ϵ, 20800), 297 nm (sh, ϵ, 10500).

nmr: $\delta_{ppm}^{DMSO\text{-}d_6}$ 3.4-4.5 (4H, m, 2-$\underline{H}$ and 3-$\underline{H}$),

4.67 (2H, s, NCH_2COO), 4.97 (1H, d, 4 Hz, 6-$\underline{H}$), 5.66 (1H, d-d, 4 & 8 Hz, 7-$\underline{H}$), 7.2-7.5 (5H, m, phenyl-$\underline{H}$), 7.64 (1H, d, 10 Hz, pyridazine-$\underline{H}$), 8.29 (1H, s, CHO), 9.29 (1H, d, 8 Hz, 7-CONH, disappeared by addition of D_2O).

Anal. Calc'd. for $C_{24}H_{20}N_6O_9S_2 \cdot 1H_2O$: C, 46.60; H, 3.58; N, 13.59; S, 10.37. Found: C, 46.70, 47.20; H, 3.25, 3.34; N, 13.37, 13.78; S, 10.84.

Example 4

BB-S488; 7-(D-Mandelamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic Acid (4)

A mixture of 7-(D-O-formylmandelamido-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid (*7*) (484 mg., 0.81 m.mole) and sodium bicarbonate (748 mg., 8.9 m.mole) in water (4 ml.) was stirred for 4 hours at room temperature, and acidified to pH 1 with dilute HCl. The precipitate (500 mg.) was collected by filtration, washed with water (2 ml.) and chromatographed on silica gel column (Wako-gel, C-200, 5 g.). The column was eluted with chloroform containing increasing methanol (3-5%) as eluent, and the fractions containing the product were combined, treated with a small amount of carbon and evaporated under reduced pressure.

The residue was triturated with ether to give 277 mg. of 7-(D-mandelamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid (BB-S488; *4*). The nmr-estimation of this product showed 10% of *7* still remained.

ir: ν_{max}^{KBr} 3600-2400, 1770, 1720, 1520, 1495, 1365, 1230 cm^{-1}.

uv: λ_{max}^{EtOH} 254 nm (ϵ, 20000), 297 nm (sh, ϵ, 9600).

Example 5

Sodium salt of BB-S488

Sodium-2-ethylhexanoate (SEH) (4.0 ml., 1 *M* solution in ethyl acetate) was added to a solution of 7-(D-mandelamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid (*4*) (2.25 g., 3.93 m.mole) in THF (200 ml.). The precipitate was collected by filtration, washed with THF (50 ml.) and dried at 60° C./1 mmHg for 3 hours. Yield of sodium 7-(D-mandelamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylate, 1.96 g. (bio-yield, 97%), M.P., 230-240° C. (dec.). The pH of the 10% aqueous solution was 3.6.

ir: ν_{max}^{KBr} 3600-3000, 1765, 1710, 1605, 1390, 1360, 1190, 1080, 1065 cm^{-1}.

uv: λ_{max}^{water} nm($E_{1\,cm}^{1\%}$) 252 (357), 310 (sh, 140).

nmr: $\delta_{ppm}^{D_2O}$ 3.43 (1H, d, 19 Hz, 2-$\underline{H}$),

3.87 (1H, d, 19 Hz, 2-$\underline{H}$), 4.15 (1H, d, 14 Hz, 3-$\underline{H}$), 4.53 (1H, d, 14 Hz, 3-$\underline{H}$), 5.16 (1H, d, 4.5 Hz, 6-$\underline{H}$), 5.36 (1H, s, PhCH), 5.73 (1H, d, 4.5 Hz, 7-$\underline{H}$), 7.13 (1H, d, 10 Hz, pyridazine-$\underline{H}$), 7.57 (5H, s, phenyl-$\underline{H}$), 7.69 (1H, d, 10 Hz, pyridazine-$\underline{H}$).

Anal. Calc'd. for $C_{23}H_{19}N_6O_8S_2Na \cdot 5/4H_2O$: C, 44.77; H, 3.51; N, 13.62; S, 10.39. Found: C, 44.93, 44.79; H, 3.31, 3.15; N, 13.41, 13.33; S, 10.19.

Example 6

Aqueous 1*N* sodium hydroxide solution was added dropwise to a suspension of 7-(D-mandelamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid (*4*)(3.51 g.) in water (20 ml.) to adjust to pH 6.0. The solution was lyophilized to yield 3.4 g. (bio-yield, 97%) of disodium 7-(D-mandelamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylate. M.p., >240° C. (dec.). The pH of the 5% aqueous solution was 5.4.

ir: ν_{KBr}^{max} 3600-3000, 1760, 1710, 1605, 1390, 1360, 1190, 1080, 1060 cm^{-1}.

uv: λ_{max}^{water} nm ($E_{1cm}^{1\%}$) 252 (320), 310 (124).

nmr: $\delta_{ppm}^{D_2O}$ 3.43 (1H, d, 19 Hz, 2-*H*),

3.90 (1H, d, 19 Hz, 2-$\underline{H}$), 4.15 (1H, d, 14 Hz, 3-$\underline{H}$), 4.53 (1H, d, 14 Hz, 3-$\underline{H}$), 4.75 (2H, s, NCH_2CO), 5.22 (1H, d, 4.5 Hz, 6-$\underline{H}$), 5.42 (1H, s, PhCH), 5.73 (1H, d, 4.5 Hz, 7-$\underline{H}$), 7.22 (1H, d, 10 Hz, pyridazine-$\underline{H}$), 7.65 (5H, s, phenyl-$\underline{H}$), 7.77 (1H, d, 10 Hz, pyridazine-$\underline{H}$).

Anal. Calc'd. for $C_{23}H_{18}N_6O_8S_2Na_2 \cdot 3/2H_2O$: C, 42.92; H, 3.29; N, 13.06; S, 9.96. Found: C, 42.90, 43.19; H, 3.06, 3.01; N, 13.04, 13.03; S, 9.97.

Example 7

Substitution of the D-mandelic acid carboxyanhydride in the procedure of Example 1 of an equimolar weight of the carboxyanhydrides prepared in similar fashion from the monosubstituted D-mandelic acids

D-2-chloro-mandelic acid,

D-3-chloro-mandelic acid,

D-4-chloro-mandelic acid,
D-2-bromo-mandelic acid,
D-3-bromo-mandelic acid,
D-4-bromo-mandelic acid,
D-2-fluoro-mandelic acid,
D-3-fluoro-mandelic acid,
D-4-fluoro-mandelic acid,
D-2-trifluoromethyl-mandelic acid,
D-3-triflurometh yl-mandelic acid,
D-4-trifluoromethyl-mandelic acid,
D-2-amino-mandelic acid,
D-3-amino-mandelic acid,
D-4-amino-mandelic acid,
D-2-nitro-mandelic acid,
D-3-nitro-mandelic acid,
D-4-nitro-mandelic acid,
D-2-hydroxy-mandelic acid,
D-3-hydroxy-mandelic acid,
D-4-hydroxy-mandelic acid,
D-2-methyl-mandelic acid,
D-3-methyl-mandelic acid,
D-4-methyl-mandelic acid,
D-2-methoxy-mandelic acid,
D-3-methoxy-mandelic acid and
D-4-methoxy-mandelic acid respectively produces
7-(D-2-chloro-mandelamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-3-chloro-mandelamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-4-chloro-mandelamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-2-bromo-mandelamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-3-bromo-mandelamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-4-bromo-mandelamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-2-fluoro-mandelamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-3-fluoro-mandelamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-4-fluoro-mandelamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-2-trifluoromethyl-mandelamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-3-trifluoromethyl-mandelamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-4-trifluoromethyl-mandelamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-2-amino-mandelamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-3-amino-mandelamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-4-amino-mandelamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-2-nitro-mandelamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-3-nitro-mandelamido-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-4-nitro-mandelamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-2-hydroxy-mandelamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-cephem-4-carboxylic acid,
7-(D-3-hydroxy-mandelamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,

7-(D-4-hydroxy-mandelamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-2-methyl-mandelamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-3-methyl-mandelamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-4-methyl-mandelamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-2-methoxy-mandelamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-3-methoxy-mandelamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid, and
7-(D-4-methoxy-mandelamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid, respectively.

Example 8

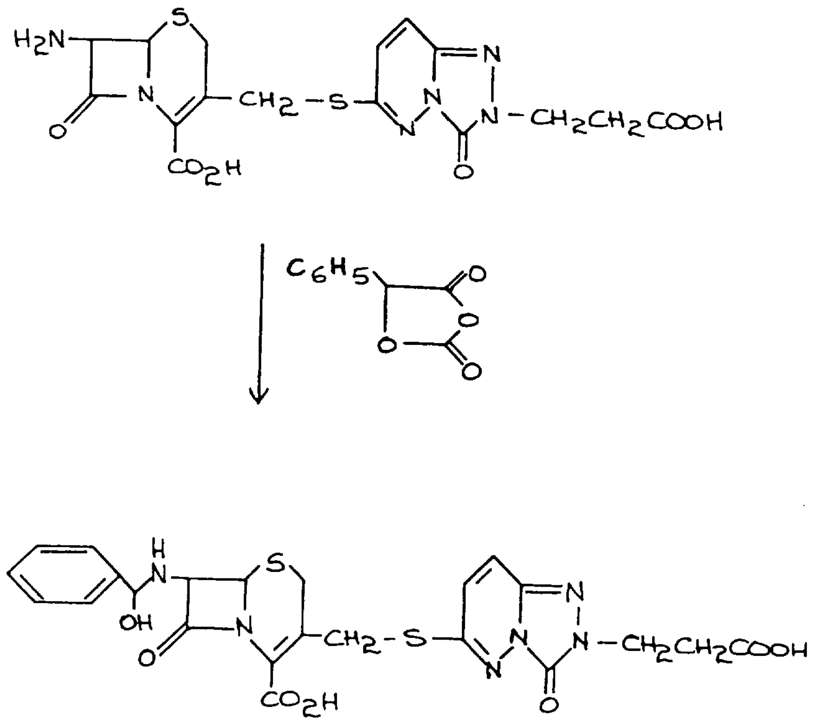
Substitution for the D-mandelic acid carboxyanhydride in the procedure of Example 1 of an equimolar weight of the carboxyanhydride prepared in similar fashion from D-2-thiopheneglycolic acid and D-3-thiopheneglycolic acid respectively produces 7-(D-α-hydroxy-2-thienylacetamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid and 7-(D-α-hydroxy-3-thienylacetamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid, respectively.

Example 9

7-(D-α-hydroxy-α-phenylacetamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid prepared from 7-D-mandelamidocephalosporanic acid.

0.27 Mole of sodium 7-D-mandelamidocephalosporanate is suspended in 1000 ml. of 0.1 M phosphate buffer of pH 6.4 and there is added 0.31 moles of 2-carboxymethyl-2,3-dihydro-6-mercapto-s-triazolo[4,3-b]pyridazin-3-one. The solution is heated at 55° C. under a nitrogen atmosphere for five hours. After one hour the pH is adjusted to 6.4 by addition of a small amount of 40% H_3PO_4. At the end of the five hour heating period the solution is cooled to 23° C. and the pH adjusted to 2 by the addition of 3 *N* HCl under a layer of ethyl acetate. The product is extracted into ethyl acetate and stirred for 15 min. at 23° C. with 2 g. of ("Darco KB") decolorizing charcoal. The mixture is then filtered through a pad of diatomaceous earth ("Celite") and the ethyl acetate removed from the filtrate under vacuum. The residue is triturated to a solid with diethyl ether, collected by filtration and dried over P_2O_5 under vacuum to yield solid 7-(D-α-hydroxy-α-phenylacetamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid.

Example 10



BB-S 527; 7-[D(-)-Mandelamido]-3-[2-(2-carboxyethyl)-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl]-3-cephem-4-carboxylic acid

To a mixture of 7-amino-3-[2-carboxyethyl)-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl]-3-cephem-4-carboxylic acid (679 mg, 1.5 m mol) and $NaHCO_3$ (445 mg, 5.3 m mol) in 50% aqueous acetone was added D-(-)-mandelic acid O-carboxyanhydride (400 mg, 2.3 m mol) at 0° C. The mixture was stirred at room temperature for 1 hour and evaporated to remove the organic solvent. The aqueous solution was washed with ether (3 × 10 ml), adjusted to pH 1 with dil. HCl and filtered to collect the crude product, which was dissolved in THF (10 ml), filtered to remove insolubles and evaporated under reduced pressure. The oily residue was triturated with ether. The solid (476 mg) was chromatographed on a column of silica gel (Wako-gle C-200, 10 g) and eluted with MeOH-$CHCl_3$ (MeOH: 0-3%). Fractions which contained the desired product were combined and evaporated to yield 287 mg (33%) of BB-S 527. M.p. >155° C (dec.).

ir: ν_{max}^{KBr} 3600 - 2400, 1780, 1720, 1550, 1520 cm^{-1}.

uv: $\lambda_{max}^{pH\ 7\ buffer}$ 253 nm (ϵ 20000), 298 nm (ϵ 9000).

Anal. Calc'd. for C_{24},$H_{22}N_6O_8S_2.3/2H_2O$: C, 46.98; H, 4.11; N, 13.70; S, 10.45. Found: C, 47.25, 47.39; H, 3.80, 3.76; N, 12.87, 12.77; S, 10.17.

The sodium salt of BB-S 527

A suspension of the free acid of BB-S 527 (240 mg, 0.4 m mol) in water (5 ml) was adjusted at pH 6.8 with 1 N-NaOH (0.7 ml) to give the clear solution, which was freeze-dried to leave 234 mg (91%, bio-yield) of the sodium salt of BB-S 527 as pale yellow powder. M.p. >210°C (dec.).

ir: ν_{max}^{KBr} 3600 - 2800, 1770, 1710, 1600 cm^{-1}.

uv: $\lambda_{max}^{pH\ 7\ Buffer}$ 253 nm (ϵ 21000) 298 nm (ϵ 9300).

Anal. Calc'd. for $C_{24}H_{20}N_6O_8S_2Na_2 \cdot 2H_2O$: C, 43.24; H, 3.63; N, 12.61; S, 9.62. Found: C, 43.39, 43.43; H, 3.20, 3.36; N, 12.63, 12.68; S, 9.42, 9.22.

In vitro antibacterial activity of BB-S 527 compared with BB-S 488 and cefamandole (determined by Steer's agar dilution method on Mueller-Hinton agar plate)

Organism	*BB-S 527*	*MIC (mcg/ml)* *BB-S 488*	*cefamandole*
S. aureus Smith	1.6	0.8	0.2
S. aureus	0.4	0.4	0.1
S. aureus BX-1633	3.1	3.1	0.4
St. faecalis	>100	>100	>100
E. coli NIHJ	0.4	0.2	0.05
E. coli ATCC 8739	12.5	6.3	3.1
E. coli Juhl	0.4	0.2	0.8
E. coli BX-1373	6.3	3.1	3.1
E. coli	0.1	0.1	0.1
E. coli	0.1	0.05	0.1
E. coli	6.3	3.1	1.6
Kl. pneumoniae	6.3	3.1	3.1
Kl. pneumoniae	0.2	0.1	0.8
Kl. pneumoniae	0.8	0.4	0.8
Kl. pneumoniae	0.4	0.2	0.8
Pr. vulgaris	0.1	0.1	0.2
Pr. vulgaris	12.5	0.8	50
Pr. mirabilis	0.2	0.05	0.8
Pr. mirabilis	0.1	0.05	0.2
Pr. morganii	>100	>100	>100
Pr. morganii	0.4	0.2	0.8
Pr. rettgeri	0.2	0.2	0.4
Ps. aeruginosa	>100	>100	>100
Ps. aeruginosa	>100	>100	>100
Shig. dysenteriae	0.025	0.025	0.1
Shig. flexneri	50	25	6.3
Shig. sonnei	0.1	0.05	0.2
Serr. marcescens	>100	>100	100
Enterob. cloacae	6.3	3.1	3.1
Sal. enteritidis	0.05	0.025	0.05
Sal. typhosa	0.1	0.05	0.1
B. anthracis	0.4	0.2	0.4

Example 11

Substitution for the D-mandelic acid carboxyanhydride in the procedure of Example 10 of an equimolar weight of the carboxyanhydrides prepared in similar fashion from the monosubstituted D-mandelic acids
D-2-chloro-mandelic acid,
D-3-chloro-mandelic acid,
D-4-chloro-mandelic acid,
D-2-bromo-mandelic acid,
D-3-bromo-mandelic acid,
D-4-bromo-mandelic acid,
D-2-fluoro-mandelic acid,
D-3-fluoro-mandelic acid,
D-4-fluoro-mandelic acid,
D-2-trifluoromethyl-mandelic acid,
D-3-trifluoromethyl-mandelic acid,
D-4-trifluoromethyl-mandelic acid,
D-2-amino-mandelic acid,
D-3-amino-mandelic acid,
D-4-amino-mandelic acid,
D-2-nitro-mandelic acid,
D-3-nitro-mandelic acid,
D-4-nitro-mandelic acid,
D-2-hydroxy-mandelic acid,
D-3-hydroxy-mandelic acid,
D-4-hydroxy-mandelic acid,
D-2-methyl-mandelic acid,
D-3-methyl-mandelic acid,
D-4-methyl-mandelic acid,

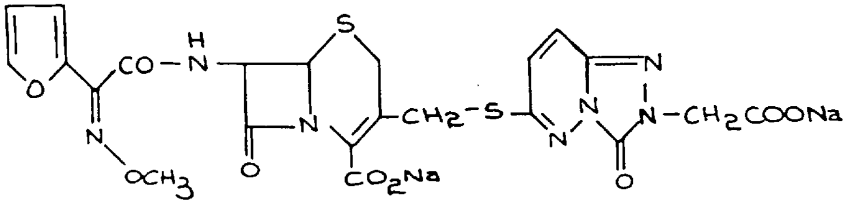
D-2-methoxy-mandelic acid,
D-3-methoxy-mandelic acid and
D-4-methoxy-mandelic acid respectively produces
7-(D-2-chloro-mandelamido)-3-(2-carboxyethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-3-chloro-mandelamido)-3-(2-carboxyethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-4-chloro-mandelamido)-3-(2-carboxyethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-2-bromo-mandelamido)-3-(2-carboxyethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-3-bromo-mandelamido)-3-(2-carboxyethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-4-bromo-mandelamido)-3-(2-carboxyethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-2-fluoro-mandelamido)-3-(2-carboxyethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-3-fluoro-mandelamido)-3-(2-carboxyethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-4-fluoro-mandelamido)-3-(2-carboxyethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-2-trifluoromethyl-mandelamido)-3-(2-carboxyethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-3-trifluoromethyl-mandelamido)-3-(2-carboxyethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-4-trifluoromethyl-mandelamido)-3-(2-carboxyethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-2-amino-mandelamido)-3-(2-carboxyethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-3-amino-mandelamido)-3-(2-carboxyethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-4-amino-mandelamido)-3-(2-carboxyethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-2-nitro-mandelamido)-3-(2-carboxyethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-3-nitro-mandelamido)-3-(2-carboxyethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-4-nitro-mandelamido)-3-(2-carboxyethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-2-hydroxy-mandelamido)-3-(2-carboxyethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-3-hydroxy-mandelamido)-3-(2-carboxyethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-4-hydroxy-mandelamido)-3-(2-carboxyethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-2-methyl-mandelamido)-3-(2-carboxyethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-3-methyl-mandelamido)-3-(2-carboxyethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-4-methyl-mandelamido)-3-(2-carboxyethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-2-methoxy-mandelamido)-3-(2-carboxyethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(D-3-methoxy-mandelamido)-3-(2-carboxyethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid, and
7-(D-4-methoxy-mandelamido)-3-(2-carboxyethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid, respectively.

Example 12

Substitution for the D-mandelic acid carboxyanhydride in the procedure of Example 10 of an equimolar weight of the carboxyanhydride prepared in similar fashion from D-2-thiopheneglycolic acid and D-3-thiopheneglycolic acid respectively produces 7-(D-α-hydroxy-2-thienylacetamido)-3-(2-carboxyethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl]-3-cephem-4-carboxylic acid and 7-(D-α-hydroxy-3-thienylacetamido)-3-(2-carboxyethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-

carboxylic acid, respectively.

Example 13



7-[(2Z)-2-Methoxyimino(fur-2-yl)acetamido]-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic Acid Disodium Salt; BB-S511.

To a solution of (2Z)-2-methoxyimino(fur-2-yl)-acetic acid (507 mg., 3 m.moles) and triethylamine (0.42 ml., 3 m.moles) in dichloromethane (6 ml.) was added oxalyl chloride (0.26 ml., 3 m.moles) at 0-5° C. and the mixture was stirred for 30 minutes. The mixture was evaporated at reduced pressure to give an oily residue of the acid chloride which was dissolved in dry acetone (10 ml.). After filtration the acetone solution was added to a mixture of 7-amino-3-(2-carboxyethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid (1.31 g., 3 m.moles) and NaHCO$_3$ (504 mg., 6 m.moles) in water (20 ml.) at 0-5° C. The mixture was stirred at 0-5° C. for 2 hours with the acetone being removed under reduced pressure. The aqueous solution was washed with ether (2 × 50 ml.) and adjusted to pH 1-2 with conc. HCl to afford a precipitate which was collected by filtration, washed with water and dried *in vacuo*. The solid was dissolved in THF (tetrahydrofuran) (40 ml.) and filtered. To the filtrate was added 1 *M*-SEH (sodium ethylhexanoate) in ethyl acetate (3 ml.) and the resulting precipitate was collected by filtration and dried *in vacuo*. Yield of 7-[(2Z)-2-methoxyimino(fur-2-yl)acetamido]-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid disodium salt was 1.0 g. (54%) mp >210° C. (dec.).

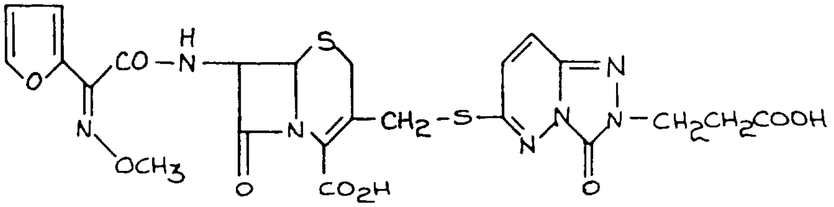
ir: $\nu^{\text{KBr}}_{\text{max}}$ 1770, 1710, 1680, 1610, 1550 cm^{-1}.

uv: $\lambda^{\text{pH 7 buffer}}_{\text{max}}$ 257 nm (ε 25000), 277 nm (ε 24000).

nmr: $\delta^{\text{DMSO-d}_6}_{\text{ppm}}$ 7.70 (1H, br, furan-Hα), 7.52 (1H, d, J=9.5 Hz, pyridazine-H), 6.87 (1H, br, furan-Hα), 7.52 (1H, d, J=9.5 Hz, pyridazine-H), 6.5-6.6 (2H, m, furan-Hβ), 5.58 (1H, m, 7-H), 5.00 (1H, d, J=4.5 Hz, 6-H), 3.84 (3H, s, OC$\underline{H}_3$).

Anal. Calc'd. for $C_{22}H_{17}N_7O_9S_2Na_2 \cdot H_2O$: C, 40.55; H, 2.94; N, 15.05; S, 9.84. Found: C, 40.81, 41.02; H, 3.08, 3.22; N, 14.69, 14.86; S, 9.70, 9.62.

Example 14



7-[2Z)-2-Methoxyimino(fur-2-yl)acetamido]-3-[2-(2-carboxyethyl)-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl]-3-cephem-4-carboxylic Acid Sodium Salt; BB-S526.

The acid chloride prepared from (2Z)-2-methoxyimino(fur-2-yl)acetic acid (169 mg., 1 m.mole) was dissolved in dry acetone (5 ml.) and filtered to remove insolubles. The filtrate was added to a solution of 7-amino-3-[2-(2-carboxyethyl)-2,3-dihydro-s-triazolo-[4,3-b]pyridazin-3-on-6-ylthiomethyl]-3-cephem-4-carboxylic acid, (452 mg., 1 m.mole) and $NaHCO_3$ (336 mg., 4 m.mole) in water (10 ml.). The reaction mixture was stirred for 2 hours in an ice-water bath. Acetone was removed at reduced pressure. The aqueous solution was washed with ether (2 × 10 ml.) and adjusted to pH 1-2 with conc. HCl. The resulting precipitate was collected by filtration, washed with water and dried under reduced pressure. A solution of the precipitate in THF (10 ml.) was treated with active carbon. A SEH solution in ethyl acetate (1 *M*, 0.8 ml.) was added to the THF solution to give 7-[(2Z)-2-methoxyimino(fur-2-yl)acetamido]-3-[2-(2-carboxyethyl)-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl]-3-cephem-4-carboxylic acid sodium salt which was collected by filtration. Yield: 410 mg. (66% as mono Na salt). Mp. >205° C. (dec.).

ir: ν_{max}^{KBr} 3600-2800, 1770, 1710, 1600, 1550 cm^{-1}.

uv: $\lambda_{max}^{pH\ 7\ buffer}$ 257 nm (ϵ 27000), 276 (ϵ 26100).

nmr: $\delta_{ppm}^{DMSO\text{-}d_6+D_2O}$ 2.70, t, J=6 Hz, $C\underline{H}_2$),

3.3-4.5 (9H, m, 2-H, 3-$C\underline{H}_2$, CH_2 and $OC\underline{H}_3$), 4.96 (1H, d, J=5.5 Hz, 6-H), 5.53 (1H, d, J=5.5 Hz, 7-H), 6.55 (2H, m, furan-H), 6.92 (1H, d, J=10 Hz, pyridazine-H), 7.53 (1H, d, J=10 Hz, pyridazine-H), 7.68 (1H, br, furan-H).

Anal. Calc'd. for $C_{23}H_{20}N_7O_9S_2Na{\cdot}H_2O$: C, 42.92; H, 3.45; N, 15.23; S, 9.96. Found: C, 43.08, 42.77; H, 3.20, 3.03; N, 14.96, 14.76; S, 9.96.

TABLE 7

In vitro Activity Using Mueller-Hinton Agar
By the Serial Dilution Method

		Geometric Mean of MIC (Mcg./ml)	
	BB-S511 (Ex. 13)	*BB-S526 (Ex. 14)*	*Cefuroxime*
S. aureus (3 strains)	1.97	1.6	1.24
E. coli (7)	0.58	0.78	1.28
Kl. pneumoniae (4)	0.47	0.93	3.1
Proteus (6)	0.021	0.061	0.88
Shig.(3), Serr.(1) Enterab.(1), Sal.(2)	1.11	2.41	4.06
B. anthracis (1)			
S. pyogenes (5)	0.032	0.032	0.025
S. viridans (5)	0.15	0.4	0.1
D. pneumoniae (5)	0.037	0.056	0.0125
N. meningitidis (5)	2.37	3.60	1.6
N. gonorrhoeae (5)	1.36	1.6	0.4
H. influenzae (7)	0.64	0.71	1.16

Cefuroxime is sodium 6R, 7R-3-carbamoyloxymethyl-7-(2Z)-2-methoxyimino(fur-2-yl)acetamidoceph-3-em-4-carboxylate.

TABLE 8

Geometric Means of MIC's Against 3 strains of S. aureus and 27 strains of Gram-negative Bacteria
(mcg./ml., Mueller-Hinton Agar)

	No. of Strains	*BB-S511*	*BL-S786*
S. aureus	1	1.6	1.6
S. aureus, Penicillin-R	2	0.6	1.6
E. coli	6	0.2	0.2
E. coli, Cephalosporin-R	1	6.3	12.5
K. pneumoniae	4	0.7	0.3
Indole (-) Proteus	2	0.1	0.2
Indole(+) Proteus	3	0.05	0.3
Indole(+) Proteus, Cephalosporin-R	2	6.3	50.1
S. marcescens	1	25	>100
E. cloacae	1	3.1	1.6
Shigella, Salmonella	5	0.3	0.5
P. aeruginosa	2	>100	>100

BL-S786 is 7-[α-(2-aminomethylphenyl)acetamido]-3-[(1-carboxymethyltetrazol-5-ylthio)methyl]-3-cephem-4-carboxylic acid.

TABLE 9

Geometric Means of MIC's Against 18 Strains of S. marcescens

BB-S511	*BL-S786*
5.4	85.9

Example 15

Substitution of an equimolar weight of 2-ethoxy-imino-2-(fur-2-yl)acetic acid for the 2-methoxyiminofuryl acetic acid used in the procedures of Examples 13 and 14 produces 7-(2-ethoxyimino-2-furylacetamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid and 7-(2-ethoxyimino-2-furylacetamido)-3-[2-(2-carboxyethyl)-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl]-3-cephem-4-carboxylic acid, respectively.

Example 16

Substitution of an equimolar weight of 2-n-propoxyimino-2-(fur-2-yl)acetic acid for the 2-methoxyiminofuryl acetic acid used in the procedures of Examples 13 and 14 produces 7-(2-n-propoxyimino-2-furylacetamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid and 7-(2-n-propoxyimino-2-furylacetamido)-3-[2-(2-carboxyethyl)-2-3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl]-3-cephem-4-carboxylic acid, respectively.

Example 17

Substitution of an equimolar weight of 2-n-butoxyimino-2-(fur-2-yl)acetic acid for the 2-methoxyiminofuryl acetic acid used in the procedures of Examples 13 and 14 produces 7-(2-n-butoxyimino-2-furylacetamido)-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid and 7-(2-n-butoxyimino-2-furylacetamido)-3-[2-(2-carboxyethyl)-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl]-3-cephem-4-carboxylic acid, respectively.

Example 18

The products of Examples 13-17 are prepared as *syn* isomers essentially free of the corresponding *anti* isomers by the use in the procedures of those examples of purified *syn* isomers of the appropriate 2-alkoxyimino-2-(fur-2-yl)acetic acid. Conversion of part of the *syn* isomer to *anti* isomer during preparation of the acid chloride from the acid is substantially avoided by minimizing its exposure to hydrogen chloride, e.g. by first converting the acid to its anhydrous sodium salt and by treating that salt with oxalyl chloride under anhydrous conditions in the presence of a hydrogen ion acceptor such as dimethylformamide.

Such syn isomers are also named as (2Z)-2-alkoxyimino-2-(fur-2-yl)acetic acids.

Example 19

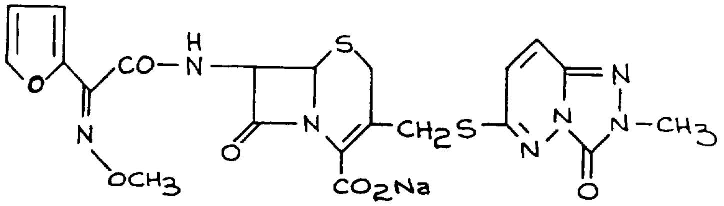
An injectable pharmaceutical composition is formed by adding sterile water or sterile saline solution (2 ml.) to 100-500 mgm. of 7-[(2Z)-2-methoxyimino-(fur-2-yl)acetamido]-3-(2-carboxymethyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid disodium salt.

Pharmaceutical compositions of the sodium and potassium salts of the other compounds of the present invention, preferably in the form of the pure *syn* isomer, are formulated in a similar manner.

When the compounds are first prepared in the form of the free acid they are converted to the desired, highly water soluble potassium salt by treatment with potassium 2-ethylhexanoate using the procedure of Example 13.

It is occasionally advantageous to have admixed with said solid cephalosporin as a stabilizing and/or solubilizing agent a sterile, anhydrous solid such as sodium carbonate, potassium carbonate or lithium carbonate (e.g. in about 5 or 6 percent by weight of the weight of the cephalosporin) or such as L-lysine, arginine or histidine (e.g. in about 20-50% by weight of the weight of the cephalosporin) or such as a sodium, potassium or calcium salt of levulinic acid, citric acid, ascorbic acid, tartaric acid or pyruvic acid (e.g. in about 25-200% by weight of the weight of the cephalosporin) or such as sodium bicarbonate, ammonium carbamate alkali metal or ammonium phosphates or N-methyl-glucamine (per U.K. 1,380,741).

Example 20



BB-S510

BB-S510; 7-[(2Z)-2-Methoxyimino(fur-2-yl)acetamido]-3-(2-methyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic Acid. Sodium Salt.

To a solution of (2Z)-2-methoxyimino(fur-2-yl)acetic acid (253 mg., 1.5 m.mol.) and triethylamine (0.2 ml., 1.5 m.mol.) in dichloromethane (3 ml.) was added oxalyl chloride (0.13 ml., 1.5 m.mol.) at 0-5° C. and the mixture was stirred for 30 minutes and evaporated at reduced pressure to give the acid chloride as an oil which was dissolved in dry acetone (5 ml.) and filtered to remove insolubles. The acetone solution was added to a mixture of 7-amino-3-(2-methyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid (591 mg., 1.5 m.mol.) and $NaHCO_3$ (504 mg., 6 m.mol.) in water (10 ml.) at 0-5° C. The reaction mixture was stirred at 0-5° C. for 3 hours. Acetone was removed at reduced pressure and the residual aqueous solution was washed with ether (2 × 30 ml.) and adjusted to pH 1-2 with concentrated HCl. The precipitate which was collected by filtration, washed with water and dried *in vacuo*, was dissolved in THF (30 ml.) and filtered to remove insolubles. To the THF solution was added a solution of sodium 2-ethylhexanoate (SEH, 1 *M*, 1.5 ml.) in ethyl acetate and the resulting precipitate was collected by filtration and dried *in vacuo*. Yield: 492 mg. of 7-[(2Z)-2-methoxyimino(fur-2-yl)acetamido]-3-(2-methyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid sodium salt (58%). M.P. >180° C. (dec.).

ir: ν_{max}^{KBr} 1770, 1720, 1670, 1600, 1550 cm^{-1}.

uv: $\lambda_{max}^{pH7Buffer}$ 257 nm (ϵ 22600), 277 nm (ϵ 22300).

nmr: $\delta_{ppm}^{D_2O}$ 7.53 (1H, d, J=1.5 Hz, furan-Hα), 7.35 (1H, d, J=9.5 Hz, pyridazine-H), 6.90 (1H, d, J=9.5 Hz, pyridazine-H), 6.72 (1H, d, J=3.0 Hz, furan-Hβ), 6.48 (1H, q, J=1.5 and 3.0 Hz, furan-Hβ), 5.72 (1H, d, J=4.5 Hz, 7-H), 5.14 (1H, d, J=4.5 Hz, 6-H), 2.94 (3H, s, O-C$\underline{H}_3$), 3.61 (3H, s, N-C$\underline{H}_3$).

Anal. Calc'd. for $C_{21}H_{18}N_7O_7S_2Na \cdot 1/2THF \cdot H_2O$: C, 44.44; H, 3.89; S, 10.32. Found: C, 44.89; H, 3.92; S, 9.67.

TABLE 10

In Vitro Activity Using Mueller-Hinton Agar By the Serial Dilution Method

	Geometric Mean of MIC (Mcg./ml.)		
	BB-S510 (Ex. 20)	BB-S515	Cefuroxime
S. aureus (3 strains)	0.62	2.48	1.24
E. coli (7)	2.11	2.33	1.28
Kl. pneumoniae (4)	6.3	3.1	3.1
Proteus (6)	1.39	1.11	0.88
Shig.(3), Serr.(1) Enterab.(1), Sal.(2)	6.26	5.26	4.06
B. anthracis (1)			
S. pyogenes (5)	0.0125	0.032	0.025
S. viridans (5)	0.13	0.59	0.1
D. pneumoniae (5)	0.021	0.1	0.0125
N. meningitidis (5)	1.03	5.45	1.6
N. gonorrhoeae (5)	0.26	2.07	0.4
H. influenzae (7)	0.35	2.11	1.16

Cefuroxime is sodium 6R,7R-3-carbamoyloxymethyl-7-(2Z)-2-methoxyimino(fur-2-yl)acetamidoceph-3-em-4-carboxylate.

TABLE 11

Geometric Means of MIC's Against 3 Strains of S. aureus and 27 Strains of Gram-negative Bacteria

(mcg./ml., Mueller-Hinton Agar)

	No. of Strains	*BB-S510*	*BL-S786*
S. aureus	1	0.1	1.6
S. aureus, Penicillin-R	2	0.1	1.6
E. coli	6	0.1	0.2
E. coli, Cephalosporin-R	1	3.1	12.5
K. pneumoniae	4	2.6	0.3
Indole (-) Proteus	2	1.1	0.2
Indole(+) Proteus	3	0.2	0.3
Indole(+) Proteus, Cephalosporin-R	2	6.3	50.1
S. marcescens	1	6.3	>100
E. cloacae	1	3.1	1.6
Shigella, Salmonella	5	0.5	0.5
P. aeruginosa	2	>100	>100

BL-S786 is 7-[α-(2-aminomethylphenyl)acetamido]-3-[(1-carboxymethyltetrazol-5-ylthio)methyl]-3-cephem-4-carboxylic acid.

TABLE 12

Geometric Means of MIC's Against 18 Strains of S. marcescens

BB-S510	*BL-S786*
7.9	85.9

Example 21

Substitution of an equimolar weight of 2-ethoxyimino-2-(fur-2-yl)acetic acid for the 2-methoxyiminofuryl acetic acid used in the procedure of EXAMPLE ¼? PRODUCES ⅞-(2-ethoxyimino-2-furylacetamido)-3-(2-methyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid.

Example 22

Substitution of an equimolar weight of 2-n-propoxyimino-2-(fur-2-yl)acetic acid for the 2-methoxyiminofuryl acetic acid used in the procedure of Example 20 produces 7-(2-n-propoxyimino-2-furylacetamido)-3-(2-methyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid.

Example 23

Substitution of an equimolar weight of 2-n-butoxyimino-2-(fur-2-yl)acetic acid for the 2-methoxyiminofuryl acetic acid used in the procedure of Example 20 produces 7-(2-n-butoxyimino-2-furylacetamido)-3-(2-methyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxlic acid.

Example 24

The products of Examples 20-23 were prepared as *syn* isomers essentially free of the corresponding *anti* isomers by the use in the procedures of those examples of purified *syn* isomers of the appropriate 2-alkoxyimino-2-(fur-2-yl)acetic acid. Conversion of part of the *syn* isomer to *anti* isomer during preparation of the acid chloride from the acid is substantially avoided by minimizing its exposure to hydrogen chloride, e.g. by first converting the acid to its anhydrous sodium salt and by treating that salt with oxalyl chloride under anhydrous conditions in the presence of a hydrogen ion acceptor such as dimethylformamide.

Such syn isomers are also names as (2Z)-2-alkoxyimino-2-(fur-2-yl)acetic acids.

Example 25

An injectable pharmaceutical composition is formed by adding sterile water or sterile saline solution (2 ml.) to 100-500 mgm. of 7-[(2Z)-2-methoxyimino(fur-2-yl)acetamido]-3-(2-methyl-2,3-dihydro-s-triazolo[4,3-b]pyridazin-3-on-6-ylthiomethyl)-3-cephem-4-carboxylic acid disodium salt.

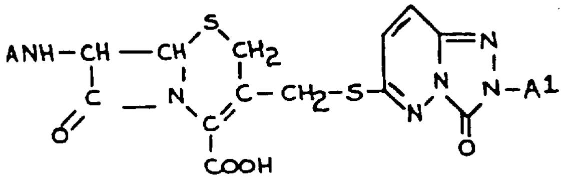
Pharmaceutical compositions of the sodium and potassium salts of the other compounds of the present invention, preferably in the form of the pure *syn* isomer, are formulated in a similar manner.

When the compounds are first prepared in the form of the free acid they are converted to the desired, highly water soluble potassium salt by treatment with potassium 2-ethylhexanoate using the procedure of Example 20.

It is occasionally advantageous to have admixed with said solid cephalosporin as a stabilizing and/or solubilizing agent a sterile, anhydrous solid such as sodium carbonate, potassium carbonate or lithium carbonate (e.g. in about 5 or 6 percent by weight of the weight of the cephalosporin) or such as L-lysine, arginine or histidine (e.g. in about 20-50% by weight of the weight of the cephalosporin) or such as a sodium, potassium or calcium salt of levulinic acid, citric acid, ascorbic acid, tartaric acid or pyruvic acid (e.g. in about 25-200% by weight of the weight of the cephalosporin) or such as sodium bicarbonate, ammonium carbamate, alkali metal or ammonium phosphates or N-methyl-glucamine (per U.K. 1,380,741).

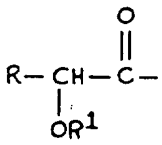
WHAT WE CLAIM IS:

1. A compound having the formula

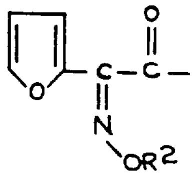


I

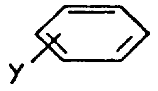
wherein A is



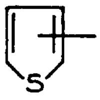
or



wherein R^1 is hydrogen or formyl;
R is

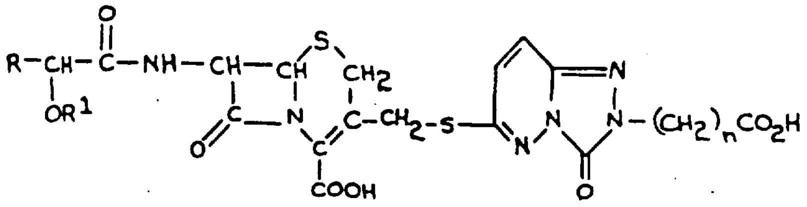


or



Y is hydrogen, chlorine, bromine, fluorine, trifluoromethyl, amino, nitro, hydroxy, (lower)alkyl of 1 to 4 carbon atoms or (lower)alkoxy of 1 to 4 carbon atoms; R^2 is (lower)alkyl of 1 to 4 carbon atoms and wherein A^1 is methyl or $-(CH_2)_nCOOH$ and n is one or two; or an easily hydrolyzed ester or nontoxic pharmaceutically acceptable salt thereof, or the benzaldehyde or salicylaldehyde Schiff base or acetaldehyde or acetone derivative of such a compound when it contains an amino group.

2. A compound according to claim 1 having the D-configuration in the 7-side chain and the formula



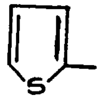
II

wherein n is one or two, R^1 is hydrogen or formyl and R is

and Y is hydrogen, chlorine, bromine, fluorine, trifluoromethyl, amino, nitro, hydroxy, lower alkyl of 1 to 4 carbon atoms or lower alkoxy of 1 to 4 carbon atoms.

3. A compound according to Claim 2, wherein R is chlorophenyl, bromophenyl, trifluoromethylphenyl, tolyl or methoxyphenyl.

4. A compound according to Claim 2, wherein R is

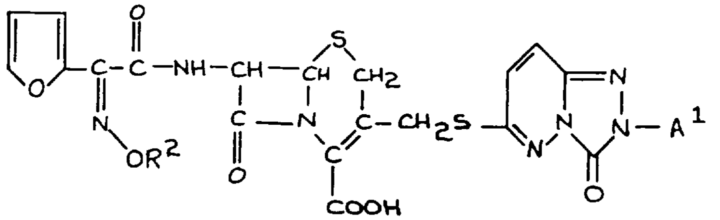


5. A compound according to Claim 2, wherein R is phenyl.

6. A compound according to Claims 2, 3, 4, or 5 wherein R^1 is hydrogen.

7. A compound according to any of Claims 2, 3, 4 or 5 wherein R^1 is formyl.

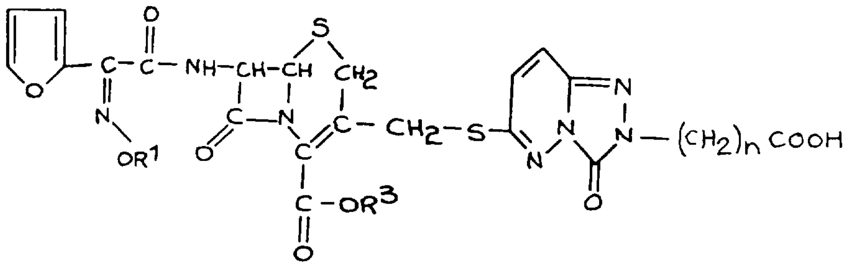
8. A compound according to Claim 1 having the formula III



III

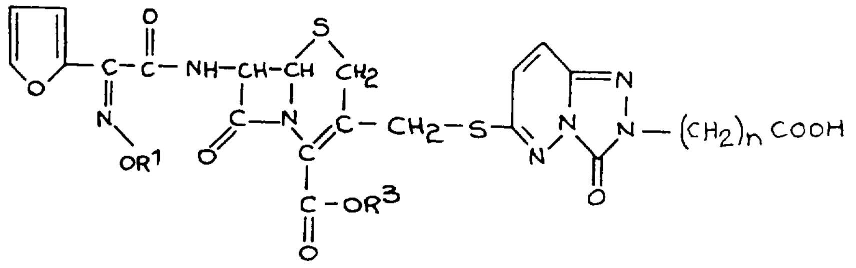
wherein A^1 is methyl or $-(CH_2)_nCOOH$ and n is one or two and R^2 is alkyl containing 1 to 4 carbon atoms, the easily hydrolyzed esters and the non-toxic pharmaceutically acceptable salts of those acids.

9. A compound according to Claim 8 having the formula



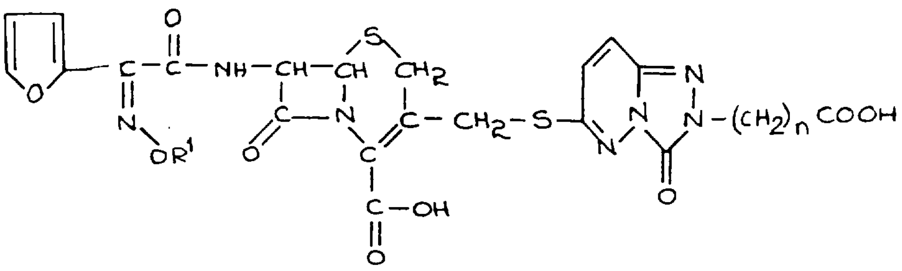
wherein R^1 is alkyl containing 1 to 4 carbon atoms, n is one or two and R^3 is hydrogen or a conventional, pharmaceutically acceptable, easily hydrolyzed ester forming group; or a non-toxic, pharmaceutically acceptable salt thereof, said compound being at least 75% by weight in the form of its *syn* isomer.

10. A *syn* isomer of a compound according to Claim 9 having the formula



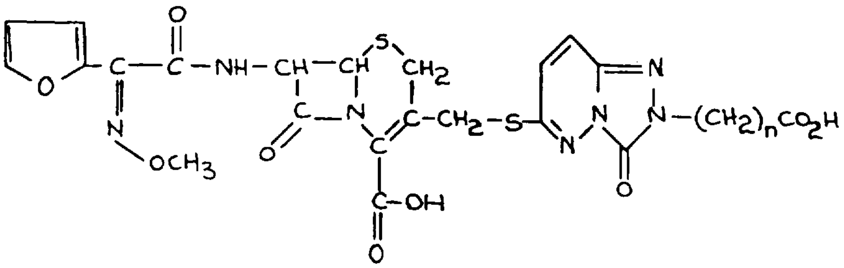
wherein R^1 is alkyl containing 1 to 4 carbon atoms, n is one or two and R^3 is hydrogen, pivaloyloxymethyl, acetoxymethyl, methoxymethyl, acetonyl, phenacyl, p-nitrobenzyl, β,β,β-trichloroethyl, 3-phthalidyl or 5-indanyl or a non-toxic, pharmaceutically acceptable salt thereof.

11. A *syn* isomer of a compound according to Claim 9 having the formula



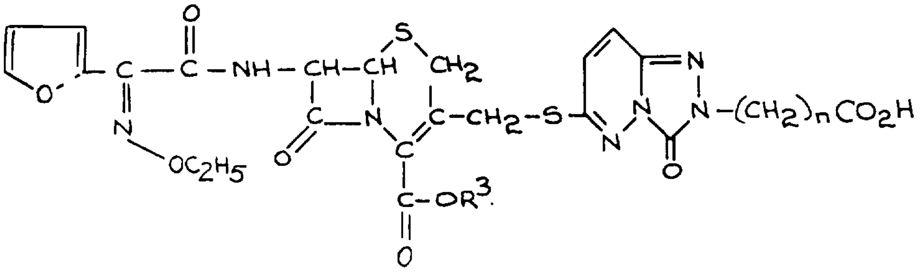
wherein R^1 is alkyl containing 1 to 4 carbon atoms and n is one or two or a non-toxic pharmaceutically acceptable salt thereof.

12. A *syn* isomer of a compound according to Claim 11 having the formula



wherein n is one or two or a non-toxic, pharmaceutically acceptable salt thereof.

13. A *syn* isomer of a compound according to Claim 9 having the formula

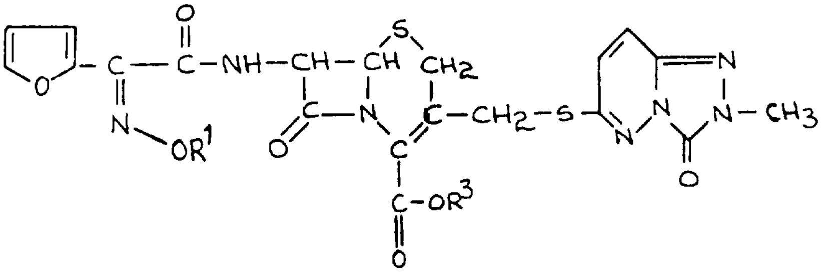


wherein R^3 is hydrogen or a conventional, pharmaceutically acceptable, easily hydrolyzed ester forming group and n is one or two or a non-toxic, pharmaceutically acceptable salt thereof.

14. A compound of any of the Claims 8 to 13, wherein n is one.

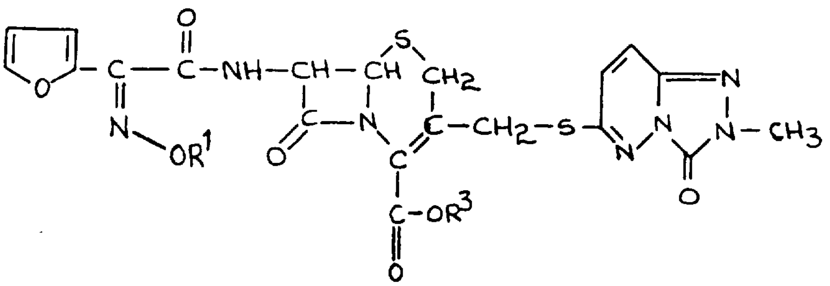
15. A compound of any of the Claims 8 to 13, wherein n is two.

16. A compound according to Claim 8 having the formula



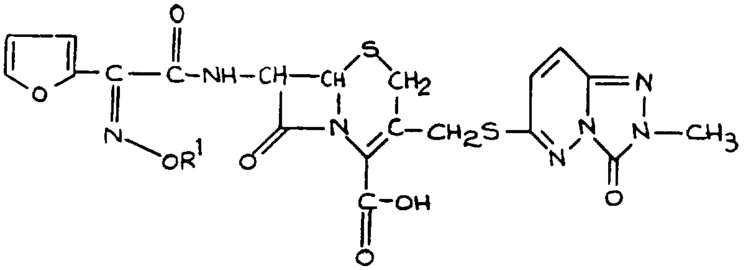
wherein R^1 is alkyl containing 1 to 4 carbon atoms and R^3 is hydrogen or a conventional, pharmaceutically acceptable, easily hydrolyzed ester forming group; or a non-toxic, pharmaceutically acceptable salt thereof, said compound being at least 75% by weight in the form of its *syn* isomer.

17. A *syn* isomer of a compound according to Claim 16 having the formula



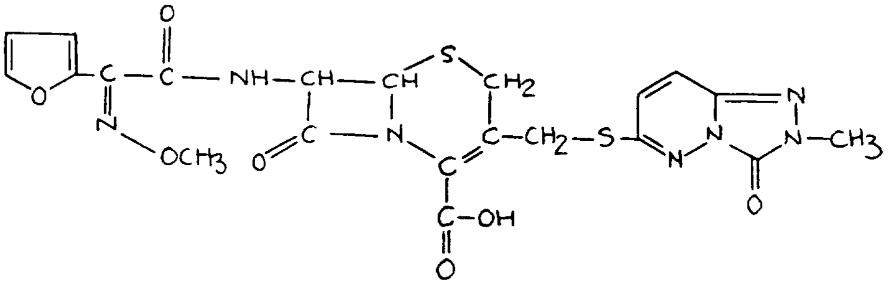
wherein R^1 is alkyl containing 1 to 4 carbon atoms and R^3 is hydrogen, pivaloyloxymethyl, acetoxymethyl, methoxymethyl, acetonyl, phenacyl, p-nitrobenzyl, β,β,β-trichloroethyl, 3-phthalidyl or 5-indanyl or a non-toxic pharmaceutically acceptable salt thereof.

18. A *syn* isomer of a compound according to Claim 16 having the formula



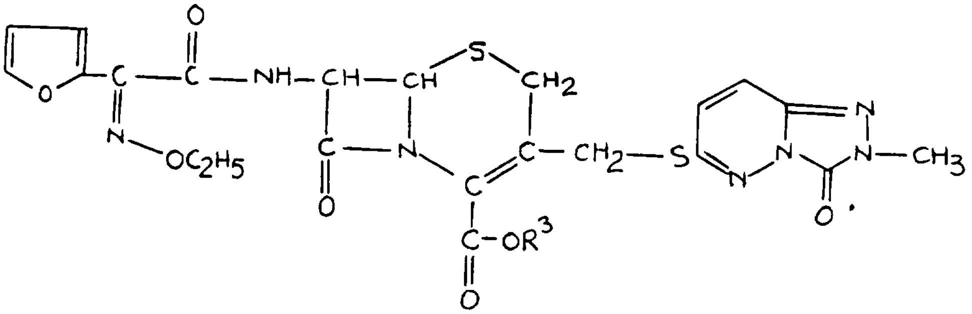
wherein R^1 is alkyl containing 1 to 4 carbon atoms or a non-toxic pharmaceutically acceptable salt thereof.

19. The *syn* isomer of a compound according to Claim 16 having the formula



or a non-toxic, pharmaceutically acceptable salt thereof.

20. The *syn* isomer of a compound according to Claim 16 having the formula



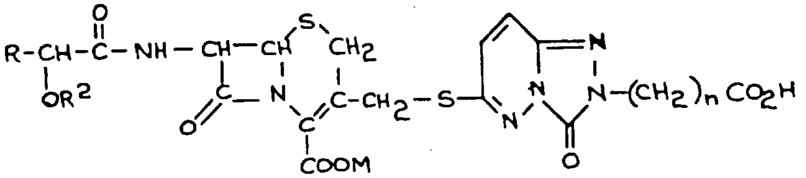
wherein R^3 is hydrogen or a conventional, pharmaceutically acceptable, easily hydrolyzed ester forming group, or a non-toxic, pharmaceutically acceptable salt thereof.

21. A compound according to any one of the preceding claims in the form of the sodium or potassium salt.

22. A compound according to any one of the preceding claims in the form of the

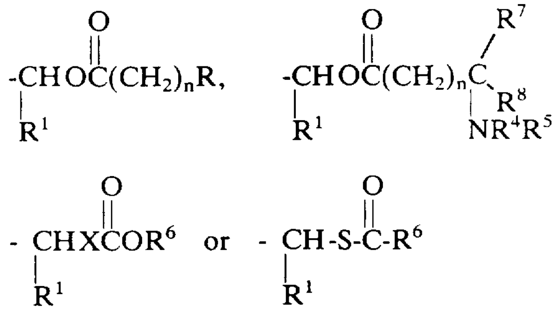
pivaloyloxymethyl, acetoxymethyl, acetonyl, phenacyl or methoxymethyl ester.

23. A compound according to Claim 1 having the formula



wherein n is one or two, R^2 is hydrogen or formyl and R is

and Y is hydrogen, chlorine, bromine, fluorine, trifluoromethyl, amino, nitro, hydroxy, lower alkyl of 1 to 4 carbon atoms or lower alkoxy of 1 to 4 carbon atoms and M is

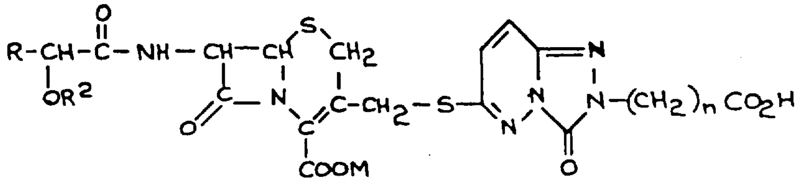


$$-\underset{R^1}{CH}O\overset{O}{\overset{\|}{C}}(CH_2)_nR, \quad -\underset{R^1}{CH}O\overset{O}{\overset{\|}{C}}(CH_2)_n\underset{NR^4R^5}{\overset{R^7}{C}}R^8$$

$$-\underset{R^1}{CH}X\overset{O}{\overset{\|}{C}}OR^6 \text{ or } -\underset{R^1}{CH}\text{-}S\text{-}\overset{O}{\overset{\|}{C}}\text{-}R^6$$

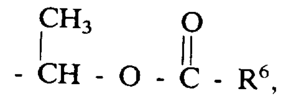
n is 0 to 4; R is hydrogen, alkyl having 1 to 8 carbon atoms, cycloalkyl of 3 to 6 carbon atoms, phenyl, C_1-C_4 phenalkyl, pyridyl, thienyl, or pyrrolyl; R^1 is hydrogen, methyl or ethyl; R^7 and R^8 are each hydrogen, alkyl having 1 to 6 carbon atoms, phenyl, pyridyl or thienyl; R^4 and R^5 are each hydrogen or alkyl of 1 to 4 carbon atoms; R^6 is alkyl having 1 to 4 carbon atoms, phenyl, phenalkyl having 1 to 4 carbon atoms, pyridyl, thiadiazolyl, amino or C_1-C_4 alkylamino; X is NH or oxygen; and each phenyl group is unsubstituted or substituted with one or two substituents selected from alkyl having 1 to 6 carbon atoms, alkoxy having 1 to 4 carbon atoms, hydroxy, amino NHR^1, $N(R^1)_2$, nitro, fluoro, chloro, bromo or carboxy, or a non-toxic, pharmaceutically acceptable salt thereof.

24. A compound according to Claim 1 having the formula

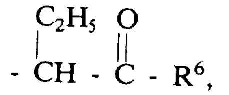


wherein n is one or two, R^2 is hydrogen or formyl and R is

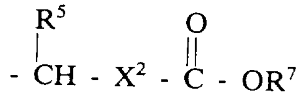
and Y is hydrogen, chlorine, bromine, fluorine, trifluoromethyl, amino, nitro, hydroxy, lower alkyl of 1 to 4 carbon atoms or lower alkoxy of 1 to 4 carbon atoms and M is selected from



$$-\overset{\overset{\displaystyle CH_3}{|}}{C}H-O-\overset{\overset{\displaystyle O}{||}}{C}-R^6,$$



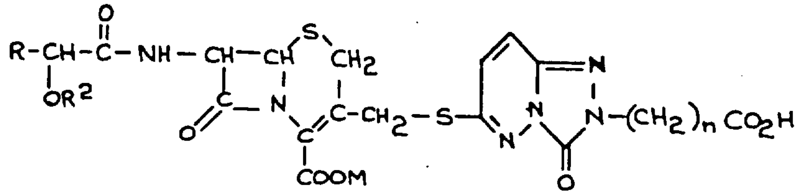
$$-\overset{\overset{\displaystyle C_2H_5}{|}}{C}H-\overset{\overset{\displaystyle O}{||}}{C}-R^6,$$



$$-\overset{\overset{\displaystyle R^5}{|}}{C}H-X^2-\overset{\overset{\displaystyle O}{||}}{C}-OR^7$$

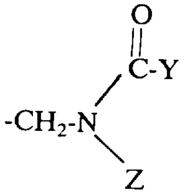
wherein R^5 is a hydrogen atom, a methyl or an ethyl group; X^2 is -O-, -NH-; R^6 is a basic group; R^7 is an alkyl group, a cycloalkyl group, an aryl group, an aralkyl group or a heterocyclic group, which groups may be substituted with one or more groups selected from amino groups, substituted amino groups, halogen atoms, nitro groups, or alkoxy groups; or a non-toxic pharmaceutically acceptable salt thereof.

25. A compound according to Claim 1 having the formula



wherein n is one or two, R^2 is hydrogen or formyl and R is

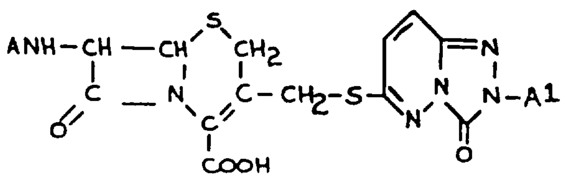
and Y is hydrogen, chlorine, bromine, fluorine, trifluoromethyl, amino, nitro, hydroxy, lower alkyl of 1 to 4 carbon atoms or lower alkoxy of 1 to 4 carbon atoms and M is



$$-CH_2-N\begin{matrix}\diagup \overset{\overset{\displaystyle O}{||}}{C}-Y\\ \diagdown Z\end{matrix}$$

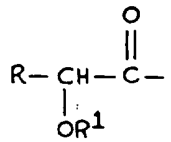
wherein Y is alkyl of one to six carbon atoms, phenyl, benzyl, alkoxy of one to six carbon atoms, or benzyloxy; Z is alkyl of one to six carbon atoms, phenylbenzyl, alkoxy of one to six carbon atoms, cyclopentyl, cyclohexyl and phenyl, or Y+Z taken together are a 3-benzoxazolidine ring; or a non-toxic, pharmaceutically acceptable salt thereof.

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



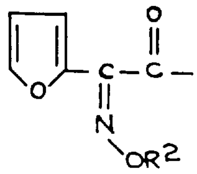
I

wherein A is

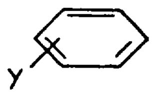


$$R-\underset{\displaystyle OR^1}{CH}-\overset{\displaystyle O}{\overset{\|}{C}}-$$

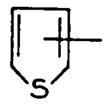
or



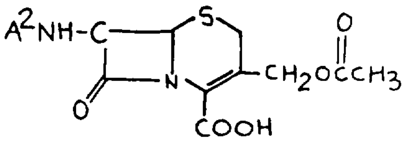
wherein R^1 is hydrogen or formyl;
R is



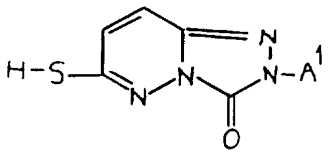
or



Y is hydrogen, chlorine, bromine, fluorine, trifluoromethyl, amino, nitro, hydroxy, (lower)alkyl of 1 to 4 carbon atoms or (lower)alkoxy of 1 to 4 carbon atoms;
R^2 is (lower)alkyl of 1 to 4 carbon atoms, and wherein A^1 is methyl or $-(CH_2)_nCOOH$ and n is one or two; or an easily hydrolyzed ester of non-toxic pharmaceutically acceptable salt thereof;
characterized by reacting a compound of the formula



in which A^2 is H or A as defined above or a salt or easily hydrolyzable ester thereof with a compound of the formula



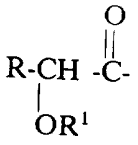
wherein A^1 is as defined above, and, when A^2 is H, treating the resulting compound with an acylating agent of the formula

A—X

in which X is halide or a functional equivalent thereof and A is as defined above, which when the A group contains a free amino or hydroxyl, said groups are blocked by conventional protecting groups and subsequently removed to afford a compound of the formula I, and, if desired, converting a resulting free acid of a compound of the formula I to the corresponding ester or non-toxic pharmaceutically acceptable salt thereof, and, if desired, converting a resulting salt or easily hydrolyzable ester of a compound of the formula I said compound to the corresponding free acid of the formula I.

27. The process according to Claim 26 wherein a resulting free acid of the formula I is converted to an ester selected from the pivaloyloxymethyl-, acetoxymethyl-, methoxymethyl-, acetonyl-, phenacyl-, p-nitrobenzyl-, β,β,β-trichloroethyl-, 3-phthalidyl- or 5-indanyl-oxy.

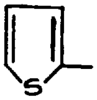
28. The process according to Claim 26 or 27 wherein A is



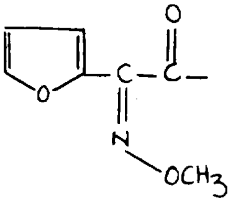
$$R-\underset{\displaystyle OR^1}{CH}-\overset{\displaystyle O}{\overset{\|}{C}}-$$

wherein R^1 is hydrogen or formyl and R is chlorophenyl, bromophenyl, trifluoromethyl phenyl, tolyl or methoxyphenyl.

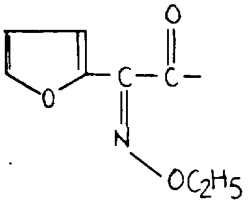
29. The process according to Claim 27 or 28 wherein R is



30. The process according to Claim 26 or 27 wherein A is



or



31. The process according to any of Claims 26 to 30 wherein A^1 is methyl.

32. The process according to any of Claims 26 to 30 wherein A^1 is $-(CH_2)_nCOOH$; where n is one or two.

33. The process of Claim 32 where n is one.

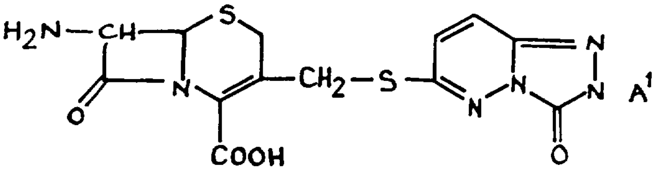
34. The process of Claim 32 where n is two.

35. The process of any one of Claims 26 to 34 where A has the D-configuration.

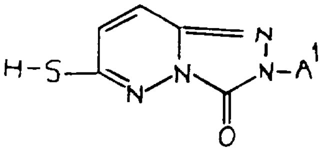
36. The process according to any one of Claims 26, 27 and 31 to 35 further comprising converting the resulting compound, when it has an amino group, to the corresponding benzaldehyde or salicylaldehyde Schiff base.

37. The process according to any one of Claims 26, 27 and 31 to 35 comprising treating the resulting compound, when it has an amino group, with acetaldehyde or acetone to afford a corresponding derivative thereof.

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

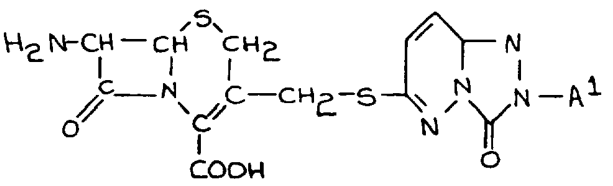


wherein A^1 is methyl or $-(CH_2)_nCOOH$ and n is one or two; characterized by reacting 7-amino-cephalosporanic acid or a salt or easily hydrolyzable ester or Schiff base thereof with a compound of the formula



where A^1 is as defined above.

39. A process for the production of the antibacterial agents of formula I which comprises reacting a compound of the formula



IV

where A^1 is as hereinbefore defined or a salt or easily hydrolyzed ester or Schiff base thereof with an organic monocarboxylic acid chloride or a functional equivalent thereof as an acylating agent corresponding to the acid AOH.

40. A process according to Claim 39 substantially as described herein and exemplified.

41. A compound according to Claim 1 substantially as described herein.

42. A compound according to Claim 1 substantially as described herein with reference to any one of Examples 1 to 24.

43. A process according to Claim 28 substantially as described herein and exemplified.

44. Compounds when prepared by the process of any one of Claims 26 to 40 or 43.

45. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and an antibacterially effective amount of a compound in accordance with any one of Claims 1 to 25, 41, 42 or 44.

46. A pharmaceutical composition according to Claim 45 further comprising a semi-synthetic penicillin or another cephalosporin or a cephamycin or a β-lactamase inhibitor or an aminoglycoside antibiotic.

47. A pharmaceutical composition substantially as herein described and exemplified.

48. A method of treating bacterial infections comprising administering by injection to an infected warm-blooded non-human animal, an effective but non-toxic dose of a compound in accordance with any one of Claims 1 to 25, 42 or 44.

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