

PATENT SPECIFICATION

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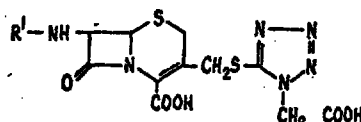
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 and ALPHONSE P. GRANATEK

(54) TETRAZOLETHIOMETHYL CEPHALOSPORIN COMPOUNDS

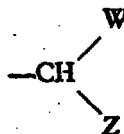
(71) We, BRISTOL-MYERS COMPANY, a Corporation organised and existing under the laws of the State of Delaware, United States of America, having offices at 345 Park Avenue, New York, New York 10022, United States of America, do hereby declare the invention for which we pray that a patent may be granted 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 compounds having the structure:



wherein R¹ is acyl or hydrogen and an ester or a nontoxic pharmaceutically acceptable salt thereof.

Said ester of the compound of the formula I include those having, as the whole or part of the ester moiety derived from an alcohol, a group of the formula



wherein, when W represents hydrogen, Z represents (lower) alkanoyl, benzoyl, naphthoyl, furyl, thenoyl, nitrobenzoyl, methylbenzoyl, halobenzoyl, phenylbenzoyl, 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. Preferred embodiments of this invention include the pivaloyloxymethyl, acetoxymethyl, methoxymethyl, acetonyl, phenacyl, p-nitrobenzyl, β,β,β -trichloroethyl, 3-phthalidyl or 5-indanyl ester of compound I.

SPECIFICATION AMENDED · SEE ATTACHED SLIP

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. Acyl (R^1) includes, but is not limited to, the groups having the structures:

(i) $R^u C_n H_{2n} CO$ — where R^u is aryl (carbocyclic or heterocyclic), cycloalkyl, substituted aryl, substituted cycloalkyl, or a non-aromatic or mesoionic heterocyclic group, and n is an integer from 1—4. Examples of this group include phenylacetyl; substituted phenylacetyl, e.g. fluorophenylacetyl, nitrophenylacetyl, aminophenylacetyl, β -(*o*-aminomethylphenyl)-propionyl, *o*-, *m*- and *p*-guanidino-phenylacetyl, *o*-, *m*- and *p*-aminomethylphenylacetyl, acetoxypheylacetyl, methoxypheylacetyl, methylphenylacetyl, or hydroxyphenylacetyl; N,N -bis-(2-chloroethyl)aminophenylpropionyl; thien-2- and -3-acetyl; 4-isoxazolyl- and substituted 4-isoxazolylacetyl; 1-cyclohexenylacetyl, 2-aminomethyl-1-cyclohexenylacetyl, 1,4-cyclohexadienylacetyl, 2-aminomethyl-1,4-cyclohexadienylacetyl; pyridylacetyl; tetrazolylacetyl (other heterocyclic-acetyl groups of this type are disclosed in U.S. 3,819,623 and 3,516,997) or a sydnonacetyl group as disclosed in U.S. 3,681,328; 3,530,123 and 3,563,983. The substituted 4-isoxazolyl group may be a 3-aryl-5-methyl isoxazol-4-yl group, the aryl group being, e.g. phenyl or halophenyl, e.g. chloro- or bromo- phenyl. An acyl group of this type is 3-*o*-chlorophenyl-5-methyl isoxazol-4-yl-acetyl and another is that in which isoxazol is replaced by isothiazole as disclosed in U.S. 3,551,440. Other acyl groups of this type include cyanoacetyl (and similar compounds disclosed in U.S. 3,719,673), 3,5,7-triaza-1-azonia-1-adamantyl)acetyl (as disclosed in U.S. 3,720,669), *m*-aminopyridiniumacetyl (as disclosed in U.S. 3,757,013), *o*-, *m*- and *p*-(2'-aminoethoxy)phenylacetyl (as disclosed in U.S. 3,759,905), 4,5-dimethoxycarbonyl-1,2,3-triazol-1-ylacetyl or 4-cyano-1,2,3-triazol-1-ylacetyl (as disclosed in U.S. 3,821,206), imidazol-(1)-acetyl (as disclosed in U.S. 3,632,810), *p*-aminomethylphenylacetyl (as disclosed in U.S. 3,382,241), *o*-aminomethyl-*p*-hydroxyphenylacetyl (as disclosed in U.S. 3,823,141), β -(*o*-aminomethylphenyl)propionyl (as disclosed in U.S. 3,813,391), α -amino-2,4,6-cycloheptatrienyl-acetyl (as disclosed in U.S. 3,539,562) and lower alkoxy-carbonyl-acetyl) as disclosed in U.S. 3,557,104);

(ii) $C_n H_{2n+1} CO$ — where n is an integer from 1—7. The alkyl group may be straight or branched, and if desired, may be interrupted by an oxygen or sulphur atom or substituted by, e.g. a cyano group. Examples of such groups include cyanoacetyl, hexanoyl, heptanoyl, octanoyl, butylthioacetyl and trifluoromethylthioacetyl;

(iii) $C_n H_{2n-1} CO$ — where n is an integer from 2—7. The alkenyl group may be straight or branched and, if desired, may be interrupted by an oxygen or a sulphur atom. An example of such is allylthioacetyl;



where R^u has the meaning defined under (i) and in addition may be benzyl, and R^v and R^w , which may be the same or different, each represent hydrogen, phenyl, benzyl, phenethyl or lower alkyl. Examples of such groups include phenoxyacetyl, 2-phenoxy-2-phenylacetyl, 2-phenoxypropionyl, 2-phenoxybutyryl, benzyloxycarbonyl, 2-methyl-2-phenoxypropionyl, *p*-cresoxyacetyl and *p*-methylthiophenoxyacetyl;

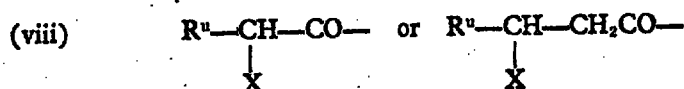


where R^u has the meaning defined under (i) and, in addition, may be benzyl and R^v and R^w have the meanings defined under (iv). Examples of such groups include *S*-phenylthioacetyl, *S*-chlorophenylthioacetyl, *S*-fluorophenylthioacetyl, pyridylthioacetyl, and *S*-benzylthioacetyl and *o*-aminomethylphenylthioacetyl, *p*-aminomethylphenylthioacetyl (as disclosed in U.S. 3,663,540), cyanomethylthioacetyl (as disclosed in France 2,194,417), 4-pyridylthioacetyl (as disclosed in U.S. 3,503,967), and heterocyclylmercaptoacetyl (as disclosed in U.S. 3,627,760);

(vi) $R^u Z (CH_2)_m CO$ — where R^u has the meaning defined under (i) and, in addition, may be benzyl, Z is an oxygen or sulphur atom and m is an integer from 2—5.

An example of such a group is S-benzylthiopropionyl;

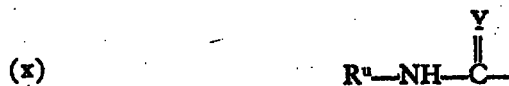
(vii) R^uCO- where R^u has the meaning defined under (i). Examples of such groups include benzoyl, substituted benzoyl (e.g. aminobenzoyl), 4-isoxazolyl- and substituted 4-isoxazolyl carbonyl, cyclopentanecarbonyl, sydnone carbonyl, naphthoyl and substituted naphthoyl (e.g. 2-ethoxynaphthoyl), quinoxalinylicarbonyl and substituted quinoxalinylicarbonyl (e.g. 3-carboxy-2-quinoxalinylicarbonyl). Other possible substituents for benzoyl include alkyl, alkoxy, phenyl or phenyl substituted with carboxy, alkylamido, cycloalkylamido, allylamido, phenyl-(lower)alkylamido, morpholinocarbonyl, pyrrolidinocarbonyl, piperidinocarbonyl, tetrahydropyridino, furfuryl-amido or N-alkyl-N-anilino, or derivatives thereof, and such substituents may be in the 2- or 2- and 6-positions. Examples of such substituted benzoyl groups are 2,6-dimethoxybenzoyl, 2-biphenylcarbonyl, 2-methylaminobenzoyl and 2-carboxybenzoyl. Where the group R^u represents a substituted 4-isoxazolyl group, the substituents may be as set out above under (i). Examples of such 4-isoxazole groups are 3-phenyl-5-methylisoxazol-4-yl carbonyl, 3-o-chlorophenyl-5-methyl-isoxazol-4-yl carbonyl and 3-(2,6-dichlorophenyl)-5-methylisoxazol-4-ylcarbonyl;



where R^u has the meaning defined under (i) and X is hydrazino, guanidino, ureido, thioureido and substituted thioureido as disclosed in U.S. 3,741,962, allophanamido (as in U.S. 3,483,188) 3-guanyl-1-ureido (see U.S. 3,579,501), cyanamino (see U.S. 3,796,709), amino, substituted amino (e.g. acylamido or a group obtained by reacting the amino group and/or group(s) of the 7-side chain with an aldehyde or ketone, e.g. formaldehyde, acetaldehyde, butyraldehyde, acetylacetone, methyl acetoacetamide, benzaldehyde, salicylaldehyde, acetone, methyl ethyl ketone or ethyl acetoacetate), hydroxy, carboxy (as disclosed in U.S. 3,282,926 and U.S. 3,819,601), esterified carboxy (as disclosed, for example, in U.S. 3,635,961), triazolyl, tetrazolyl, cyano, halogeno, acyloxy, (e.g. formyloxy or lower alkanoyloxy) or etherified hydroxy group. Examples of such acyl groups are α -aminophenylacetyl, α -carboxyphenylacetyl, 2,2-dimethyl-5-oxo-4-phenyl-1-imidazolidinyl, α -amino-p-hydroxyphenylacetyl, α -amino-p-acetoxyphenylacetyl, α -hydroxyphenylacetyl and α -formyloxyphenylacetyl or other acyl groups of this type as disclosed, for example, in U.S. 3,812,116 and U.S. 3,821,017, and α -amino-2- or 3-thienylacetyl (see U.S. 3,342,677) and α -amino-3- or 4- or 5-isothiazolacetyl (see U.S. 3,579,506) and other α -amino- and α -hydroxy-heterocyclyl-acetyl groups as disclosed, for example, in U.S. 3,821,207;



where R^z , R^v and R^s , which may be the same or different, may each represent lower alkyl, phenyl or substituted phenyl. An example of such an acyl group is triphenyl-carbonyl;



wherein R^u has the meaning defined under (i) and in addition may be hydrogen, lower alkyl or halogen substituted lower alkyl, and Y represents oxygen or sulphur. An example of such a group is $Cl(CH_2)_2NHCO$;

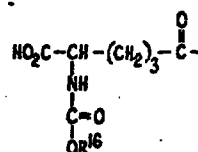


where X has the meaning defined under (viii) above and n is an integer of from 1 to 4. An example of such an acyl group is 1-amino-cyclohexanecarbonyl;

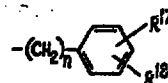
(xii) Amino acyl, for example $R^wCH(NH_2)-(CH_2)_nCO-$ where n is an integer from 1—10, or $H_2N-C_nH_{2n}Ar(CH_2)_mCO-$ where m is zero or an integer from 1—10, and n is 0, 1, or 2 R^w is a hydrogen atom or an alkyl, aralkyl or carboxy group or a group as defined under R^u above, and Ar is an arylene group, e.g. p-phenylene or 1,4-naphthylene. Examples of such groups are disclosed in British patent No. 1,054,806. A group of this type is the p-aminophenylacetyl group. Other acyl groups of this type include those, e.g. δ -aminoadipoyl derived from naturally occurring amino acids and derivatives thereof, e.g. N-benzoyl- δ -aminoadipoyl;

(xiii) Substituted glyoxyl groups of the formula $R^v.CO.CO-$ where R^v is an aliphatic, araliphatic or aromatic group, e.g. a thienyl group, a phenyl group, or a mono-, di- or tri-substituted phenyl group, the substituents being, for example, one or more halogen atoms (F, Cl, Br, or I), methoxy groups, methyl groups, or amino groups, or a fused benzene ring as disclosed, for example, in U.S. 3,546,219 and 3,573,294;

(xiv)

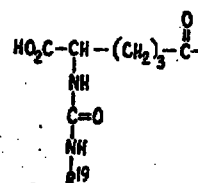


wherein R^{16} is (lower)alkyl or an aralkyl group of the formula

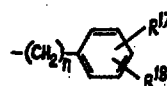


wherein n is an integer of 1 to 6 and R^{17} and R^{18} are alike or different and each is H, Cl, Br, F, NO_2 , (lower)alkyl or (lower)alkoxy;

(xv)

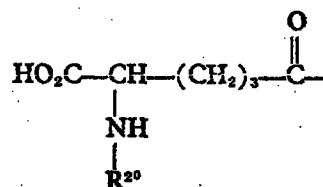


wherein R^{19} is (lower)alkyl or a group of the formula

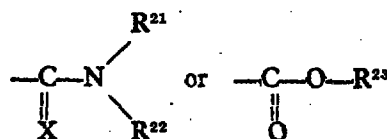


wherein n is an integer of 0 to 6 and R^{17} and R^{18} are alike or different and each is H, Cl, Br, F, NO_2 , (lower)alkyl or (lower)alkoxy;

(xvi)

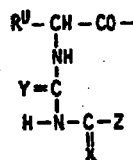


wherein R^{20} is an α -halo or α,α -dihalo C_2-C_4 alkanoyl or R^{20} is N-isobornyloxy-carbonyl as disclosed in U.S. 3,819,619 or R^{20} is the group of the formula

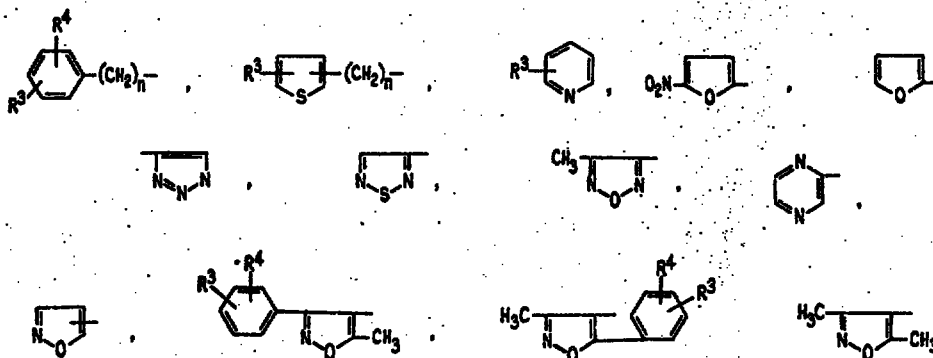


in which R^{21} and R^{22} are each hydrogen, lower alkyl, phenyl, halophenyl, tolyl, lower alkoxyphenyl, or naphthyl, X is oxygen or sulfur, and R^{23} is lower alkyl as disclosed in U.S. 3,819,621 or R^{20} is hydrogen (as in Cephalosporin C);

(xvii)

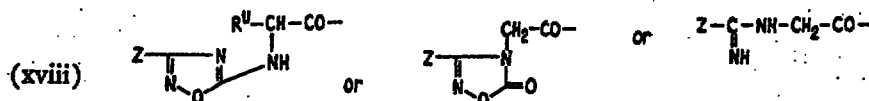


where R^u has the meaning defined under (i), X represents oxygen or imino, Y represents oxygen or sulfur and Z represents (lower)alkyl, cycloalkyl having four, five, six or seven carbon atoms, monohalo(lower)alkyl, dichloromethyl, trichloromethyl, (lower)alkenyl of two to six carbon atoms,



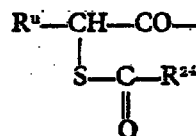
n is an integer from 0 to 3 inclusive and each of R^3 and R^4 is hydrogen, nitro, di(lower)alkylamine, (lower)alkanoylamino, (lower)alkanoyloxy, (lower)-alkyl (comprising straight and branched chain saturated aliphatic groups having from one to six carbon atoms inclusive), (lower)-alkoxy, sulfamyl, chloro, iodo, bromo, fluoro or trifluoromethyl. In preferred embodiments R^u is phenyl, p-hydroxyphenyl or 2- or 3-thienyl and Z is 2-furyl or phenyl.

Examples are disclosed in U.S. 3,687,949 and U.S. 3,646,024;



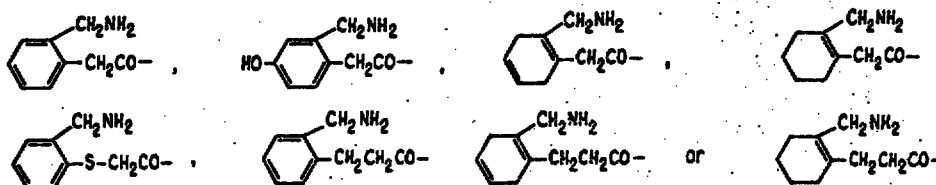
wherein R^u has the meaning defined in (i) and Z has the meaning defined in (xvii). Examples are disclosed in U.S. 3,626,024 and 3,692,779;

(xix)

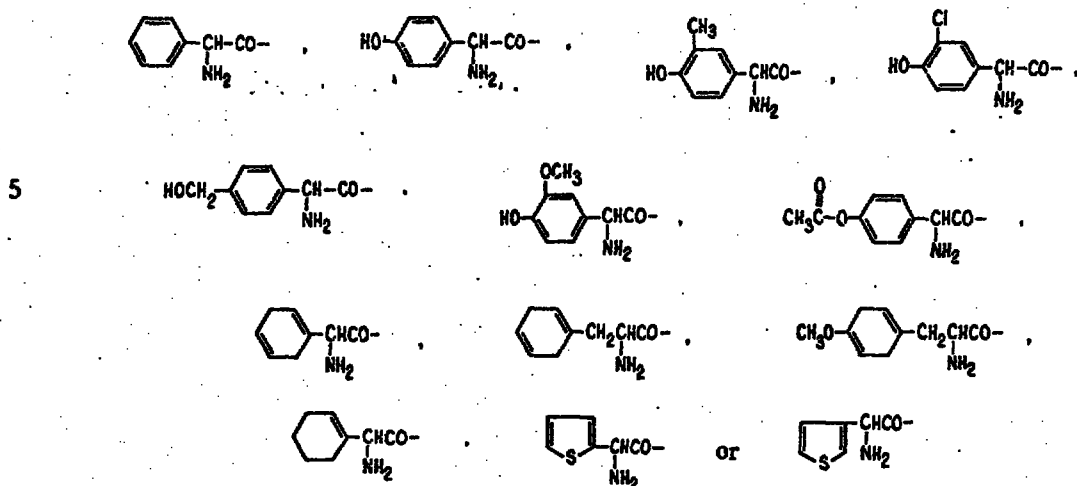


where R^u has the meaning defined in (i) and R^{24} is lower alkyl, cycloalkyl, aryl or certain heterocyclic groups. Examples are disclosed in U.S. 3,778,436.

A preferred embodiment of the present invention consists of the compounds of Formula I wherein R^1 has the structure

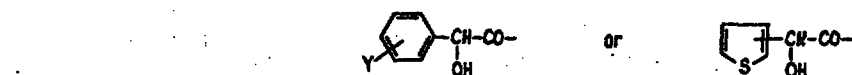


Another preferred embodiment of the present invention consists of the compounds of Formula I having the D configuration in the 7-side chain wherein R¹ has the structure



10 A preferred embodiment of the present invention comprises the compounds of Formula I where R¹ is D-(-)- α -amino-phenylacetyl, D-(-)- α -amino-*p*-hydroxyphenylacetyl or D-(-)- α -amino-*p*-acetoxyphenylacetyl.

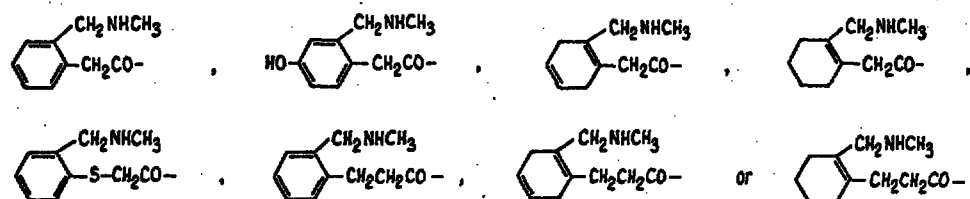
Another preferred embodiment of the present invention consists of the compounds of Formula I having the D configuration in the 7-side chain wherein R¹ has the structure



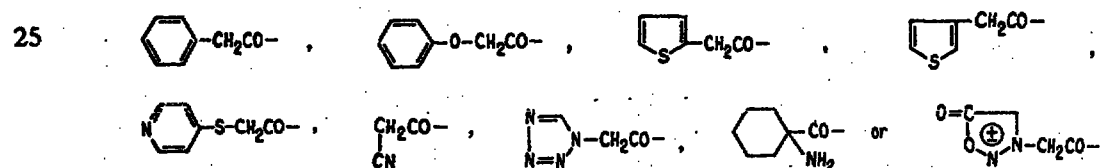
and Y is hydrogen, chlorine, bromine, fluorine, trifluoromethyl, amino, nitro, hydroxy, lower alkyl or 1-4 carbon atoms or lower alkoxy or 1-4 carbon atoms.

A further preferred embodiment of the present invention consists of compounds of Formula I wherein R¹ is D-(-)- α -hydroxyphenylacetyl.

20 Another preferred embodiment of the present invention consists of the compounds of Formula I wherein R¹ has the structure



Another preferred embodiment of the present invention consists of the compounds of Formula I wherein R¹ has the structure



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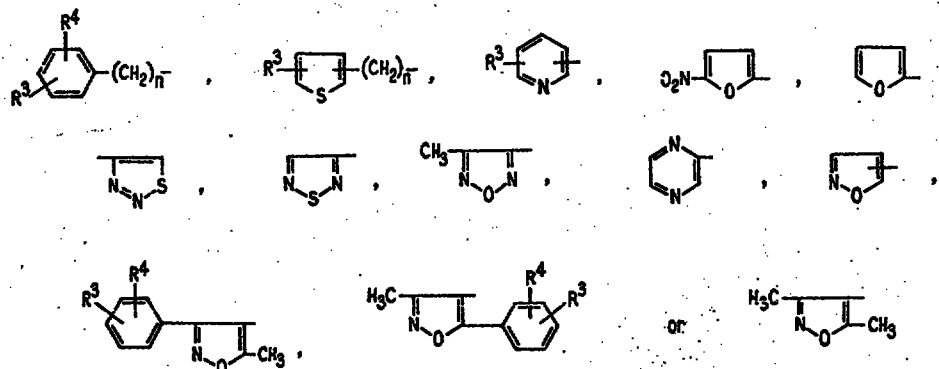

$$\frac{dy}{ds}$$

15



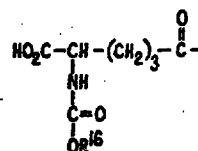
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in which Z represents (lower)alkyl, cycloalkyl having 4, 5, 6 or 7 carbon atoms, monohalo(lower)alkyl, dichloromethyl, trichloromethyl, (lower)alkenyl of 2 to 6 carbon atoms,

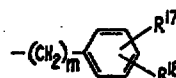


n is an integer from 0 to 3 inclusive and each of R³ and R⁴ is hydrogen, nitro, di(lower)-alkylamino, (lower)alkanoylamino, (lower)alkanoyloxy, (lower)alkyl, (lower)alkoxy, sulfamyl, chloro, iodo, bromo, fluoro or trifluoromethyl. In the most preferred embodiments Z is phenyl, 2-thienyl, 3-thienyl, 2-furyl or 5-nitro-2-furyl.

Another preferred embodiment of the present invention consists of the compounds having the Formula I wherein R¹ has the formula

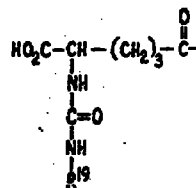


wherein R¹⁶ is (lower)alkyl or an aralkyl group of the formula



wherein m is an integer of 0 to 6 and R¹⁷ and R¹⁸ are alike or different and each is H, Cl, Br, F, NO₂, (lower)alkyl or (lower)alkoxy.

Another preferred embodiment of the present invention consists of the compounds having the formula I wherein R¹ has the formula



wherein R¹⁹ is (lower)alkyl or a group of the formula



wherein m is an integer of 0 to 6 and R¹⁷ and R¹⁸ are alike or different and each is H, Cl, Br, F, NO₂, (lower)alkyl or (lower)alkoxy.

Another preferred embodiment of the present invention consists of the compounds of the Formula I and pivaloyloxymethyl, acetoxymethyl, methoxymethyl, acetoxy, phenacyl, p-nitrobenzyl or β,β,β-trichloroethyl esters thereof wherein R¹ has the structure

$$\text{C}_6\text{H}_4\begin{cases} \text{CH}_2\text{NH}-\text{C}(=\text{Y})\text{NH}-\text{C}(=\text{X})- \\ \text{CH}_2\text{CO}- \end{cases}$$
$$\text{C}_6\text{H}_4\begin{cases} \text{CH}_2\text{NH}-\overset{\text{Y}}{\overset{\text{||}}{\text{C}}}-\text{NH}-\overset{\text{X}}{\overset{\text{||}}{\text{C}}}-\text{Z} \\ \text{CH}_2\text{CH}_2\text{CO}- \end{cases}$$
$$\text{CH}_2\text{NH}-\overset{\text{Y}}{\overset{\parallel}{\text{C}}}-\text{NH}-\overset{\text{X}}{\overset{\parallel}{\text{C}}}-\text{Z}$$

$$\text{CH}_2\text{CH}_2\text{CO}-$$

5

C1=CC=C(C=C1)OC1=CN(C=C1)OC[C@H]1C=CC(=O)N1

10

15

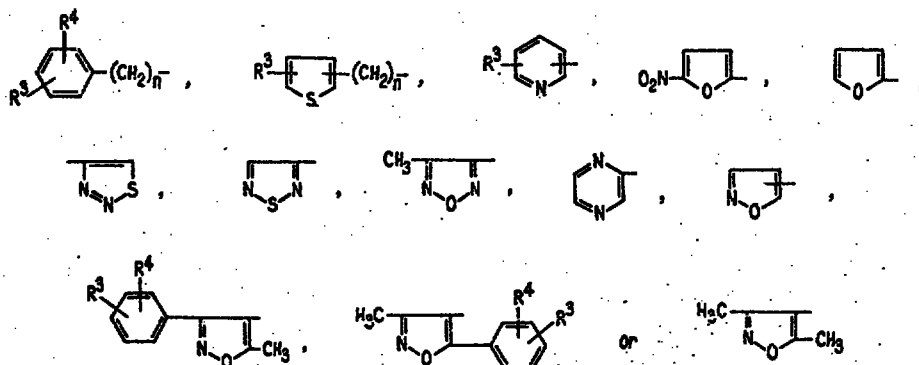
$$\text{HO}-\text{C}_6\text{H}_3(\text{Cl})-\text{CH}(\text{NH}-\text{C}(=\text{X})-\text{Z})-\text{CO}-$$
$$\text{C}_6\text{H}_5-\text{CH}_2-\underset{\text{NH}}{\underset{\text{Y}=\text{C}}{\text{CH}}}-\text{CO}-\underset{\text{X}}{\underset{\text{NH}}{\text{C}}}=\text{Z}$$

or

$\text{Y}=\text{C}-\text{NH}-\text{C}=\text{X}$

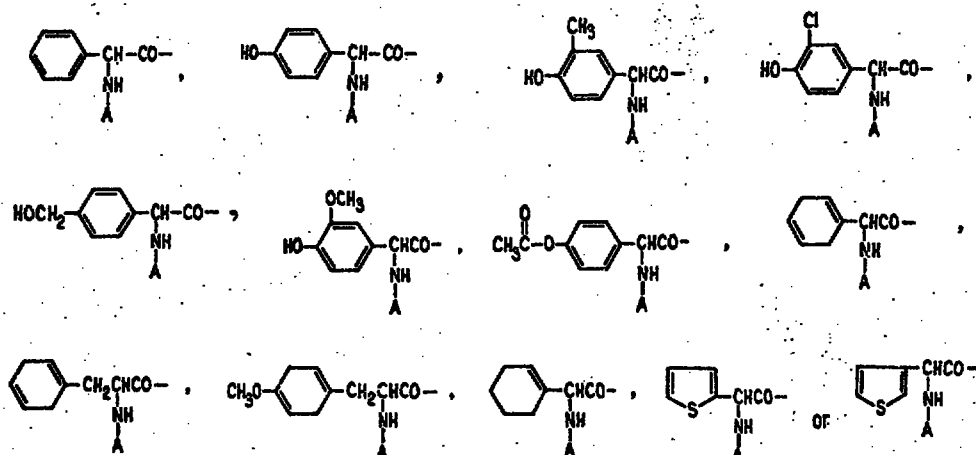
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amino, (lower)alkyl, cycloalkyl having 4, 5, 6 or 7 carbon atoms, monohalo(lower)alkyl, dichloromethyl, trichloromethyl, (lower)alkenyl of 2 to 6 carbon atoms,



n is an integer from 0 to 3 inclusive and each of R³ and R⁴ is hydrogen, nitro, di(lower)alkylamino, (lower)alkanoylamino, (lower)alkanoyloxy, (lower)alkyl, (lower)alkoxy, sulfamyl, chloro, iodo, bromo, fluoro or trifluoromethyl.

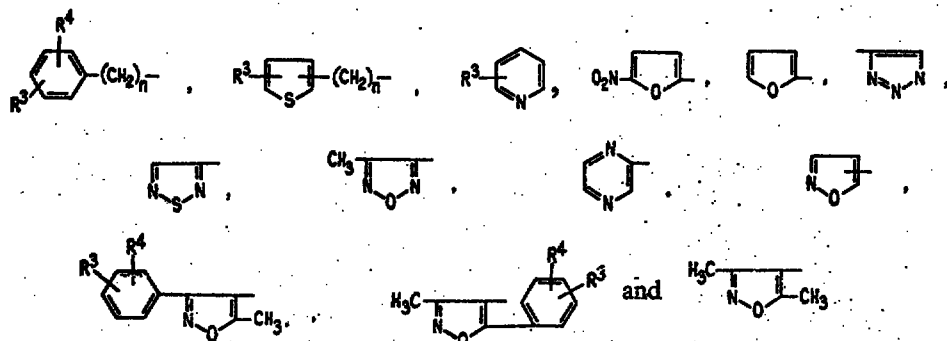
Another preferred embodiment of the present invention consists of the compounds having the Formula I wherein R¹ has the D configuration at the alpha carbon atom and has the formula



15 wherein A represents



in which Z represents (lower)alkyl, cycloalkyl having four, five, six or seven carbon atoms, monohalo(lower)alkyl, dichloromethyl, trichloromethyl, (lower)alkenyl of two to six carbon atoms,



(A)

	10	11	1,525,626	11
yl,			n is an integer from 0 to 3 inclusive and each of R ³ and R ⁴ is hydrogen, nitro, di(lower)alkylamine, (lower)alkanoylamino, (lower)alkanoyloxy, (lower)-alkyl (comprising straight and branched chain saturated aliphatic groups having from one to six carbon atoms inclusive), (lower)-alkoxy, sulfamyl, chloro, iodo, bromo, fluoro or trifluoromethyl.	
		5	Another preferred embodiment of the present invention consists of the compounds having the Formula I and pivaloyloxymethyl, acetoxymethyl, methoxymethyl, acetonyl, phenacyl, p-nitrobenzyl or β,β,β -trichloroethyl esters thereof wherein R ¹ represents phenylacetyl,	5
		10	o-, m- and p-fluorophenylacetyl,	10
			o-, m- and p-nitrophenylacetyl,	
			o-, m- and p-guanidino-phenylacetyl,	
			o-, m- and p-acetoxypheylacetyl,	
		15	o-, m- and p-methoxyphenylacetyl,	15
	5		o-, m- and p-methylphenylacetyl,	
			o-, m- and p-hydroxyphenylacetyl,	
			N,N'-bis-(2-chloroethyl)aminophenylpropionyl,	
			thien-2-acetyl,	
		20	thien-3-acetyl,	20
ro,			4-isoxazolylacetyl,	
yl,			1-cyclohexenylacetyl,	
ids			1,4-cyclohexadienylacetyl,	
om	10		pyridylacetyl,	
			sydnoneacetyl,	
		25	3-o-chlorophenyl-5-methyl-isoxazol-4-yl-acetyl,	25
			cyanoacetyl,	
			3,5,7-triaza-1-azonia-1-adamantyl)acetyl,	
			m-aminopyridiniumacetyl,	
		30	o-, m- and p-(2'-aminoethoxy)phenylacetyl,	30
			4,5-dimethoxycarbonyl-1,2,3-triazol-1-ylacetyl,	
			4-cyano-1,2,3-triazol-1-ylacetyl,	
			imidazol-(1)-acetyl,	
			m- and p-aminomethylphenylacetyl,	
		35	α -amino-2,4,6-cycloheptatrienyl-acetyl,	35
			lower alkoxy carbonylacetyl,	
			hexanoyl,	
			heptanoyl,	
			octanoyl,	
co-		40	butylthioacetyl,	40
			trifluoromethylthioacetyl,	
			allylthioacetyl,	
			phenoxyacetyl,	
	15	45	2-phenoxy-2-phenylacetyl,	45
			2-phenoxypropionyl,	
			2-phenoxybutyryl,	
			benzyloxycarbonyl,	
			2-methyl-2-phenoxypropionyl,	
			p-cresoxyacetyl,	
			p-methylthiophenoxyacetyl,	
on		50	S-phenylthioacetyl,	50
wo			S-chlorophenylthioacetyl,	
			S-fluorophenylthioacetyl,	
			pyridylthioacetyl,	
			S-benzylthioacetyl,	
	20	55	p-aminomethylphenylthioacetyl,	55
			cyanomethylthioacetyl,	
			4-pyridylthioacetyl,	
			S-benzylthiopropionyl,	
			benzoyl,	
		60	aminobenzoyl,	60
			4-isoxazolyl-carbonyl,	
			cyclopentane-carbonyl,	
			sydnone-carbonyl,	
			naphthoyl,	
		65	2-ethoxynaphthoyl,	65

- quinoxaliny-carbonyl,
 3-carboxy-2-quinoxaliny-carbonyl,
 2,6-dimethoxybenzoyl,
 2-biphenylcarbonyl,
 2-methylaminobenzoyl,
 2-carboxybenzoyl,
 3-phenyl-5-methylisoxazol-4-yl-carbonyl,
 3-o-chlorophenyl-5-methyl-isoxazol-4-yl-carbonyl or
 3-2',6'-dichlorophenyl-5-methylisoxazol-4-ylcarbonyl.

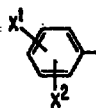
Another preferred embodiment of the present invention consists of the compounds having the Formula I wherein R^1 is



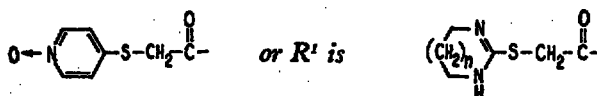
wherein Z is amino(lower)alkyl, N-(lower)alkyl-amino(lower)alkyl, or N,N-di(lower)-alkylamino(lower)-alkyl or R^1 is



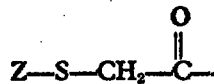
wherein Z is aminophenyl, aminophenyl(lower)-alkyl, N-(lower)alkylaminophenyl, N,N-di(lower)-alkylaminophenyl, N-(lower)alkylaminophenyl(lower)-alkyl, N,N-di-(lower)alkylaminophenyl(lower)alkyl, phenylamino(lower)alkyl, phenyl(lower)alkyl-amino-(lower)alkyl, substituted phenylamino(lower)alkyl or substituted phenyl(lower)-alkylamino(lower)alkyl, wherein substituted phenyl is defined as a group having the formula



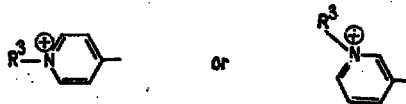
in which X^1 and X^2 are alike or different and are selected from the group consisting of hydrogen, fluoro, chloro, bromo, (lower)alkyl, (lower)alkoxy, nitro, amino, tri-fluoromethyl and phenyl or R^1 is



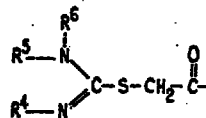
wherein n is 1 or 2 or R^1 is



wherein Z is a radical of the formula



in which R^3 is (lower)alkyl, (lower)alkenyl or (lower)alkynyl or R^1 is



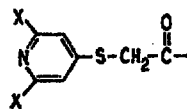
wherein formula R^4 , R^5 and R^6 are each (lower)alkyl, (lower)alkynyl, (lower)alkenyl or (lower)cycloalkyl and R^5 may also be hydrogen; or R^1 is

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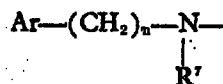
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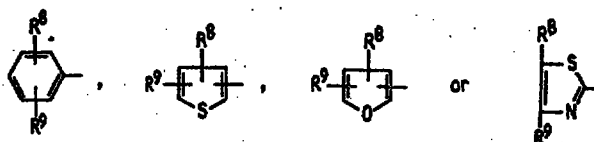
in which X is fluoro or chloro or R' is



wherein X is oxygen or sulfur; B represents di(lower)alkylamino wherein the alkyl group may be alike or different, piperidino, methylpiperidino, dimethylpiperidino, pyrrolidino, methylpyrrolidino, dimethylpyrrolidino, morpholino, methylmorpholino, dimethylmorpholino, N'-(lower)alkylpiperazino, N'-(lower)alkyl-methylpiperazino, N'-(lower)alkyl-dimethylpiperazino, trimethyleneimino, hexamethyleneimino or a radical of the formula

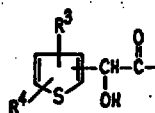


wherein n is zero, one, two or three, R' is (lower)alkyl and Ar is a radical of the formula



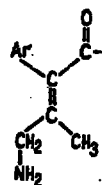
wherein R⁸ and R⁹ are each hydrogen, fluoro, chloro, bromo, (lower)alkyl or (lower)-alkoxy.

Another preferred embodiment of the present invention consists of the compounds having the Formula I wherein R¹ has the formula

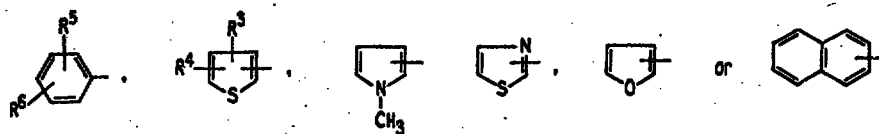


wherein R³ and R⁴ are each hydrogen, chloro, bromo, fluoro, iodo, nitro (lower)alkyl, (lower)alkoxy, (lower)alkylthio, di(lower)alkylamino, (lower)-alkanoylamino, (lower)-alkanoyl or (lower)alkyl-sulfonyl.

Another preferred embodiment of the present invention consists of the compounds having the Formula I wherein R¹ has the formula

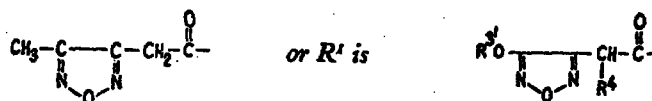


wherein Ar is

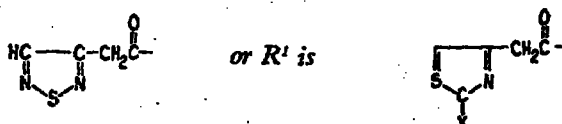


in which R⁵ and R⁶ are each hydrogen, fluoro, chloro, methoxy or methyl and R³ and R⁴ are each hydrogen, bromo or chloro.

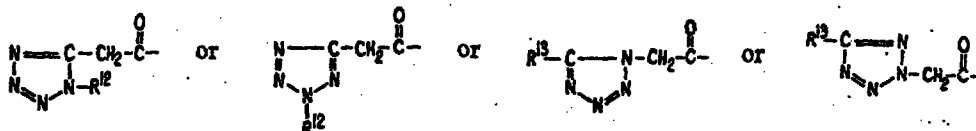
Another preferred embodiment of the present invention consists of the compounds having the Formula I wherein R^1 is



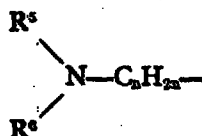
5 wherein R^3 and R^4 are each a member selected from the group consisting of hydrogen and (lower)alkyl or R^1 is



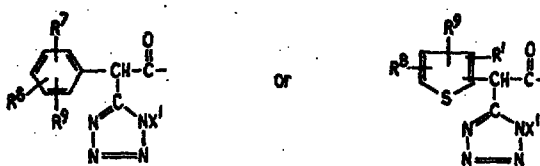
wherein X represents a member selected from the group consisting of hydrogen and (lower)alkyl or R^1 is



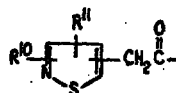
10 wherein R^{12} is hydrogen or (lower)alkyl; R^{13} is hydrogen, chloro, bromo, iodo, fluoro, trifluoromethyl, (lower)alkyl, (lower)alkoxy, (lower)alkylthio, carboxymethoxy, (lower)alkylsulfonyl, phenyl, benzyl, phenoxy, benzyloxy, and radicals of the formula



15 wherein R^5 and R^6 each represent hydrogen, (lower)alkyl, phenyl, benzyl, cycloalkyl having from three to seven carbon atoms, inclusive, and when taken together with $>\text{N}-$, constitute a heterocyclic ring selected from the group consisting of pyrrolidino, piperidino, morpholino, 1,2,5,6-tetrahydropyridino, N-(lower)alkylpiperazino and hexamethyleneimino and n is an integer from 0 to 3 inclusive; or R^1 is

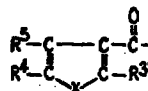


20 wherein R^7 , R^8 and R^9 each represents a member selected from the group consisting of hydrogen, nitro, di(lower)alkylamino, (lower)alkanoylamino, (lower)alkanoyloxy, (lower)alkyl, (lower)alkoxy, sulfamyl, chloro, iodo, bromo, fluoro and trifluoromethyl; X and X' are alike or different and each represents hydrogen or R^1 is



25 wherein R^{10} and R^{11} each represent a member selected from the group consisting of hydrogen, chloro, bromo, iodo and methyl.

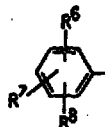
Another preferred embodiment of the present invention consists of the compounds having the Formula I wherein R^1 is



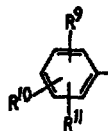
wherein X is a divalent radical selected from the group consisting of



wherein R^3 and R^4 each represent a member selected from the group consisting of (lower)alkyl, chloro, bromo and the radical having the formula

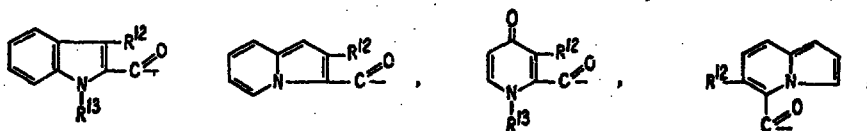
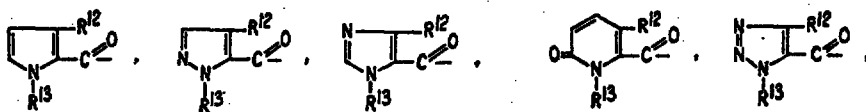


wherein R^6 , R^7 and R^8 each represent a member selected from the group consisting of hydrogen, fluoro, bromo and chloro, and R^5 represents a member selected from the group consisting of hydrogen, (lower)alkyl and the radical having the formula

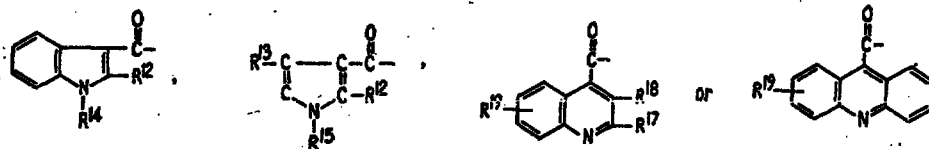
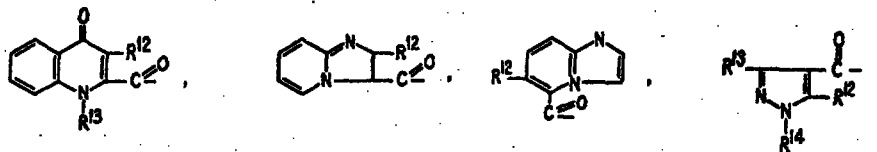


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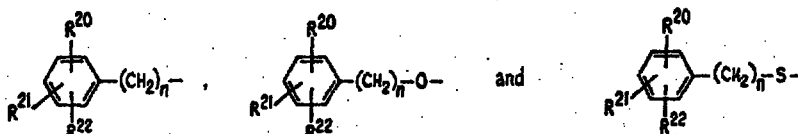
wherein R^9 , R^{10} and R^{11} each represent a member selected from the group consisting of hydrogen, fluoro, bromo and chloro and R^4 and R^5 bonded together to form a benz group or R^4 is



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wherein R^{12} and R^{13} each represent a member selected from the group consisting of nitro, (lower)alkyl, (lower)alkoxy, (lower)alkanoylamino, chloro, bromo, iodo, fluoro, hydroxy, (lower)alkylthio, cyclohexyl, cyclopentyl, cycloheptyl, (lower)alkoxycarbonyl, mercapto, (lower)alkylsulfonyl, (lower)alkanoyl, (lower)alkanoyloxy and the three radicals having the formulae

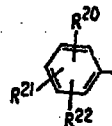


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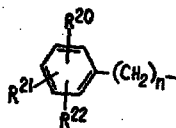
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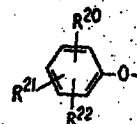
- wherein n is an integer from zero to three inclusive and R^{20} , R^{21} and R^{22} each represent a member selected from the group consisting of hydrogen, fluoro, bromo, chloro, iodo, trifluoromethyl, (lower)alkyl, (lower)alkoxy, hydroxy, nitro and amino; R^{14} represents a member selected from the group consisting of hydrogen, (lower)alkyl and the radical having the formula



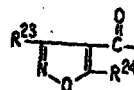
- wherein R^{20} , R^{21} and R^{22} have the meaning defined above; R^{15} represents a member selected from the group consisting of hydrogen and (lower)alkyl; R^{16} represents a member selected from the group consisting of hydrogen and R^{12} as defined above; R^{17} and R^{18} each represent a member selected from the group consisting of (lower)alkyl, (lower)alkoxy, chloro, bromo, iodo, fluoro, cyclohexyl, cyclopentyl, cycloheptyl and the two radicals having the formulae



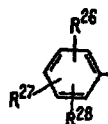
and



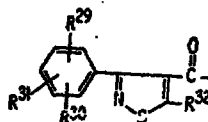
- wherein n is an integer from zero to three inclusive and R^{20} , R^{21} and R^{22} have the meaning defined above; and R^{19} represents a member selected from the group consisting of hydrogen and R^{17} as defined above; or R^1 is



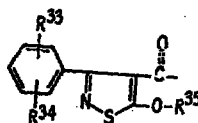
- wherein R^{23} and R^{24} each represent a member selected from the group consisting of (lower)alkyl, (lower)alkylthio, benzylthio, cyclohexyl, cyclopentyl, cycloheptyl, benzyl, styryl, phenylethyl, phenylpropyl, furyl, thienyl, naphthyl and a member selected from the group consisting of a radical having the formula



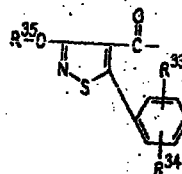
- wherein R^{26} , R^{27} and R^{28} each represent a member selected from the group consisting of hydrogen, fluoro, chloro, bromo, iodo, trifluoromethyl, (lower)alkyl, (lower)alkoxy, nitro, methylsulfonyl, cyano, di(lower)alkylamino and methylmercapto; or R^1 is



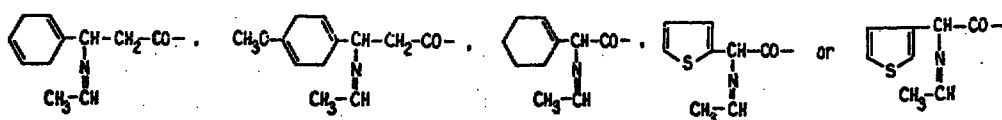
- wherein R^{29} , R^{30} and R^{31} represent hydrogen, chloro, bromo, iodo, trifluoromethyl, fluoro, methylsulfonyl, nitro, (lower)alkyl or (lower)alkoxy, and R^{32} represents (lower)alkyl or R^1 is



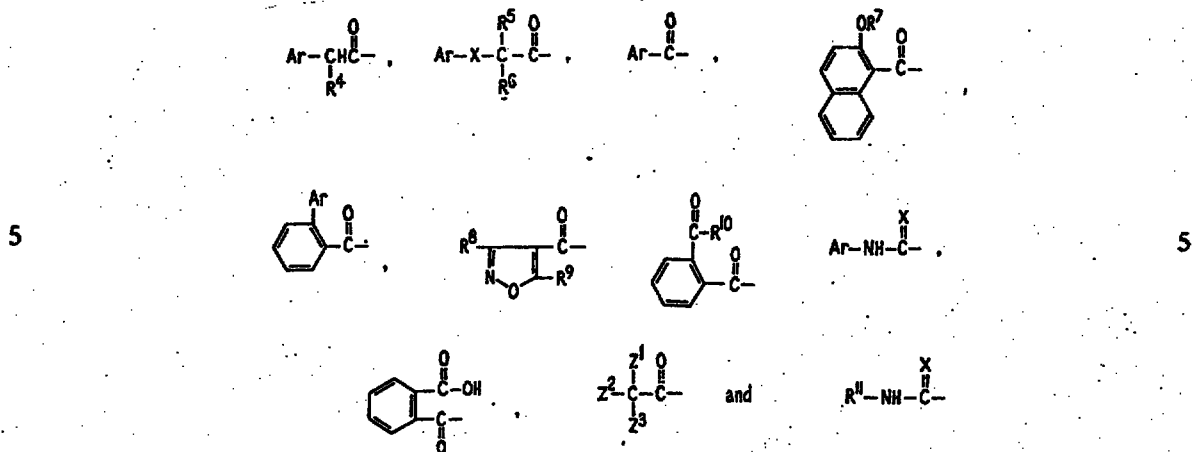
or



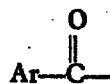
- wherein R^{33} and R^{34} are each hydrogen, chloro, bromo, iodo, (lower)alkyl, (lower)alkoxy or trifluoromethyl and R^{35} represents (lower)alkyl.



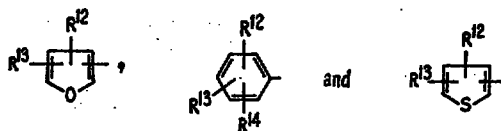
Another preferred embodiment of the present invention consists of the compounds having the Formula I wherein R^1 has the formula



wherein R^4 represents a member selected from the group consisting of hydrogen, amino, carbobenzoxyamino, phenyl, fluoro, chloro, bromo, iodo, hydroxy, (lower)alkanoyloxy and (lower)alkoxy; X represents a member selected from the group consisting of oxygen and sulfur; R^5 and R^6 each represent a member selected from the group consisting of hydrogen, phenyl, benzyl, phenethyl and (lower)alkyl; R^7 represents (lower)alkyl; R^8 and R^9 each represent a member selected from the group consisting of (lower)alkyl, (lower)alkylthio, benzylthio, cyclohexyl, cyclopentyl, cycloheptyl, benzyl, phenethyl, phenylpropyl, furyl, thienyl, naphthyl and Ar—; R^{10} represents a member selected from the group consisting of (lower)alkylamino; di(lower)alkylamino, cycloalkylamino having from 3 to 7 carbon atoms inclusive, allylamino, diallylamino, phenyl-(lower)alkylamino, morpholino, (lower)alkylamino, pyrrolidino, (lower)alkylpyrrolidino, di(lower)alkylpyrrolidino, N,N-hexamethyleneimino, piperidino, (lower)alkylpiperidino, di(lower)alkylpiperidino, 1,2,5,6-tetrahydropyridino, N-(lower)alkylpiperazino, N-(lower)alkylpiperazino, N-phenylpiperazino, N-(lower)alkyl(lower)alkylpiperazino, N-(lower)alkyl-di-(lower)alkylpiperazino, furfurylamino, tetrahydrofurfurylamino, N-(lower)alkyl-N-furfurylamino, N-alkyl-N-anilino and (lower)alkoxyanilino; Z^1 , Z^2 and Z^3 each represent a member selected from the group consisting of (lower)alkyl, and Ar—; R^{11} represents a member selected from the group consisting of (lower)alkyl, (lower)cycloalkyl, naphthyl, benzyl, phenethyl and

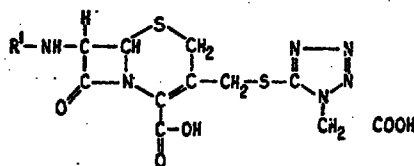


and Ar represent a monovalent radical having one of the formulae

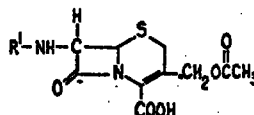


wherein R^{12} , R^{13} and R^{14} are each a member selected from the group consisting of hydrogen, chloro, bromo, iodo, trifluoromethyl, phenyl, (lower)alkyl and (lower)alkoxy, but only one R group may represent phenyl.

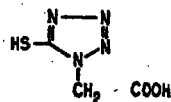
The present invention also provides the process for the preparation of a compound of the formula



wherein R^1 is acyl, or an ester or a non-toxic pharmaceutically acceptable salt thereof characterized by reacting a compound of the formula



in which R^1 is H or acyl, or a salt or easily hydrolyzable ester thereof with a compound of the formula

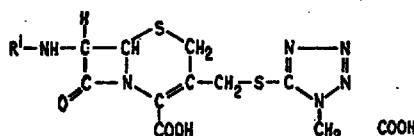


and, when R^1 is H, treating the resulting compound with an acylating agent of the formula

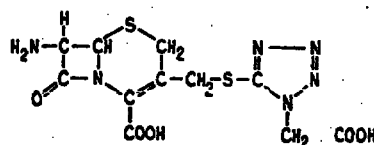


in which X is halide or a functional equivalent thereof and R^1 is acyl, which when the acyl group contains free amino or hydroxyl, said groups are blocked by conventional protective groups and subsequently removed to afford a compound of the Formula I, and, if desired, converting in a resulting free acid, salt or easily hydrolyzable ester of a compound of the Formula I said resulting compound to the corresponding ester or non-toxic pharmaceutically acceptable salt thereof, and, if desired, converting in 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.

The present invention also provides the process for the production of the anti-bacterial agents having the structure



wherein R^1 is acyl which comprises reacting a compound of the formula



or a salt or easily hydrolyzed ester or 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-amino-penicillanic acid and used in Great Britain 1,073,530 and particularly the pivaloyloxymethyl, acetoxyethyl, 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.

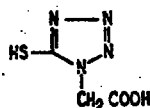
Such functional equivalents include the corresponding acid anhydrides, including mixed anhydrides and particularly the mixed anhydrides prepared from stronger acids such as the lower aliphatic monoesters of carbonic acid, or alkyl and aryl sulfonic acids and of more hindered acids such as diphenylacetic acid. A particularly useful anhydride is an N-carboxy-anhydride (also called a Leuch's anhydride; see U.S.

3,080,356 and 3,206,455) including but not limited to D-mandelic acid carboxy-anhydride (U.S. 3,167,549) or the corresponding substituted D-mandelic acid carboxy-anhydride. 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 II after first reacting said free acid with N,N'-dimethylchloroformiminium chloride [cf. Great Britain 1,008,170 and Novak and Weichert, *Experientia* XXI, 6, 360 (1965)] or by the use of enzymes or of an N,N'-carbonyldiimidazole 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-cyclohexyl-N'-(2-morpholinoethyl)carbodiimide; cf. Sheehan and Hess, *J. Amer. Chem. Soc.*, 77, 1967 (1955)], or of alkylamine 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. E. 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'-carbonyldiimidazole is reacted with a carboxylic acid in equimolar proportions at room temperature in tetrahydrofuran, 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 II. 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 microorganisms, 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—325 (1971) and in U.S. 3,682,777.

For the coupling of the organic carboxylic acid, including but not limited to a substituted or unsubstituted D-mandelic acid (with or without a protecting group on the α -hydroxyl), as described above with compound II) 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.

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 of a 7-acylaminocephalosporanic acid (prepared by substituting 7-aminocephalosporanic acid for the 3-thiolated-7-aminocephalosporanic acids in the acylation procedures described herein and elsewhere reported) with a thiol having the formula



and then removing the protecting group if any is present, as on an α -hydroxy or α -amino or the like or on the carboxyl group or both. 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

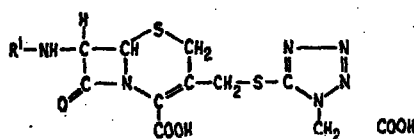
about 50° to 100° C. in the presence of a mild base such as sodium bicarbonate, e.g. preferably near neutrality such as at about pH 6. An excess of the thiol is preferably employed. The reaction product is isolated by careful acidification of the reaction mixture followed by extraction with a water-immiscible organic solvent. To provide some specific examples for purposes of illustration but not of limitation, substituted or unsubstituted D-mandelamido-cephalosporanic acids are prepared by the procedures described generally or specifically in *J. Med. Chem.* 17(1), 34-41 (1974) and the references cited therein. As noted above, the preparation of many other 7-acylamido-cephalosporanic acids is described in the patent and scientific literature, e.g. in U.S. Class 260-243C.

When the organic carboxylic acid contains a functional group such as amino or hydroxyl it is often desirable to first block (or protect) the amino or hydroxy group, then carry out the coupling reaction and finally subject the resulting compound to chemical removal of the protecting group, that is, subjecting the resulting compound to elimination reaction of the protecting group.

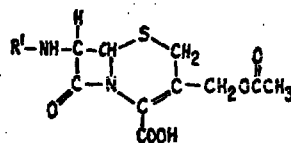
There are particularly included within the scope of the present invention the antibacterial agents which are prepared in the above-described acylation process by the use therein of the organic monocarboxylic acids or their acid chlorides or other equivalents which have previously been used to acylate 6-amino-penicillanic acid as described, for example U.S. patents 2,941,995; 2,951,839; 2,985,648; 2,996,501; 3,007,920; 3,025,290; 3,028,379; 3,035,047; 3,040,033; 3,041,332; 3,041,333; 3,043,831; 3,053,831; 3,071,575; 3,071,576; 3,079,305; 3,079,306; 3,080,356; 3,082,204; 3,093,547; 3,093,633; 3,116,285; 3,117,119; 3,118,877; 3,120,512; 3,120,513; 3,130,514; 3,127,394; 3,140,282; 3,040,032; 3,142,673; 3,147,247; 3,174,964; 3,180,863; 3,198,804; 3,202,653; 3,202,654; 3,202,655; 3,210,337; 3,157,639; 3,134,767; 3,132,136; in British Patent Specifications 874,414; 874,416; 876,516; 876,662; 877,120; 877,323; 877,531; 878,233; 880,042; 880,400; 882,335; 888,110; 888,552; 889,066; 889,069; 889,070; 889,168; 889,231; 890,201; 891,174; 891,279; 891,586; 891,777; 891,938; 893,518; 894,247; 894,457; 894,460; 896,072; 899,199; 900,666; 902,703; 903,785; 904,576; 905,778; 906,383; 908,787; 914,419; 916,097; 916,204; 916,205; 916,488; 918,169; 920,176; 920,177; 920,300; 921,513; 922,278; 924,037; 925,281; 931,567; 932,644; 938,066; 938,321; 939,708; 940,488; 943,608; 944,417; in numerous Belgian Patents, e.g. 593,222; 595,171; 597,857; 602,494; 603,703; 609,039; 616,419; 617,187; and in South African Patent Applications, e.g. 60/2882; 60/3057; 60/3748; 61/1649; R61/2751; 62/54; 62/4920; 63/1612 and 63/2423.

The term "(lower)alkyl" as used herein means both straight and branched chain aliphatic hydrocarbon radicals having from one to ten carbon atoms such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, t-butyl, amyl, hexyl, 2-ethylhexyl, heptyl, decyl, etc. Similarly, where the term "(lower)" is used as part of the description of another group, e.g. "(lower)alkoxy", it refers to the alkyl portion of such group which is therefore as described above in connection with "(lower)alkyl".

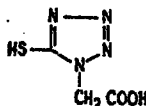
The present invention thus also provides the process for the production of the antibacterial agents having the structure



wherein R¹ is acyl
which comprises reacting a compound having the formula

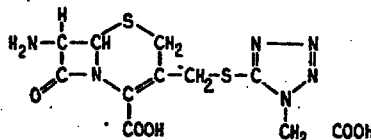


wherein R¹ is acyl (including cephalosporin C itself) with a compound having the formula

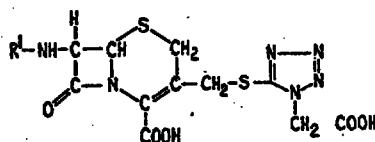


5 In the case of the compounds having a substituted amino group in the 7-side chain it is often desirable to prepare first the compound containing an unsubstituted (free or primary) amino group and then react that product with the appropriate reagent to produce final products of the type illustrated above in the definitions of acyl groups under viii, xvii and xviii as appropriate.

The present invention also provides the process for the production of the novel and valuable intermediate having the structure

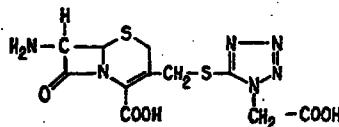


10 which comprises removing the 7-side chain from a reagent having the structure

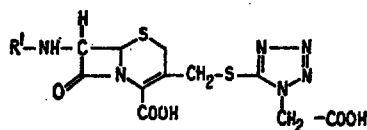


15 wherein the acyl group (R¹) is one of those defined above in xiv, xv and xvi by converting said reagent, preferably in silylated form, to an imino-chloride (as with PCl₅) and thence to an imino-ether (as with methanol) and thence to the desired product by hydrolysis (as with water). Details for the conduct of such reactions are known to the art and taught, for example, in U.S. 3,575,970; 3,573,295 and 3,573,296.

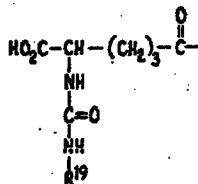
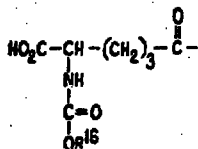
The present invention also provides the process for the production of the novel and valuable intermediate having the formula



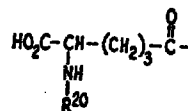
20 characterized by the consecutive steps of
(a) treating a silyl ester of a compound of the formula



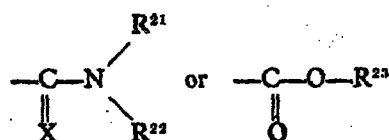
in which n is 1 to 9 and R¹ is of the formulae



or



25 wherein R¹⁶ and R¹⁹ are each (lower)alkyl or an aralkyl group having 1 to 6 carbon atoms in the alkyl portion and R²⁰ is hydrogen, an α-halo or α,α-dihalo C₂-C₄ alkanoyl, N-isobornyloxycarbonyl or a group of the formulae

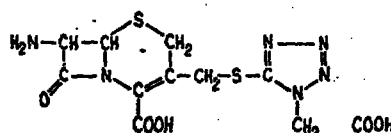


in which R^{21} and R^{22} are each H, (lower)alkyl, phenyl halophenyl, tolyl, (lower), alkoxyphenyl, or naphthyl, X is oxygen or sulfur, and R^{23} is (lower)alkyl with PCl_5 to afford the corresponding imino-chloride;

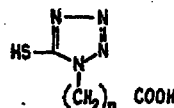
(b) treating said imino-chloride with methanol; and

(c) hydrolyzing the resulting imino-ether to the desired product.

The present invention also provides the process for the production of the novel and valuable intermediate having the structure



which comprises reacting 7-aminocephalosporanic acid or a salt or easily hydrolyzed ester or Schiff base thereof with a compound having the formula



in which n is 1 to 9, also called HSR² herein.

Such salts include the nontoxic carboxylic acid salts thereof, including nontoxic metallic salts such as sodium, potassium, calcium and aluminum, the ammonium salt and substituted ammonium salts, e.g. salts of such nontoxic amines as trialkylamines including triethylamine, procaine, dibenzylamine, N-benzyl-beta-phenethylamine, 1-phenamine, N,N'-dibenzylethylenediamine, dehydroabietylamine, N,N'-bis-dehydroabietylthylenediamine, N-(lower)alkylpiperidine, e.g. N-ethylpiperidine, and other amines which have been used to form salts with benzylpenicillin; and the nontoxic acid addition salts thereof (i.e., the amine salts) including the mineral acid addition salts such as the hydrochloride, hydrobromide, hydroiodide, sulfate, sulfamate and phosphate and the organic acid addition salts such as the maleate, acetate, citrate, oxalate, succinate, benzoate, tartrate, fumarate, malate, mandelate, ascorbate and the like.

Also included in this invention are the compounds (used as either intermediates or metabolic precursors) in which the amino group is "blocked" by substituents such as 2-iodoethoxycarbonyl (U.K. 1,349,673), t-butoxycarbonyl, carbobenzyloxy, formyl, o-nitrophenylsulfenyl, β,β,β -trichloroethoxycarbonyl, 4-oxo-2-pentenyl-2, 1-carbomethoxy-1-propenyl-2- and the like. Particularly included in such blocking groups are the ketones (especially acetone) and aldehydes (especially formaldehyde and acetaldehyde) disclosed, for example, in U.S. patents 3,198,804 and 3,347,851 and the β -ketoesters and β -diketones disclosed, for example, in U.S. patent 3,325,479 and the β -ketoamides disclosed in Japan 71/24714 (Farmdoc 47,321S).

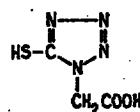
The preferred esters of the cephalosporins of the present invention are the pivaloyloxymethyl, acetoxymethyl, methoxymethyl, acetoryl and phenacyl esters. All are useful intermediates in the production of the cephalosporin having a free carboxyl group and the first three are also of interest because on oral administration they provide different rates and amounts of absorption and give differing concentrations of the active antibacterial agent in blood and tissues.

As indicated above, these five esters of 7-aminocephalosporanic 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.

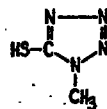
These esters of 7-aminocephalosporanic acid are then reacted with the nucleophile HSR² in the same manner as is illustrated herein for 7-aminocephalosporanic acid itself. The 3-thiolated ester of 7-aminocephalosporanic acid is then coupled with the organic carboxylic acid, e.g. D-(—)-2-phenylglycine, as before. Before or after removal of any blocking group, e.g. on an amino or hydroxy group in the 7-sidechain, the ester of the cephalosporin so obtained is, if not used per se, converted to its free acid, including its zwitterion (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).

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.

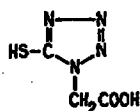
The valuable intermediate having the structure



can be prepared by a process which comprises forming in an anhydrous solvent the lithio derivative of a thiol of the formula



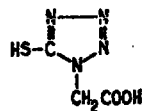
and reacting said lithio derivative with carbon dioxide to form a product which is then hydrolyzed to give the compound of the formula



In the treatment of bacterial infections in man, the compounds of this invention are administered parenterally or orally 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 in the form of liquid preparations such as solutions or suspensions.

STARTING MATERIALS.

A. 1-Carboxymethyl-5-mercaptotetrazole.



a) Recrystallization of 1-methyl-5-mercaptotetrazole. Procedure:

1. One hundred and ten grams of 1-methyl-5-mercaptotetrazole is slurried in 350 ml. of boiling chloroform. A near solution is obtained.

2. The hot solution (50—60°) is rapidly filtered by vacuum through a heated Buchner funnel (11 cm. SS No. 604 paper containing $\frac{1}{2}$ to $\frac{3}{4}$ inch of packed filter aid ("Supercel" registered Trade Mark). The filter pad is washed with 50 ml. of 50—60° C. chloroform which is added to the filtrate.

3. The filtrate is cooled to approximately 0—6° C. and kept at 0—6° C. for 2 hours. The crystals which have formed are collected by filtration at 0—6° C. and washed with 60 ml. of 0—6° C. chloroform which is added to the filtrate. The crystals (cut A) are air dried at 37—45° C. for 18 hours.

4. The filtrate is concentrated on the rotary vacuum evaporator (60° C. bath) to approximately one-half volume. This slurry is cooled to 0—6° C. and kept at 0—6° C. for 2 hours. The crystals are collected by filtration at 0—6° C., washed with 40 ml. of 0—6° C. chloroform which is added to the filtrate. The crystals (cut B) are air dried at 37—45° C. for 18 hours. Crystal cuts A and B are composited to give an approximate 65% weight yield.

5. The filtrate of cut B, Step 4 may be reworked twice as described in Step 4 to obtain an additional 15% recovery.

b) Preparation of the Di-sodium Salt of 1-carboxymethyl-5-mercaptopotetrazole.
Procedure:

1. Five hundred ml. of substantially dry and pure tetrahydrofuran in a 2-liter 3 neck flask with stirrer is cooled in a salt-acetone-ice bath to approximately -10° C. Dry nitrogen gas is blown on the liquid surface.

2. Five hundred ml. of 15.06% (1.6 N) butyl lithium in hexane (Foote Mineral Co.) is added over a ten minute period under dry nitrogen and stirring to the tetrahydrofuran. The near solution is cooled to -5 to -10° C.

3. Forty-six and four tenths gram (46.4 g.) of 1-methyl-5-mercaptopotetrazole (recrystallized as above) is dissolved in 200 ml. of substantially pure and dry tetrahydrofuran. The solution is filtered if cloudy and then cooled to 5 to 10° C.

4. The cooled solution of step 3 is added over 10 minutes with stirring and under dry nitrogen to the butyl lithium solution. The temperature should be maintained at -5° C. to +10° C. maximum. Precipitates may form.

5. The mixture is stirred under dry nitrogen and 0° C. to +10° C. for one half hour.

6. Anhydrous carbon dioxide gas is bubbled through at a rapid rate and with rapid stirring for 15—30 minutes at approximately ambient temperature (0 to 10° C.) to no higher than +20° C.

7. The white precipitate which forms is suitably collected by filtration in an area of low humidity. The precipitate is washed with about 75 ml. of tetrahydrofuran.

8. The precipitate is dissolved in 250 ml. of water (pH 8.5—9.5). A second layer of tetrahydrofuran may be present. This may be removed in the vacuum rotary evaporator (50° C. bath).

9. The aqueous solution is adjusted to pH 1.6—2.0 with concentrated hydrochloric acid.

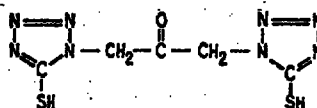
10. The acid aqueous solution is extracted twice with 250 ml. portions of ethyl acetate. Each 250 ml. ethyl acetate extract is back extracted with 100 ml. portions of water. The water extracts are discarded. The ethyl acetate extracts (free of any water layer) are filtered and composited.

11. The combined ethyl acetate extracts are concentrated to dryness on the vacuum rotary evaporator (60° C. bath).

12. The crystals in the flask are boiled with 300 ml. of chloroform for about 2 minutes. The hot slurry (50—60° C.) is vacuum filtered through a heated Buchner funnel (11 cm—SS—604 paper). The crystals are washed with about 75 ml. of 50° C. chloroform. The crystals are air dried at room temperature for about 3 hours and then made about 100—200 mesh.

13. The 100—200 mesh crystals are treated with boiling chloroform exactly as described in step 12 (the hot chloroform removes most of the unreacted 1-methyl-5-mercaptopotetrazole). Yield: approximately 45 to 50 grams of crystalline 1-carboxymethyl-5-mercaptopotetrazole. These crystals may contain 0.02 to 0.05 moles of 1-methyl-5-mercaptopotetrazole.

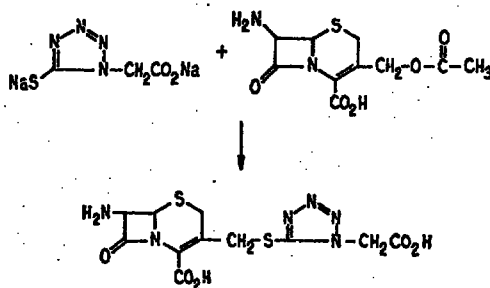
14. The crystals of step 13 are slurried with 250 ml. of ethyl ether at room temperature for 3—5 minutes. The mixture is filtered. The insolubles (0.5—5%) may be a contaminating symmetrical mercaptopotetrazole ketone of the following tentative structure:



CAUTION: This compound EXPLODES at approximately 205—210° C.

15. The ether filtrate of step 14 is evaporated to dryness on the vacuum rotary evaporator (50° C. bath). Approximately 42 to 48 grams of crystalline 1-carboxymethyl-5-mercaptotetrazole containing approximately 0.01—0.05 mole of 1-methyl-5-mercaptotetrazole is recovered.
- 5 16. The crystals are dissolved in 420 ml. of absolute ethanol (approximately 100 mg./ml.). The solution is warmed to 50—60° C.
- 10 17. To the hot solution of step 16, 310 ml. of a 41% sodium 2-ethylhexanoate (SEH) solution in isopropanol is added with very rapid stirring over a 10 minute period. A crystalline precipitate forms. The mixture is slurried at 50—60° C. for 20 minutes.
- 10 18. The mixture is filtered hot (50—60° C.) through a heated Buchner funnel (11 cm-SS—No. 604 paper). The crystals are washed with 75 ml. of 50° C. ethanol.
- 15 19. The ethanol damp crystals of step 18 are slurried in 200—300 ml. of ethanol. The slurry is passed through a 200 mesh screen. The slurry is heated to 50—60° C. for 5 minutes with rapid stirring (unreacted sodium 1-methyl-5-mercaptotetrazole is very soluble in hot ethanol).
- 20 20. The crystals are collected at 50—60° C. on a 11 cm-SS No. 604 paper in a heated Buchner funnel. The crystals are washed with 75—100 ml. of ethanol and vacuum dried at 50—60° C. for 24—48 hours. Yield: 40—48 grams of di-sodium 1-carboxymethyl-5-mercaptotetrazole (free of 1-methyl-5-mercaptotetrazole as observed by NMR).

B. 7-Amino-3-(1-carboxymethyltetrazol-5-yl-thiomethyl)-3-cephem-4-carboxylic acid.



- 25 1. Into a 3 necked flask set up with an agitator, a temperature regulator, thermometer and a nitrogen inlet tube, place 18 grams (0.066 mole) of 7-aminocephalosporanic acid, and 300 ml. of 0.1 M pH 6.4 phosphate buffer (20.7 grams of sodium phosphate, monobasic .1H₂O + 8.5 grams of sodium phosphate, dibasic, anhydrous, q.s. to 2 liters).
- 30 2. With agitation of the mixture described in step 1, add 1.5 grams of sodium bisulfite and 16 grams (0.078 moles) of 1-carboxymethyl-5-mercaptotetrazole disodium.
- 30 3. With agitation continuing, bubble nitrogen through the mixture for 10 minutes.
- 30 4. Maintaining agitation and nitrogen inflow, heat the slurry over a 20 minute period to 56° C. During this time interval, 6.5 grams of sodium bicarbonate is added in small increments.
- 35 5. With continued agitation and nitrogen inflow, maintain the temperature of the solution at 56° C. for 4 hours. The pH should remain at between 6.2—6.6.
- 35 6. Cool the reaction mixture in an ice bath to 5° C.
- 40 7. Add 50 ml. of a 1:1 phosphoric acid/water solution to the mixture or concentrated HCl to a pH of 2.0—3.0.
- 40 8. Collect the product by filtration. Wash the filter cake with 20 ml. of cold water followed by 200 ml. of cold methanol.
- 40 9. Air dry the solid to constant weight. (A typical run produced 14.5 grams of product.) This product may vary in color from yellow to dark brown.
- 45 10. Pass the product through a 200 mesh stainless steel screen.
- 45 11. Suspend 10 grams of the 200 mesh powder in 200 ml. of n-propanol with rapid stirring.
- 45 12. Add 2.0 ml. of concentrated hydrochloric acid and stir vigorously for 0.5 hour at room temperature.
- 50 13. Filter the slurry. Wash the brown solids with 20 ml. of n-propanol and add the wash to the filtrate (save the filter cake for possible recovery of additional product).
- 50 14. Add 1.5 grams of charcoal ("Darco G—60" — "Darco" is a registered Trade Mark) to the n-propanol filtrate of step 13. Slurry for 0.5 hour. Remove the carbon by filtration. Wash the carbon with 20 ml. of n-propanol and add the wash to the filtrate.

15. With rapid stirring, add triethylamine to the n-propanol filtrate to an apparent pH of 3.0. Crystals form. Slurry for 10 minutes.

16. Collect the white crystals by filtration and wash with 30 ml. of n-propanol, 50 ml. of methanol, and vacuum dry at 40° C. for 24 hours. Yield: 4 to 8 grams of 7-amino-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid.

17. An alternative procedure for the purification of 7-amino-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid follows:

a) Slurry 10 grams of the 200 mesh product (from step 10) in 75 ml. of 1 N hydrochloric acid for 10—15 minutes at room temperature. Filter to remove dark brown solids.

b) Add 2.5 grams of charcoal ("Darco G-60") and slurry for 0.5 hour.

c) Remove the carbon by filtration. Wash the carbon with 15 ml. of water and add the wash to the filtrate.

d) With rapid stirring, add concentrated ammonium hydroxide to the filtrate to pH 2.5—3.0. Crystals form.

e) Slurry the crystal mass for 25 minutes. Remove the crystals by filtration. Wash the crystals with 30 ml. of water, 50 ml. of methanol, and vacuum dry at room temperature. Yield: 4—7 grams of near white crystals.

The other reagents used to prepare the compounds of the present invention are synthesized either as described in the art (e.g. as in the patents and publications noted above) or by strictly analogous procedures. For example when use is made of a D-(—)- α -amino acid it is prepared according to the procedures set forth in U.S. patents 3,198,804; 3,342,677 or 3,634,418 or by Friis et al., Acta Chem. Scand. 17, 2391—2396 (1966) or by Neims et al., Biochemistry (Wash.) 5, 203—213 (1966) or in other publications on this subject. For convenience and purposes of illustration, however, there are given below some specific examples of such syntheses to prepare carboxylic acids containing a free amino group which is "blocked" with tert.-butoxycarbonyl.

C. 2-(tert.-Butoxycarbonylaminoethyl)-1,4-cyclohexadienylacetic acid.

A solution of 16.5 g. (0.1 mole) of o-aminomethylphenylacetic acid in 1.5 l of liquid ammonia (which has been treated with 50 mg. of Li to remove a trace of moisture) was slowly diluted with 500 ml. of dry t-BuOH. To the solution was added in small portions 3.4 g. (0.5 atom) of Li over a period of 4 hours and the mixture was stirred for 16 hours at room temperature removing the liquid ammonia in a hood and finally evaporated to dryness below 40° C. The residue was dissolved in 500 ml. of water and the solution was chromatographed on a column of IR—120 (H⁺, 700 ml.) resin and eluted with 1% NH₄OH solution. Ninhydrin positive fractions of the eluate were combined and evaporated to dryness. The residue was washed with four 50 ml. portions of hot acetone and recrystallized from 500 ml. of ethanol-water (1:1) to give 11.2 g. (67%) of colorless needles, o-(2-aminomethyl-1,4-cyclohexadienyl)acetic acid. M.p. 183° C.

IR: $\gamma_{\max}^{\text{nuj}}$ 1630, 1520, 1380, 1356 cm⁻¹.

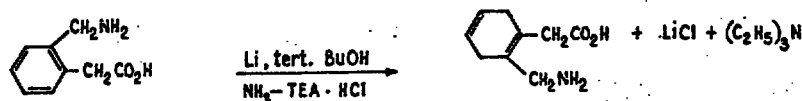
NMR: δ D₂O + K₂CO₃ 2.72 (4H, s, H₂C[⌢]),

3.01 (2H, s, CH₂CO), 3.20 (2H, s, CH₂—N), 5.78 (2H, s, >C=),

Anal. Calcd. for C₉H₁₃NO₂: C, 64.65; H, 7.84; N, 8.38.

Found: C, 64.77; H, 8.06; N, 8.44.

Improved Procedure for the Preparation of o-(2-aminomethyl-1,4-cyclohexadienyl)-acetic acid.



The procedure used by Welch, Dolfini and Giarrusso in U.S. patent 3,720,665 (Example 1) to make D-2-amino-2-(1,4-cyclohexadienyl)acetic acid was adapted. A solution of 830 ml. of distilled liquid ammonia was dried with 40 mg. of lithium under an argon atmosphere. To this stirred solution was added 11.0 g. (0.07 mole) of 2-aminomethylphenylacetic acid and 340 ml. of tert. butyl alcohol. A total of 1.6 g. (0.225 mole) of lithium was added to the vigorously stirred solution over a period

of 2 hours. The grey mixture was then treated with 35 g. (0.215 mole) of triethylamine (TEA) hydrochloride and stirred overnight at room temperature for 18 hours. The *tert.* butyl alcohol was removed at 40° (15 mm.) to yield a white residue which was dried *in vacuo* over P₂O₅ overnight. The solid was dissolved in 30 ml. of 1:1 methanol-water and added with stirring to 3.5 l. of 1:1 chloroform-acetone at 5°. The mixture was stirred for 20 min. and the amino acid, α -(2-aminomethyl-1,4-cyclohexadienyl)-acetic acid, was collected and dried for 16 hours *in vacuo* over P₂O₅ to yield 6.3 g. (58%) of white crystals, m.p. 190° decomp. The IR and NMR spectra were consistent for the structure.

A solution of 19.31 g. (0.135 m) of *tert.*-butoxycarbonylazide in 152 ml. of tetrahydrofuran (THF) was added to a stirred solution of 14.89 g. (0.09 m) of 2-amino-methyl-1,4-cyclohexadienylacetic acid and 7.20 g. (0.18 m) of sodium hydroxide in 281 ml. of water. The solution was stirred for 18 hr. at 25° and then filtered through diatomaceous earth (Super-cel). The THF was removed at 40° (15 mm) and the residual solution was washed with ether (2 $\times$ 175 ml.) and acidified with 6 N hydrochloric acid (HCl). The mixture was stirred in an ice-bath and the precipitate was collected and dried for 18 hr. *in vacuo* over P₂O₅ at 25° to yield 17.3 g. (72.6%) of 2-(*tert.*-butoxycarbonylamino-methyl)-1,4-cyclohexadienylacetic acid as a white powder. The IR and NMR spectra were consistent for the structure.

D. D-(—)-*p*-Hydroxy- α -*tert.*-butoxycarboxamidophenylacetic acid.

In a mortar 5.65 g. (0.14 m) of magnesium oxide and 11.7 g. (0.07 m) of D-(—)-*p*-hydroxyphenylglycine were triturated and added to 177 ml. of 50% dioxane. Slowly 20.0 g. (0.14 m) of *tert.*-butoxycarbonylazide was added to the rapidly stirring mixture and stirred for 20 hours at 45° to 50°. The mixture was then diluted out with 710 ml. of ice water and 177 ml. of ethyl acetate. The mixture was filtered and the filtrate separated into 2 phases. The organic phase was washed 3 $\times$ 50 ml. with 3% sodium bicarbonate solution and 3 $\times$ 50 ml. with water. All the water phases were combined, cooled and adjusted to pH 5.0 with conc. hydrochloric acid. The product was extracted 3 $\times$ 125 ml. into ethyl acetate. The ethyl acetate was washed 2 $\times$ 50 ml. with water, dried over magnesium sulfate and then evaporated at 35° at 15 mm to an oil. The oil was triturated with petroleum ether to yield D-(—)-*p*-hydroxy- α -*tert.*-butoxycarboxamidophenylacetic acid as a white solid. This product was collected and dried 18 hr. *in vacuo* over P₂O₅ at 25° to yield 13.5 g. (72.2%) of off-white powder. M.p. 102°. The IR and NMR spectra were consistent for the structure.

E. D-(—)- α -*tert.*-Butoxycarboxamidophenylacetic acid.

The procedure followed was that used above for D-(—)-*p*-hydroxy- α -*tert.*-butoxycarboxamidophenylacetic acid. Two crops of sample were obtained to yield 5.2 g. (30%) M.p. 82°. The IR and NMR spectra were consistent for the structure.

F. 2-(*tert.*-Butoxycarbonylamino-methyl)-1,2-cyclohexenyl-acetic acid which is also named [2-(*N*-*t*-Butoxycarbonylamino)methyl-1-cyclohexen-1-yl]acetic acid.

a) α -[2-(*t*-Butoxycarbonylamino-methyl)-1,4-cyclohexadienyl]acetic acid (1).
To a stirred solution of 8.0 g. (0.048 mole) of α -(2-aminomethyl-1,4-cyclohexadienyl)acetic acid and 3.8 g. (0.096 mole) of NaOH in 150 ml. of water was added a solution of 10.3 g. (0.072 mole) of *t*-butoxycarbonylazide in 80 ml. of THF and the mixture was stirred for 18 hours at room temperature. The tetrahydrofuran (THF) was removed under reduced pressure and the residual solution was washed with ether (2 $\times$ 100 ml.), acidified with 6 N HCl and extracted with ether (3 $\times$ 100 ml.). The combined extracts were washed with water (2 $\times$ 100 ml.) and a saturated NaCl solution (100 ml.), dried with Na₂SO₄ and evaporated to dryness. The oily residue was triturated with *n*-hexane to give 10.5 g (82%) of colorless powder 1 melting at 113°C.

IR: $\gamma_{\text{max}}^{\text{nuj}}$ 3370, 1715, 1640, 1530, 1280, 1160 cm⁻¹.

NMR: $\delta_{\text{ppm}}^{\text{CDCl}_3}$ 1.45 (9H, s, *t*-Bu-H), 2.73 (4H, s, H₂C=C), 3.16 (2H, s, CH₂CO), 3.76 (2H, d, 6Hz, CH₂N) 4.90 (1H, m, NH), 5.66 (2H, s, H), 10.6 (1H, br-s, COOH).

Anal. Calcd. for C₁₄H₂₁NO₄: C, 62.90; H, 7.92; N, 5.24.

Found: C, 63.13; H, 8.21; N, 5.26.

b) [2-(N-*t*-Butoxycarbonylamino)methyl-1-cyclohexen-1-yl]acetic acid (2).

A solution of [2-(N-*t*-butoxycarbonylamino)methyl-1,4-cyclohexadien-1-yl]acetic acid (1), (1.33 g., 5 mmoles) in 3% ammonium hydroxide (10 ml.) was hydrogenated at 40 psi with palladium on charcoal (10%, 0.2 g.). A theoretical amount of hydrogen was taken up in 3 hours. The catalyst was removed and the filtrate was acidified to pH 2 with dil. HCl and extracted with ethyl acetate (2 × 50 ml.). The combined extracts were washed with water (20 ml.), dried with Na₂SO₄ and evaporated under reduced pressure to afford an oil (1.34 g.) which solidified on standing for several days. Recrystallization from *n*-hexane/ethyl acetate gave 1.2 g. (90%) of 2 as colorless prisms melting at 118–119° C.

IR: γ_{max} nujol 3450, 1730, 1660, 1510 cm⁻¹.

NMR: δ CDCl₃ ppm 1.58 (9H, s, *t*-butyl-*H*), 1.50–1.90 (4H, m, —CH₂—), 1.90–2.20 (4H, m, allylic methylene-*H*), 3.18 (2H, s, CH₂—CO), 3.78 (2H, d, 6 Hz, CH₂—N), 5.00 (1H, br-s, NH), 8.98 (1H, br-s, COCH). ("Nujol" is a registered Trade Mark.)

Anal. Calcd. for C₁₄H₂₃NO₄: C, 62.43; H, 8.61; N, 5.20.
Found: C, 62.12; H, 8.77; N, 5.37.

G. 2-*t*-Butoxycarbonylamino-methyl-4-hydroxyphenylacetic acid is prepared, for example, according to U.S. 3,823,141.

H. *o*-(*tert*-Butoxycarbonylamino-methyl)phenylthioacetic acid is prepared, for example, according to U.S. 3,657,232 and see also U.S. 3,813,390.

I. β -[*o*-(*tert*-Butoxycarbonylamino-methyl)phenyl]-propionic acid is prepared, for example, according to U.S. 3,813,391.

J. *D*-(—)-*N*-*t*-Butoxycarbonyl-2-(3'-methyl-4'-hydroxyphenyl)glycine.

2-(3'-Methyl-4'-hydroxyphenyl)glycine.

A solution of 59.02 g. (0.6 mole) of 75% glyoxylic acid in 100 ml. of water was added to a suspension of 54.6 g. (0.5 mole) of 2-methylphenol and 140 ml. of conc. ammonium hydroxide in 400 ml. of water at room temperature. The temperature of the mixture rose to 37°. The mixture was stirred at room temperature for 65 hours. The solution, initially at pH 10.1, was adjusted to pH 6.8 with 6 *N* hydrochloric acid causing the product to crystallize. The product was collected by filtration, washed with water and dried *in vacuo* over phosphorous pentoxide giving 31.5 g. (34.8%) of 2-(3'-methyl-4'-hydroxyphenyl)glycine; decomp. 196–199°. The infrared and nuclear magnetic resonance spectra were consistent for the desired product. Reference: Belgium patent number 774,029 to Beecham Group Limited, 1972 (Farmdoc 27, 122T) which reports m.p. 205–207° C.

Anal. Calcd. for C₉H₁₁NO₃: C, 59.66; H, 6.13; N, 7.73.

Found: C, 57.68; H, 6.23; N, 7.47; H₂O, 2.34.

Found, corrected for 2.34% H₂O: C, 59.06; H, 6.12; N, 7.67.

D,L-*N*-Chloroacetyl-2-(3'-methyl-4'-hydroxyphenyl)glycine.

A suspension of 20.2 g. (0.112 mole) of *D,L*-2-(3'-methyl-4'-hydroxyphenyl)glycine in 175 ml. of water was adjusted to pH 10.3 with 20% sodium hydroxide causing a solution. The solution was cooled in an ice bath. Chloroacetic anhydride (38.2 g., 0.224 mole) was added all at once and the pH of the reaction mixture was maintained at pH 10 by the addition of 20% sodium hydroxide until no further pH change was detected. The reaction mixture was stirred an additional 10 min. in the cold. The reaction mixture was then acidified to pH 2.0 with 6*N* hydrochloric acid causing the product to crystallize. The product was collected by filtration, washed with water and air dried. Recrystallization from 200 ml. of hot water gave 13.7 g. (47.4%) of *D,L*-*N*-chloroacetyl-2-(3'-methyl-4'-hydroxyphenyl)glycine. The infrared and nuclear magnetic resonance spectra were consistent for the desired product.

Anal. Calcd. for C₁₁H₁₂NO₃Cl · H₂O: C, 47.92; H, 5.118; N, 5.081.

Found: C, 48.11; H, 5.16; N, 5.15.

D-(-)-N-Chloroacetyl-2-(3'-methyl-4'-hydroxyphenyl)glycine.

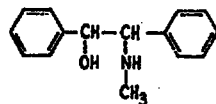
D,L-N-Chloroacetyl-2-(3'-methyl-4'-hydroxyphenyl)glycine (5.0 g., 0.0194 mole) and L-ephedrine acetate (6.1 g., 0.0213 mole) was dissolved in 50 ml. of isopropyl alcohol by heating on a steam bath. Water (50 ml.) was added and upon cooling, the L-ephedrine salt crystallized. The salt was collected by filtration and air dried.

The salt was suspended in 30 ml. of water and 50 ml. of methylene chloride and the mixture adjusted to pH 10.0 with 20% sodium hydroxide. The phases were separated and the aqueous phase was extracted twice more with methylene chloride.

The aqueous solution was then adjusted to pH 2.0 with 6 N hydrochloric acid causing the product to crystallize. The product was collected by filtration and dried *in vacuo* over phosphorus pentoxide affording 0.9 g. (36.1%) of D-(-)-N-chloroacetyl-2-(3'-methyl-4'-hydroxyphenyl)glycine; m.p. 170—172°, $[\alpha]_D^{24} = 185.9^\circ$ (C 1, 95% EtOH). The infrared and nuclear magnetic resonance spectra were consistent for the desired product.

Anal. Calcd. for $C_{11}H_{12}NO_3Cl$: C, 51.27; H, 4.696; N, 5.436.
Found: C, 51.21; H, 4.77; N, 5.29.

1,2-Diphenyl-2-methylaminoethanol, commonly called ephedrine (per Federal Register, June 7, 1951), has the structure



The compound is also named N-methyl-1,2-diphenyl-2-hydroxy-ethylamine or alpha, beta-diphenyl-beta-hydroxy-N-methyl-ethylamine or 1,2-diphenyl-2-methylamino-1-ethanol.

This invention utilizes only the levo-erythroisomer. Methods for its preparation and reaction with penicillin G were described in U.S. patents 2,645,638 (V. V. Young) and 2,768,081 (F. H. Buckwalter). The latter reviews earlier literature as does W. B. Wheatley et al., J. Org. Chem. 18(11), 1564—1571 (1953). It was used to resolve racemic phenoxymethyl penicillin by Sheehan et al., J. Am Chem. Soc., 81, 3089—3094 (1959); see especially p. 3091.

D-(-)-2-(3-Methyl-4'-hydroxyphenyl)glycine.

D-(-)-N-Chloroacetyl-2-(3'-methyl-4'-hydroxyphenyl)glycine (11.1 g., 0.0431 mole) was combined with 100 ml. of 2 N hydrochloric acid and the mixture was refluxed for 1.5 hours. The solution was cooled and the pH adjusted to 5.0 with 20% sodium hydroxide causing the product to crystallize. The product was collected by filtration, washed with water and dried *in vacuo* over phosphorus pentoxide giving 7.4 g. (94.7%) of D-(-)-2-(3'-methyl-4'-hydroxyphenyl)glycine; decomp. 205—209°, $[\alpha]_D^{24} = -152.6^\circ$ (C 1, 1 N HCl). The infrared and nuclear magnetic resonance spectra were consistent for the desired product.

Anal. Calcd. for $C_9H_{11}NO_3$: C, 59.66; H, 6.13; N, 7.73.

Found: C, 58.62; H, 5.49; N, 7.78; H_2O , 1.46.

Found, corrected for 1.46% H_2O : C, 59.48; H, 5.41; N, 7.84.

D-(-)-N-t-Butoxycarbonyl-2-(3'-methyl-4'-hydroxyphenyl)glycine.

To a slurry of 7.2 g. (0.0397 mole) of D-(-)-2-(3'-methyl-4'-hydroxyphenyl)glycine and 3.2 g. (0.08 mole) of powdered magnesium oxide stirred in 100 ml. of 50% dioxane at room temperature, 9.7 g. (0.068 mole) of t-butoxycarbonyl azide was added dropwise. The reaction mixture was then heated to 42—45° under a nitrogen atmosphere for 19 hours. The mixture was then diluted with 100 ml. of ice water. The solution was layered with ethyl acetate and filtered to remove some insoluble material that had separated. The aqueous phase of the filtrate was separated and extracted twice more with ethyl acetate. The aqueous solution was then adjusted to pH 5.0 with 42% phosphoric acid and extracted 5 times with ethyl acetate. The combined organic extracts were washed 3 times with water, dried over sodium sulfate and the solvent removed at reduced pressure leaving an oil. The oil dried *in vacuo* over phosphorus pentoxide resulting in 10.6 g. (95%) of D-(-)-N-t-butoxycarbonyl-2-(3'-methyl-4'-hydroxyphenyl)glycine. The infrared spectrum was consistent for the desired structure.

K. *D*-(—)-*N*-*t*-Butoxycarbonyl-2-(3'-methoxy-4'-hydroxyphenyl)glycine.
2-(3'-Methoxy-4'-hydroxyphenyl)glycine.

A solution of 59.2 g. (0.6 mole) of 75% glyoxylic acid in 100 ml. of water was added to a suspension of 62.07 g. (0.5 mole) of 2-methoxyphenyl and 140 ml. of concentrated ammonium hydroxide in 400 ml. of water at room temperature. The temperature of the mixture rose to 35°. The mixture was stirred at room temperature for 65 hours. The product that had crystallized was collected by filtration, washed with water, then acetone and dried *in vacuo* over phosphorus pentoxide giving 57.4 g. (58.2%) of 2-(3'-methoxy-4'-hydroxyphenyl)glycine; decomp. 218—220° (Lit. 240°). The infrared and nuclear magnetic resonance spectra were consistent for the desired product.

Anal. Calcd. for $C_{11}H_{11}NO_4$: C, 54.82; H, 5.62; N, 7.10.

Found: C, 53.77; H, 5.91; N, 6.97; H_2O , 1.13.

Found, corrected for 1.13% H_2O : C, 54.38; H, 5.85; N, 7.05.

Reference: B. Block, *Z. Physiol. Chem.* 98, 226 (1917).

Resolution of 2-(3'-Methoxy-4'-hydroxyphenyl)glycine.

A. Methyl 2-(3'-Methoxy-4'-hydroxyphenyl)glycinate.

A cooled suspension of 94 g. (0.476 mole) of 2-(3'-methoxy-4'-hydroxyphenyl)glycine in 500 ml. of absolute methanol was gassed at a rapid rate with HCl for 20 min. At first a clear solution was obtained and then crystalline product separated in quantity. After 20 hours the methyl ester hydrochloride was filtered and washed sparingly with methanol; 99.6 g. after air drying. A cooled solution of the hydrochloride in 800 ml. of water was adjusted to pH 8 (NaOH) giving a crystalline precipitate of the ester free base; 81.3 g. The IR and NMR spectra was consistent.

Anal. Calcd. for $C_{10}H_{13}NO_4$: C, 56.86; H, 6.20; N, 6.63.

Found: C, 56.46; H, 6.28; N, 6.55; H_2O , 0.59.

B. *D*-(—)-2-(3'-Methoxy-4'-hydroxyphenyl)glycine.

A mixture of 50 g. (0.237 mole) of methyl 2-(3'-methoxy-4'-hydroxyphenyl)glycinate, 19 ml. (0.333 mole) of acetic acid and 1 l. of *i*-PrOH (isopropyl alcohol) was heated to boiling giving a partial solution. Dibenzoyl-*D*-tartaric acid monohydrate (89.2 g., 0.237 mole) was added with good stirring and then the mixture was refluxed. Soon the salt started to crystallize. The heat was shut off and the flask was allowed to cool slowly to room temperature. After cooling in an ice bath the precipitate was collected by filtration. The filtrate was concentrated to about one-third of its initial volume giving a small second crop of salt; total yield of both crops 54.1 g. after air drying (solid A; see below).

The filtrate was concentrated free of solvent. The viscous residue was combined with 300 ml. of 1N HCl and the mixture extracted with 400 ml. of $CHCl_3$. The $CHCl_3$ phase was extracted twice with 100 ml. portions of 1 N HCl. The combined HCl extracts were concentrated briefly to remove residual $CHCl_3$ and refluxed for 1 hr. The solution was concentrated to a small volume causing the amino acid HCl salt to crystallize. The product was collected by filtration and recrystallized from 50 ml. of 1N HCl. A solution of the product in 200 ml. of water was adjusted to pH 4.5 (NaOH). The mixture was heated nearly to boiling and allowed to cool to precipitate *D*-(—)-2-(3'-methoxy-4'-hydroxyphenyl)glycine as fluffy needle-like crystals. After cooling overnight, the product was collected by filtration, washed sparingly with water and methanol and dried at 40°; 8.7 g., $[\alpha]_D^{25} = -136.5^\circ$ (c 1 1N HCl). The IR and NMR spectra were fully consistent.

Anal. Calcd. for $C_{11}H_{11}NO_4 \cdot H_2O$: C, 50.23; H, 6.09; N, 6.51; H_2O , 8.37.

Found: C, 50.43; H, 6.23; N, 6.51; H_2O , 8.95.

C. *L*-(+)-2-(3'-Methoxy-4'-hydroxyphenyl)glycine.

Solid A above (54.1 g.) was suspended in 300 ml. of 1N HCl and 500 ml. of $CHCl_3$ with good agitation. The salt did not break up readily in this system, therefore, the $CHCl_3$ was separated as well as possible and 300 ml. of MIBK added with good agitation. The MIBK phase was extracted with an additional 200 ml. of 1N HCl in 3 portions. The combined and filtered HCl extracts were concentrated briefly to remove residual solvents and heated at reflux for 1 hr. to hydrolyze the ester. The reaction mixture was concentrated to a small volume. After cooling in an ice bath the crystalline amino acid HCl salt was collected by filtration. The salt was recrystallized from 75 ml. of 1 N HCl, dissolved in 500 ml. of water by warming, the solution polish filtered and adjusted to pH 4.5 (NaOH) causing the zwitterion to crystallize. The mixture was heated to boiling, filtered, and stored in the cold to precipitate the

crystalline product, L-(+)-2-(3'-methoxy-4'-hydroxyphenyl)glycine. The product was collected by filtration, washed sparingly with water and methanol and dried at 40°; 9.6 g., $[\alpha]_D^{20} = +127.2^\circ$ (c 1 1N HCl). The IR and NMR spectra were consistent. Anal. Calcd. for $C_{10}H_{11}NO_3 \cdot H_2O$: C, 50.23; H, 6.09; N, 6.51; H_2O , 8.37. Found: C, 50.53; H, 6.06; N, 6.62; H_2O , 7.46.

D-(-)-N-(t-Butoxycarbonyl)-2-(3'-methoxy-4'-hydroxyphenyl)glycine.

A mixture of 8.6 g. (0.04 mole) of D-(-)-2-(3'-methoxy-4'-hydroxyphenyl)glycine, 3.2 g. (0.08 mole) of magnesium oxide, 9.7 g. (0.68 mole) of t-butoxycarbonyl azide and 240 ml. of 1:1 dioxane-water was stirred and heated at 45—50° for 20 hours under a nitrogen atmosphere. The cooled reaction mixture was diluted with 240 ml. of ice water, filtered and extracted once with ethyl acetate. The acidified (pH 2) aqueous phase was extracted 5 times with ethyl acetate. The combined and dried (Na_2SO_4) ethyl acetate extracts were concentrated free of solvent at reduced pressure giving the product as a viscous oil; 6.3 g.

L. D-(-)-N-t-Butoxycarbonyl-2-(4-acetoxyphenyl)glycine.

This compound is prepared by substitution for the D-(-)-2-(3'-methyl-4'-hydroxyphenyl)glycine in the procedure set out above of an equimolar weight of D-(-)-2-(4-acetoxyphenyl)glycine prepared in the following manner.

Preparation of D-(-)-2-amino-2-(4-acetoxyphenyl)acetic acid.

Method A (in acetic acid as solvent).

203.5 g. (1 mole) of D-(-)-hydroxyphenylglycine chloride 800 ml. of acetic acid and 314 g. (4 moles) of acetyl chloride are stirred 48 hours at room temperature. The solid is collected, washed three times with acetone (3×250 ml.) and twice with ethanol (2×250 ml.) and dried at 40°. Yield 210 g. (85.4%). This hydrochloride is dissolved in 3.0 l of water; the solution is cooled to +5 to 10° C. and the pH adjusted to 4.5 with 20% NH_4OH . The suspension is stirred 1 hour at 5° C. and the solid collected, washed twice with water and twice with acetone, and dried at 40° C. Yield 133 g. (64% from D-(-)-p-hydroxyphenylglycine). α_D (1% HCl N_{10}) = -104.5.

Method B (in methylene chloride).

4.07 g. (0.02 Mole) of D-(-)-p-hydroxyphenylglycine hydrochloride, 30 ml. of methylene chloride and 6.28 g. (0.08 mole) of acetyl chloride are stirred 48 hours at room temperature. The solid is collected, washed twice with acetone and twice with ethanol. Yield 4.17 g. (84.5%). Anal. cl = 14.8% (calculated 14.4%).

Method C (in trifluoroacetic acid).

1.67 g. (0.01 Mole) of D-(-)-p-hydroxyphenylglycine is added with stirring, to 10 ml. of trifluoroacetic acid at room temperature. After dissolution, 1.57 g. (0.02 mole) of acetyl chloride is added. After a slightly exothermic reaction, a solid appears. The suspension is stirred 1 $\frac{1}{2}$ hr. at room temperature and the trifluoroacetic acid is removed in vacuum. The remaining solid is collected washed with methylene chloride and with ethanol. The D-(-)-2-amino-2-(4-acetoxyphenyl)acetic acid is identical to that prepared by Methods A or B. Yield: 1.9 g. (75%).

M. D-(-)-N-t-Butoxycarbonyl-2-(1'-cyclohexenyl)glycine.

This compound is prepared by substitution for the D-(-)-2-(3'-methyl-4'-hydroxyphenyl)glycine in the procedure set out above of an equimolar weight of D-(-)-2-(1'-cyclohexenyl)glycine prepared according to Belgium 773,773 (Farmdoc 25,515T) or U.S. 3,824,237.

N. D-(-)-N-t-Butoxycarbonyl-2-(3'-chloro-4'-hydroxyphenyl)glycine is prepared, for example, as disclosed in U.S. 3,489,751.

O. D-(-)-N-t-Butoxycarbonyl-2-(1',4'-cyclohexadienyl)glycine.

This compound is prepared by substitution for the D-(-)-2-(3'-methyl-4'-hydroxyphenyl)glycine in the procedure set out above of an equimolar weight of D-(-)-2-(1',4'-cyclohexadienyl)glycine (also named D-2-amino-2-(1,4-cyclohexadienyl)acetic acid) prepared according to U.S. 3,485,819.

P. D-(-)-2-tert.-Butoxycarboxamido-3-(1',4'-cyclohexadienyl)propionic acid.

This compound is prepared by substitution for the D-(-)-2-(3'-methyl-4'-hydroxyphenyl)glycine in the procedure set out above of an equimolar weight of D-2-amino-3-(1,4-cyclohexadienyl)propionic acid prepared according to U.S. 3,485,819.

Q. D-(-)-2-tert.-Butoxycarboxamido-3-(4'-methoxy-1',4'-cyclohexadienyl)propionic acid

This compound is prepared by substitution for the D-(-)-2-(3'-methyl-4'-hydroxyphenyl)glycine in the procedure set out above of an equimolar weight of D-2-

amino-3-(4'-methoxy-1,4-cyclohexadienyl)propionic acid prepared according to U.S. 3,485,819.

R. 2-*t*-Butoxycarbonylaminomethylphenyl-acetic acid is prepared, for example, according to U.S. 3,766,175.

S. *N*-tert.-Butoxycarbonyl-1-aminocyclohexanecarboxylic acid.

This compound is prepared by substitution for the D-(−)-2-(3'-methyl-4'-hydroxyphenyl)glycine in the procedure set out above of an equimolar weight of 1-amino-cyclohexanecarboxylic acid.

T. D-(−)-α-tert.-Butoxycarboxamido-3-thienyl-acetic acid is prepared, for example, according to U.S. 3,634,418, U.S. 3,198,804 and appropriate references cited therein.

U. D-(−)-α-tert.-Butoxycarboxamido-2-thienyl-acetic acid is prepared, for example, according to U.S. 3,634,418, U.S. 3,198,804 and appropriate references cited therein.

V. β-(2-tert.-Butoxycarbonylaminomethyl-1,4-cyclohexadienyl)-propionic acid.

a) β-(2-Aminomethyl-1,4-cyclohexadienyl)propionic acid.

A solution of β-(o-aminomethylphenyl)propionic acid (10.74 g., 60 mmoles) in liquid ammonia (1000 ml.) (pretreated with ca. 50 mg. of lithium to remove a trace of water) was cautiously diluted with dry *t*-BuOH (300 ml.). To the solution was added portionwise lithium (2.08 g., 0.3 atom) over a period of 3 hr. and the mixture was stirred overnight to remove liquid ammonia and evaporated to dryness. The residue was dissolved in water (200 ml.). The solution was chromatographed on a column of IR—120 (H⁺ form, 500 ml.) and eluted continuously with water (2000 ml.) and 5% ammonium hydroxide (ca. 2000 ml.). Ninhydrin positive fractions were combined, evaporated to dryness and the residue was crystallized from 90% ethanol to afford colorless needles. Yield of β-(2-aminomethyl-1,4-cyclohexadienyl)propionic acid was 9.68 g. (89%) M.p. 228—229° C.

IR: $\gamma_{\max}^{\text{nuj}}$ 2130, 1540, 1300 cm^{−1}.

NMR: $\delta_{\text{ppm}}^{\text{D}_2\text{O}}$ 2.68 (4H, s, CH₂CH₂CO), 2.74 (4H, s, ring methylene), 3.68 (2H, s, CH₂N), 5.79 (2H, s, ring vinyl-H).

Anal. Calcd. for C₁₀H₁₁NO₂: C, 66.27; H, 8.34; N, 7.73.

Found: C, 66.25; 66.31; H, 8.35, 8.52; N, 7.61, 7.71.

b) β-(2-*t*-Butoxycarbonylaminomethyl-1,4-cyclohexadienyl)propionic acid.

To a mixture of β-(2-aminomethyl-1,4-cyclohexadienyl)propionic acid (1.40 g., 7.7 mmoles) and triethylamine (2.02 g., 20 mmoles) in 50% aqueous THF (20 ml.) was added a solution of *t*-butyl azidoformate (1.43 g., 10 mmoles) in THF (5 ml.) and the reaction mixture was stirred overnight at room temperature. The mixture was washed with ether (2 × 20 ml.) and the aqueous layer was acidified to pH 2 with dil. hydrochloric acid. The mixture was extracted with chloroform (4 × 30 ml.) and the combined extracts were washed with water and dried. Evaporation of the extracts under reduced pressure afforded β-(2-tert.-butoxycarbonylaminomethyl-1,4-cyclohexadienyl)propionic acid as a colorless oil. Yield, 2.10 g. (97%).

IR: $\gamma_{\max}^{\text{liq}}$ 1700, 1510, 1240, 1160 cm^{−1}.

NMR: $\delta_{\text{ppm}}^{\text{CDCl}_3}$ 1.47 (9H, s, *t*-Bu-H), 2.45 (4H, s, CH₂CH₂), 2.69 (4H, s, ring methylene), 3.80 (2H, d, 5 Hz, CH₂N), 5.70 (2H, s, ring vinyl-H).

W. β-(2-*t*-Butoxycarbonylaminomethyl-1-cyclohexenyl)propionic acid.

β-(2-*t*-Butoxycarbonylaminomethyl-1,4-cyclohexadienyl)propionic acid (0.92 g., 3.2 mmoles) was dissolved in 3% aqueous ammonium hydroxide (20 ml.) and hydrogenated at 30 psi with palladium on carbon (10%, 0.1 g.) in a Parr apparatus for 3 hr. The mixture was filtered and the filtrate was acidified to pH 2 with dil. hydrochloric acid and extracted with ethyl acetate (3 × 30 ml.). The combined extracts were washed with a small amount of water and dried. Removal of the solvent under reduced pressure afforded β-(2-*t*-butoxycarbonylaminomethyl-1-cyclohexenyl)propionic acid as an oil; yield 0.84 g. (91%).

IR: $\gamma_{\text{cm}^{-1}}^{\text{liq}}$ 3350 (NH), 3200—2400 (COOH), 1750 (CO), 1250, 1165 (BOC).

NMR: $\gamma_{\text{ppm}}^{\text{CDCl}_3}$ 1.48 (9H, s, *t*-Bu), 1.5—1.8 (4H, m, CH₂CH₂), 1.8—2.2 (4H, m, allyl-CH₂), 2.4 (4H, CH₂CH₂—CO), 3.72 (2H, s, N—CH₂).

X. D-(—)-N-*tert.*-Butoxycarbonyl-2-(4'-hydroxymethyl-phenyl)glycine.

This compound, which is also named D-(—)-N-*t*-butoxycarbonyl-*p*-hydroxymethyl-phenylglycine, is prepared according to South Africa 73/4055; for convenience the pertinent disclosure therein is reproduced below.

5 To a solution of 1,4-benzenedicarboxaldehyde (50.0 g., 0.373 mole) in 200 ml. of dry tetrahydrofuran (THF) under nitrogen in an ice bath was added dropwise lithium tri(*t*-butoxy)aluminum hydride (104.0 g., 0.410 mole) dissolved in 500 ml. of dry tetrahydrofuran. After stirring for one half hour in an ice bath, the reaction mixture was poured into 2 l. of ice cold 2*N* hydrochloric acid. The aqueous solution was extracted with four 800 ml. portions of ether. The combined ether layers were washed with 500 ml. of ice cold 5% sodium bicarbonate solution and then with 500 ml. of saturated sodium chloride solution. After drying, the ether was removed under reduced pressure to give 46 g. of crude *p*-hydroxymethylbenzaldehyde. The crude product was chromatographed over 1 kg. of neutral alumina and the fractions eluted with ether and concentrated. Upon cooling there crystallized out *p*-hydroxymethylbenzaldehyde (17.0 g., 35% yield), m.p. 44.5–46° C.

10 To a stirred mixture of *p*-hydroxymethylbenzaldehyde (10.0 g., 0.0735 mole) and ammonium carbonate (17.1 g., 0.15 mole) in 110 ml. of 60% ethanol heated to 50° C. there was added dropwise sodium cyanide (4.0 g., 0.081 mole) dissolved in 10 ml. water. The mixture was stirred and heated to 55–60° C. for three hours and then the temperature raised to 85° C. for one hour. After cooling in an ice bath, the pH of the solution was brought to 6 by the addition of concentrated hydrochloric acid. Upon overnight cooling, the solid which had precipitated was filtered, washed with cold water, and dried. The 5-(*p*-hydroxymethylphenyl)hydantoin (1.0 g., 72% yield), m.p. 189–196° (dec.), was used to prepare the amino acid without further purification.

20 A mixture of 5-(*p*-hydroxymethylphenyl)hydantoin (10.9 g., 0.053 mole) and barium hydroxide (8 H₂O) (25.5 g., 0.081 mole) in 125 ml. water was stirred and refluxed for 18 hours. After cooling in an ice bath the reaction mixture was diluted with 125 ml. water. The solution was acidified with concentrated sulfuric acid to pH 1, the barium sulfate filtered, and the pH of the filtrate brought to 6 with lead carbonate. After filtration of the lead sulfate, the filtrate was saturated with hydrogen sulfide and the lead sulfide filtered. The aqueous solution was then concentrated to 100 ml. by azeotroping with ethanol under reduced pressure. After cooling, there was precipitated *p*-hydroxymethylphenylglycine (5.2 g., 54% yield) m.p. 228–229° C. (dec.). After recrystallization from ethanol-water the compound had m.p. 230–231° (dec.).

25 Anal. Calcd. for C₁₁H₁₁NO₃: C, 59.66; H, 6.12; N, 7.73.

Found: C, 59.46; H, 6.24; N, 7.93.

30 To a solution of *p*-hydroxymethylphenylglycine (8.0 g., 0.044 mole) and triethylamine (8.8 g., 0.087 mole) in 160 ml. water was added *t*-butoxycarbonyl azide (6.95 g., 0.049 mole) dissolved in 120 ml. tetrahydrofuran. After stirring overnight at room temperature, the reaction mixture was washed twice with 200 ml. portions of ether. The aqueous layer was covered with ether and in an ice bath was acidified to pH 3–3.5 with 3 *N* hydrochloric acid. The acidic solution was extracted three times with 200 ml. portions of ether. The combined ether layers were washed with saturated sodium chloride solution, dried, and the ether evaporated under reduced pressure. The resulting oil was triturated with chloroform-hexane and the solid filtered off to give *dl*-N-*t*-butoxycarbonyl-*p*-hydroxymethylphenylglycine (7.74 g., 63% yield), m.p. 139–141.5° (dec.).

35 Anal. Calcd. for C₁₁H₁₁NO₃: C, 59.78; H, 6.81; N, 4.98.

Found: C, 59.67; H, 6.76; N, 4.69.

40 *dl*-N-*t*-Butoxycarbonyl-*p*-hydroxymethylphenylglycine (7.560 g., 0.0269 mole) and quinine (10.199 g., 0.0269 mole) were mixed and dissolved in 110 ml. of boiling ethanol. The solution was allowed to cool to room temperature and to crystallize overnight. The salt was filtered off and the crystallization repeated three times.

45 The salt (17.76 g., m.p. 198–201° dec., [α]_D²⁵ –149.8; C=1, CH₃OH) gave after three recrystallizations resolved salt (4.6 g., m.p. 205–6° dec., [α]_D²⁵ –163.4, C=1, CH₃OH). An additional recrystallization did not increase the optical rotation.

50 The (—) quinine salt of (—)-N-*t*-butoxycarbonyl-*p*-hydroxymethylphenylglycine was suspended in 75 ml. water and 175 ml. ether in an ice bath and 3 *N* hydrochloric acid added to pH 2.5. The ether layer was removed and the aqueous layer extracted twice with 100 ml. portions of ether. The combined ether layers were washed with 100 ml. saturated sodium chloride, dried, and the ether removed under reduced pressure. The residue was triturated with chloroform-hexane and filtered to give D-(—)-N-*t*-butoxycarbonyl-*p*-hydroxymethylphenylglycine (1.68 g., 98% recovery), m.p. 111–113.5° dec., [α]_D²⁵ = –136.5 (C=1, CH₃OH).

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The use of an "en-amine" blocking group with a prospective 7-side chain containing a free amine group prior to acylation of a nucleus such as II herein is well known as from U.S. 3,223,141, U.S. 3,813,390, U.S. 3,813,391, U.S. 3,823,141 and Belgium 773,773. For convenience some specific examples follow.

5 **Sodium 2 - [N - (1 - carbethoxypropen - 2 - yl)aminomethyl] - 1,4 - cyclohexadienyl acetate (4).** 5

To a stirred solution of 460 mg. (0.02 mole) of metallic sodium in 100 ml. of absolute EtOH was added 3.34 g. (0.02 mole) of 2-aminomethyl-1,4-cyclohexadienyl-acetic acid and 3.1 g. (0.024 mole) of ethyl acetoacetate and the mixture was heated to reflux for 4 hours with stirring. The hot reaction mixture was filtered and the filtrate was allowed to keep cold overnight to give 2.0 g. of colorless needles 4 melting at 264° C. The additional product (3.3 g.) was obtained by concentration of the mother liquid. The total yield was 5.3 g. (88%). 10

IR: $\gamma_{\text{max}}^{\text{nuj}}$ 3300, 1635, 1600, 1570, 1300, 1275, 1170, 1090 cm^{-1} .

15 NMR: $\delta_{\text{ppm}}^{\text{D}_2\text{O}}$ 1.23 (3H, t, 7Hz, CH_2CH_3), 1.96 & 2.25 (3H, s, $\text{C}=\text{C}-\text{CH}_2$, cis & trans), 2.70 (4H, s, $\text{H}_2\text{C}^{\text{=}}$), 15

3.04 (2H, s, CH_2CO), 3.66 & 3.95 (2H, s, CH_2-N , cis & trans), 4.07 (2H, q, 7Hz,

CH_2CH_3), 4.45 & 4.56 (1H, s, $\text{H}^{\text{=}}$, cis & trans), 5.76 (2H, s, .

Anal. Calcd. for $\text{C}_{15}\text{H}_{20}\text{NO}_4\text{Na}$: C, 59.79; H, 6.69; N, 4.64.

Found: C, 59.69; H, 6.76; N, 4.75.

20 **Sodium D - N - (2 - methoxycarbonyl - 1 - methylvinyl) - α - amino - α - (3' - methyl - 4' - hydroxyphenyl)acetate.** 20

To a stirred solution of 3.02 g. (0.078 mole) of NaOH in 320 ml. of methanol is added 0.08 mole of D-(—)-2-(3'-methyl-4'-hydroxyphenyl)glycine and the resulting mixture is heated at reflux while a solution of 9.6 ml. (0.088 mole) of methyl acetoacetate in 80 ml. of methanol is added over a thirty minute period. After an additional 30 min. refluxing, the methanol is distilled off while toluene is added at the same rate so as to keep approximately the same internal volume. When the internal temperature reaches 100° C. the suspension is cooled in ice water for 4 hours, filtered, washed well with toluene, air dried, and vacuum dried over P_2O_5 to constant weight to yield solid sodium D - N - (2 - methoxy - carbonyl - 1 - methylvinyl) - α - amino - α - (3' - methyl - 4' - hydroxyphenyl)acetate. 25

30 **Sodium D - N - (2 - methoxycarbonyl - 1 - methylvinyl) - α - amino - α - (3' - methoxy - 4' - hydroxyphenyl)acetate.** 30

To a stirred solution of 3.02 g. (0.078 mole) of NaOH in 320 ml. of methanol is added 0.08 mole of D-(—)-2-(3'-methoxy-4'-hydroxyphenyl)glycine and the resulting mixture is heated at reflux while a solution of 9.6 ml. (0.088 mole) of methyl acetoacetate in 80 ml. of methanol is added over a thirty minute period. After an additional 30 min. refluxing, the methanol is distilled off while toluene is added at the same rate so as to keep approximately the same internal volume. When the internal temperature reaches 100° C. the suspension is cooled in ice water for 4 hours, filtered, washed well with toluene, air dried, and vacuum dried over P_2O_5 to constant weight to yield solid sodium D - N - (2 - methoxy - carbonyl - 1 - methylvinyl) - α - amino - α - (3' - methoxy-4'-hydroxyphenyl)acetate. 35

The similar use of a proton as the protecting group, as in an acid chloride hydrochloride, is equally well known and may be illustrated as follows: 45

D-(—)-2-(3'-Methoxy-4'-hydroxyphenyl)glycyl chloride hydrochloride is prepared in a high state of purity and very efficiently by the following procedure:

About 0.06 moles of D-(—)-2-(3'-methoxy-4'-hydroxyphenyl)glycine is slurried in 100 ml. of dioxane. The slurry is stirred and COCl_2 (phosgene) is passed in while the slurry temperature is held at 50—58° C. The COCl_2 is passed in for a total time of 3.5 hours. A yellow solution is obtained. The solution is purged with nitrogen to expel the excess COCl_2 . HCl gas is bubbled through the solution for 2.5 hours. The solution is stirred and a small amount is diluted with some ether to obtain some crystals which are added to the batch as seed. The solution is stirred at 20—25° C. for 16. 50

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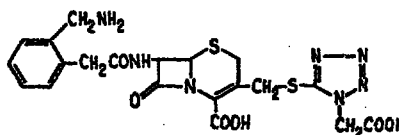
This particular mixture of secondary amines, which is sometimes referred to in these examples as "Liquid Amine Mixture No. II," is a clear amber liquid having the following physical characteristics: viscosity at 25° C. of 70 cpd., specific gravity at 20° C. of 0.826; refractive index at 25° C. of 1.4554; distillation range at 10 mm., up to 170° C. — 0.5%, 170—220° C. — 3%, 220—230° C. — 90% and above 230° C. — 6.5%.

IR—120 is also called Amberlite IR—120 and is a strong cation exchange resin containing sulfonic acid radicals. Amberlite IR—120 is a commercially available cation exchange resin of the polystyrene sulfonic acid type; it is thus a nuclear sulfonated polystyrene resin cross-lined with divinyl benzene obtained by the procedure given by Kunin, *Ion Exchange Resins*, 2nd. Edition (1958), John Wiley and Sons, Inc. Therein see pages 84 and 87 for example. ("Amberlite" is a registered Trade Mark.)

Description of the Preferred Embodiments.

Example 1.

7 - (2 - Aminomethylphenylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid.



1. Into a 3 necked flask containing 100 ml. of deionized water and set up with an agitator and thermometer, add 7.6 grams (0.021 mole) of 7-amino-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid and 3.4 grams (0.034 mole) of N-methylmorpholine. Cool to 0° C. With agitation, the solution is maintained at 0° C. using an ice bath.

2. In a separate flask set up with an agitator, add 9.6 grams (0.03 mole) of sodium 2-(1-methoxycarbonyl-1-propen-2-ylaminomethyl)phenylacetate and 184 ml. of tetrahydrofuran. With agitation, the suspension is cooled to -30° C., using a dry ice acetone bath. Maintaining agitation and temperature at -30° C., add 20 drops of dimethylbenzylamine and 4.4 grams (0.03 mole) of isobutyl chloroformate. Stir the resulting mixture for 5 minutes.

3. Add all of the mixture from step 2 to the agitation solution in step 1 at one time. The resulting solution is maintained at 3° C. with agitation for 1 hour.

4. Evaporate the tetrahydrofuran from the reaction mixture at 30° C. using vacuum (15 mm).

5. Adjust the pH of the remaining aqueous solution to 4.0 using concentrated hydrochloric acid.

6. Add 2.5 grams of charcoal ("Darco G—60") to the solution and mix for 20 minutes. Remove the carbon by filtration.

7. The filtrate is layered with 120 ml. of ethyl acetate and with agitation the pH is lowered to 3.8 with concentrated hydrochloric acid. Some light tan colored solids may separate and are removed by filtration. (Save for reworking and recovery).

8. Using an ice bath, the filtrate is cooled to 5° C. and with agitation the pH is lowered to 2.5—2.8 with concentrated hydrochloric acid. Maintain the temperature at 5° C. and continue agitation for 1 hour.

9. Collect the product by filtration. Wash the filter cake with 5 ml. of cold deionized water followed by 5 ml. of cold methanol.

10. Air dry the solid 7-(2-aminomethylphenylacetamido)-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid to constant weight. (A typical run produced 4.1 grams of product).

11. The product as obtained from step 10 is passed through a 200 mesh stainless steel screen.

12. Ten grams of this 200 mesh product is slurried in 100 ml. of chloroform. Five ml. of triethylamine is added and the mixture is heated to 50° C. with rapid stirring. The mixture is slurried at 50° C. for 5 minutes.

13. The mixture is filtered hot (7—ACA, 7-amino-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid, pigments and other impurities are soluble in hot chloroform-triethylamine solution). The filter cake is washed with 25 ml. of chloroform and air dried for 2 hours. Yield: 1—8 grams of 7-(2-aminomethylphenylacetamido)-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid.

14. The product as obtained for step 13 is passed through a 200 mesh screen.

15. Ten grams of this 200 mesh product is slurried in 75 ml. of 0.1 N hydrochloric acid for 10—15 minutes. The mixture is filtered and the filter cake is washed with 25 ml. of water, 50 ml. of methanol, and air dried at room temperature for 2—3 hours. Yield: Up to 10 g. is obtained.

16. Ten grams of 7-(2-aminomethylphenylacetamido)-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid as obtained from step 15 is slurried in 65 ml. of methanol.

a) Two ml. of concentrated hydrochloric acid is added. A solution or near solution is obtained. Stir for 5 minutes.

b) 30 ml. of water is rapidly added with vigorous stirring to the solution of a) above. An instantaneous precipitate (containing most of the color) is obtained. (A pH of 1.3 to 1.6 is required.)

c) The mixture is slurried for 1 minute and rapidly filtered. (Save solids for rework and recovery.)

d) The filtrate is seeded and moderately stirred. The onset of crystallization is about 15—30 minutes.

e) The mixture is stirred at ambient room temperature or at 4° C. for 2 hours after the onset of crystallization.

f) The crystals are removed by filtration, washed with 25 ml. of 65% water, 35% methanol mixture (v/v), 50 ml. of methanol, and vacuum dried at 50° C. for 24 hours. Yield: Up to 9 grams of purified, white 7-(2-aminomethylphenylacetamido)-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid is obtained.

17. The following are two alternative procedures for the crystallization of 7-(2-aminomethylphenylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3-cephem-4-carboxylic acid.

A)

1. Ten grams of product as obtained from step 15 is slurried in 100 ml. of methanol.

2. Two ml. of concentrated hydrochloric acid is added and a solution or near solution is obtained.

3. One and five tenths gram of charcoal ("Darco G—60") is added and the mixture is slurried for 0.5 hour.

4. The carbon is removed by filtration and washed with 20 ml. of methanol. The methanol wash is added to the filtrate.

5. One hundred and twenty ml. of water is added to the filtrate. (A small amount of precipitate may come out. This is removed by filtration and saved for rework-recovery.)

6. The solution of step 5 is rapidly stirred and adjusted to pH 2.5—3.0 with 10% sodium hydroxide. Crystals form.

7. The mixture is slurried for 0.5 hour. The crystals are removed by filtration, washed with 20 ml. of 50% methanol-water (v/v), 30 ml. of methanol and vacuum dried at 50° C. for 24 hours. Yield: Up to 9 grams of purified 7-(2-aminomethylphenylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem-4-carboxylic acid is obtained.

B)

1. Ten grams of product as obtained from step 15 is slurried in 75 ml. of water.

2. Ten percent sodium hydroxide is added to a maintained pH of 6.8—7.2. A solution or partial solution may be obtained.

3. 1.5 grams of charcoal ("Darco G—60") is added and the mixture is slurried for 0.5 hour at a maintained pH of 6.8—7.2 (continued addition of 0.1 to 1N sodium hydroxide).

4. The carbon is removed by filtration. The carbon is washed with 20 ml. of water which is added to the filtrate.

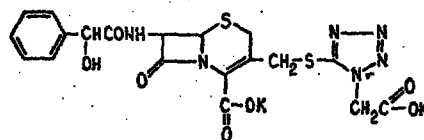
5. The pH 6.8—7.2 solution of step 4 may be crystallized at pH 2.5—3.0 as described in steps 6 and 7 of A, above or at pH 1.2—1.5 (by addition of hydrochloric acid) and as described in d, e, and f of step 16. In both instances, up to 9 grams of crystalline 7 - (2 - aminomethylphenylacetamido) - 3 - (1 - carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid is obtained. This product is frequently obtained as a crystalline monohydrate. When this compound was administered intramuscularly by injection at a dose of 10 mg/kg of body weight in mice, a blood level of 19.7 mg/ml was obtained at 15 minutes.

The protective dose in 50% of the animals challenged of title compound in mice

against a lethal intramuscular dose of *E. Coli* A15119, *K. Pneumoniae* A9977 and *E. cloacae* A21020 was 0.39–0.42, 1.5–2.5 and 1–3.2 mg/kg. respectively.

Example 2.

Dipotassium 7 - (D - α - hydroxyphenylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate.



A. 7 - (D - α - formyloxyphenylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid.

To a suspension of 500 mg. (0.0134 mole) of 7-amino-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid in 10 ml. of water at 0° was added with stirring 200 mg. of sodium bicarbonate. As soon as the solution was complete 340 mg. (0.0172 mole) of D-(-)-2-formyloxy-2-phenylacetyl chloride was added all at once in 10 ml. of acetone. As soon as a precipitate formed, solid sodium bicarbonate was added and the solution was stirred at pH 8 for 1 hr. The acetone was evaporated at 15 mm at 30°, and the solution was layered with 20 ml. of ethyl acetate and acidified with 1:1 phosphoric acid. After extraction with ethyl acetate, the mixture was filtered and the organic layer was separated and evaporated to produce what was identified as substantially pure 7-(D- α -formyloxyphenylacetamido)-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid.

B. Dipotassium 7 - (D - α - hydroxyphenylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate.

The solid isolated in step A was dissolved in 5 ml. of methanol and 5 drops of conc. hydrochloric acid. The solution was treated with carbon and heated for 3 min. on the steam-bath. The mixture was filtered and diluted with 15 ml. of water. The gummy solid was triturated with cold water and finally with anhydrous ether. The solid was dissolved in 5 ml. of acetone and was treated with 50 mg. of potassium 2-ethylhexanoate. Dipotassium 7 - (D - α - hydroxyphenylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate, as a white solid, was collected and weighed 20 mg. m.p. 175° slow decomp.

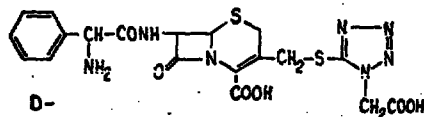
Anal. Calcd. for $C_{19}H_{16}K_2N_4O_7S_2$: C, 39.19; H, 2.77; N, 14.42. Found: C, 39.87; H, 3.50; N, 12.58.

When this compound was administered intramuscularly by injection at a dose of 10 mg/kg of body weight in mice, a blood level of 21.4 mg/ml was obtained at 15 minutes.

The protective dose of 0.8 mg/kg of the title compound was effective in 50% of the animals challenged by a lethal intramuscular dose of *E. Coli* A 15119.

Example 3.

7 - (D - α - Aminophenylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid.



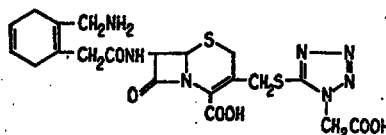
A solution of 0.55 g. (0.0022 mole) of D-(-)- α -tert.-butoxycarboxamidophenylacetic acid and 0.22 g. (0.0022 mole) of triethylamine (TEA) in 17 ml. of tetrahydrofuran (THF) at 0° was stirred vigorously with 0.300 g. (0.0022 mole) of isobutyl chloroformate. The mixture was stirred for 30 min. at 0° and a solution of 0.0022 mole of 7-amino-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid and 0.445 g. (0.0044 mole) of TEA in 6.8 ml. of 50% THF was added. The resulting solution was stirred for 2 hr. at 25° and then the THF evaporated at 40° at 15 mm to an oil. The oil was washed 2 $\times$ 50 ml. of ether, diluted in half with water and acidified to pH 3.0 with dilute hydrochloric acid. The mixture was stirred for

1 hr. in an ice-bath and the product was extracted into 75 ml. of ethyl acetate. The extract was washed with 2 x 20 ml. of water and 2 x 50 ml. of saturated sodium chloride solution. The ethyl acetate was evaporated at 35° at 15 mm. to an oil and triturated with Skellysolve B to yield 480 mg. (35.3%) of 7-(D- α -tert-butoxycarboxamidophenylacetamido)-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid. This sample was added to 1 ml. of trifluoroacetic acid and the solution was stirred for 1 hr. at 0° and then diluted with 50 ml. of ether. The salt was collected, dissolved in 10 ml. of H₂O and adjusted to pH 4.0 with dilute ammonium hydroxide (NH₄OH). The product was collected, washed with water and acetone and dried *in vacuo* over P₂O₅ for 18 hr. at 25° to yield 150 mg. (23.96%) of 7-(D- α -aminophenylacetamido)-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid; m.p. >180°, slow decomp. The IR and NMR spectra were consistent for the structure.

Anal. Calcd. for C₁₈H₁₅N₇O₆S₂ · 1 ½ H₂O: C, 42.84; H, 4.16; N, 18.41. Found: C, 43.17; H, 4.12; N, 16.74.

Example 4.

7-(2-Aminomethyl-1,4-cyclohexadienylacetamido)-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid.



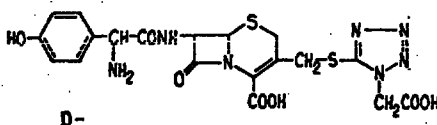
A solution of 0.80 g. (0.003 mole) of 2-t-butoxycarbonylaminoethyl-1,4-cyclohexadienylacetic acid and 0.303 g. (0.003 mole) of triethylamine in 19.2 ml. of THF was stirred at 0° and 0.41 g. (0.003 mole) of isobutyl chloroformate was added. The mixture was stirred for 30 min. at 0° and added to a solution of 0.003 mole of 7-amino-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid and 0.61 g. (0.006 mole) of TEA in 9.2 ml. of 50% THF. The resulting solution was stirred for 1 ½ hr. at 25°. The tetrahydrofuran was evaporated at 30° at 15 mm. and the residue was washed 2 x 30 with ether and then diluted in half with water. The solution was acidified to pH 3.5 with dilute hydrochloric acid and the product was collected, dried for 18 hr. *in vacuo* over P₂O₅ at 25° to yield 1.55 g. (54.0%) of white powder. A total of 3.4 ml. of trifluoroacetic acid was added to the above 7-(α -(2-t-butoxycarbonylaminoethyl-1,4-cyclohexadienylacetamido)-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid and stirred for 1 hr. at 0°. The solution was diluted with 150 ml. ether and the precipitate was collected by filtration. The trifluoroacetate salt was suspended in 3.4 ml. of water and adjusted to pH 4.5 with dilute ammonium hydroxide. The gummy residue was triturated with water, collected and washed with water and acetone. The product was dried 18 hr. *in vacuo* over P₂O₅ at 25° to yield 53 mg. (15.72%) 7-(2-aminomethyl-1,4-cyclohexadienylacetamido)-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid; m.p. >160°, slow decomp.

Anal. Calcd. for C₂₀H₂₃N₇O₆S₂ · ½ H₂O: C, 45.18; H, 4.55; N, 18.44. Found: C, 45.46; H, 4.68; N, 17.09.

The IR and NMR spectra were consistent for the structure.

Example 5.

7-(α -Amino-4-hydroxyphenylacetamido)-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid.



To a solution of 2.7 g. (0.01 mole) of D-(-)-N-tert-butoxycarbonyl-p-hydroxyphenylglycine in 92 ml. of tetrahydrofuran was added 1.1 g. (0.01 mole) of N-methylmorpholine. The solution was cooled to 0° and 1.4 g. (0.01 mole) of isobutylchloroformate was added all at once. The stirring was continued for 10 minutes and the

5 mixed anhydride solution was added to a 0° solution of 3.7 g. (0.01 mole) of 7-amino-3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid and 1.1 g. (0.01 mole) of N-methylmorpholine in 50 ml. of water. The solution was stirred for 1 hr. and the tetrahydrofuran was evaporated at 30° (15 mm) to a total volume of 45 ml. The solution was lowered to pH 2 with 1:1 phosphoric acid and extracted with ethyl acetate, washed with water and the solvent was azeotroped to a glassy solid at 30° (15 mm). The residue was triturated with ether to remove any starting acid and collected by filtration. This was hygroscopic and was transferred immediately to 5 ml. of trifluoroacetic acid and stirred for 1 hr. at 27°. The solution was diluted with 25 ml. of ether and the product was collected by filtration and suspended in 5 ml. of water. The mixture was adjusted to pH 3 with conc. ammonium hydroxide and diluted with 10 ml. of isopropanol. The light tan solid was collected by filtration and dried *in vacuo* over P₂O₅ for 24 hr. to yield 300 mg. 7-(α -amino-4-hydroxyphenylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl - 3-cephem-4-carboxylic acid. M.p. 175° slow decomp. Anal. Calcd. for C₁₈H₁₉N₇O₇S₂ $\frac{1}{2}$ i-C₃H₇O: C, 42.78; H, 4.44; N, 17.32. Found: C, 42.86; H, 4.55; N, 15.39. The IR and NMR spectra were consistent for the structure. The NMR spectrum did show the presence of .75 mole isopropyl alcohol in the product.

Example 6.

20 Substitution in the procedure of example 2 for the D-(—)-2-formyloxy-2-phenylacetyl chloride used therein of an equimolar quantity of monosubstituted D-(—)-2-formyloxy-2-phenylacetyl chloride prepared from the following monosubstituted D-mandelic acids:

25 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,
30 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,
35 D-4-trifluoromethyl-mandelic acid,
D-2-amino-mandelic acid,
D-3-amino-mandelic acid,
D-4-amino-mandelic acid,
40 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,
45 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,
50 D-4-methoxy-mandelic acid respectively produces the monosubstituted 7-(D- α -formyloxyphenylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem-4-carboxylic acid compound in step A, which is subsequently converted to

Dipotassium 7 - (D - 2 - chloro - mandelamido) - 3 - (1 - carboxymethyltetrazol-5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate,
55 Dipotassium 7 - (D - 3 - chloro - mandelamido) - 3 - (1 - carboxymethyltetrazol-5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate,
Dipotassium 7 - (D - 4 - chloro - mandelamido) - 3 - (1 - carboxymethyltetrazol-5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate,
60 Dipotassium 7 - (D - 2 - bromo - mandelamido) - 3 - (1 - carboxymethyltetrazol-5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate,
Dipotassium 7 - (D - 3 - bromo - mandelamido) - 3 - (1 - carboxymethyltetrazol-5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate,
Dipotassium 7 - (D - 4 - bromo - mandelamido) - 3 - (1 - carboxymethyltetrazol-5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate,

	Dipotassium 7 - (D - 2 - fluoro - mandelamido) - 3 - (1 - carboxymethyltetrazol-5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate,	
	Dipotassium 7 - (D - 3 - fluoro - mandelamido) - 3 - (1 - carboxymethyltetrazol-5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate,	
5	Dipotassium 7 - (D - 4 - fluoro - mandelamido) - 3 - (1 - carboxymethyltetrazol-5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate,	5
	Dipotassium 7 - (D - 2 - trifluoromethyl - mandelamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate,	
10	Dipotassium 7 - (D - 3 - trifluoromethyl - mandelamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate,	10
	Dipotassium 7 - (D - 4 - trifluoromethyl - mandelamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate,	
	Dipotassium 7 - (D - 2 - amino - mandelamido) - 3 - (1 - carboxymethyltetrazol-5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate,	
15	Dipotassium 7 - (D - 3 - amino - mandelamido) - 3 - (1 - carboxymethyltetrazol-5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate,	15
	Dipotassium 7 - (D - 4 - amino - mandelamido) - 3 - (1 - carboxymethyltetrazol-5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate,	
20	Dipotassium 7 - (D - 2 - nitro - mandelamido) - 3 - (1 - carboxymethyltetrazol-5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate,	20
	Dipotassium 7 - (D - 3 - nitro - mandelamido) - 3 - (1 - carboxymethyltetrazol-5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate,	
	Dipotassium 7 - (D - 4 - nitro - mandelamido) - 3 - (1 - carboxymethyltetrazol-5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate,	
25	Dipotassium 7 - (D - 2 - hydroxy - mandelamido) - 3 - (1 - carboxymethyltetrazol-5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate,	25
	Dipotassium 7 - (D - 3 - hydroxy - mandelamido) - 3 - (1 - carboxymethyltetrazol-5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate,	
	Dipotassium 7 - (D - 4 - hydroxy - mandelamido) - 3 - (1 - carboxymethyltetrazol-5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate,	
30	Dipotassium 7 - (D - α - formyloxyphenylacetamido) - 3 - (1 - carboxymethyltetrazol-5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate,	30
	Dipotassium 7 - (D - 2 - methyl - mandelamido) - 3 - (1 - carboxymethyltetrazol-5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate,	
35	Dipotassium 7 - (D - 3 - methyl - mandelamido) - 3 - (1 - carboxymethyltetrazol-5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate,	35
	Dipotassium 7 - (D - 4 - methyl - mandelamido) - 3 - (1 - carboxymethyltetrazol-5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate,	
40	Dipotassium 7 - (D - 2 - methoxy - mandelamido) - 3 - (1 - carboxymethyltetrazol-5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate,	40
	Dipotassium 7 - (D - 3 - methoxy - mandelamido) - 3 - (1 - carboxymethyltetrazol-5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate and	
	Dipotassium 7 - (D - 4 - methoxy - mandelamido) - 3 - (1 - carboxymethyltetrazol-5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate respectively.	

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Example 7.

Substitution for the D-mandelic acid carboxyanhydrides in the procedure of Example 2 of an equimolar weight of the carboxyanhydride prepared in similar fashion from D-2-thiopheneglycolic acid and D-3-thiopheneglycolic acid respectively produces dipotassium 7 - (D - α - hydroxy - 2 - thienylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate and dipotassium 7 - (D - α - hydroxy - 3 - thienylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - yl - thiomethyl) - 3 - cephem - 4 - carboxylate respectively.

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Example 8.

Substitution in the procedure of Example 3 for the N-tert.-butoxycarbonyl derivative of D- α -aminophenylacetic acid (also called D- α -tert.-butoxycarboxamido-phenylacetic acid) of an equimolar weight of the N-tert.-butoxycarbonyl derivative of D- α -amino-2-thienylacetic acid,
D- α -amino-3-thienylacetic acid,
D- α -amino-m-nitrophenylacetic acid,
D- α -amino-p-methylphenylacetic acid,
D- α -amino-m-methylphenylacetic acid,
D- α -amino-p-chlorophenylacetic acid,
D- α -amino-m-chlorophenylacetic acid,

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	D- α -amino-p-fluorophenylacetic acid,	
	D- α -amino-m-fluorophenylacetic acid,	
	D- α -amino-p-aminophenylacetic acid,	
5	D- α -amino-p-dimethylaminophenylacetic acid,	5
	D- α -amino-m,p-dimethoxyphenylacetic acid,	
	D- α -amino-m-iodophenylacetic acid,	
	D- α -amino-m-chloro-p-hydroxyphenylacetic acid,	
	D- α -amino-p-methoxyphenylacetic acid,	
10	D- α -amino-m-methoxyphenylacetic acid,	10
	D- α -amino-m-hydroxyphenylacetic acid,	
	D- α -amino-p-acetamidophenylacetic acid,	
	D- α -amino-m-aminophenylacetic acid, and	
	D- α -amino-m-acetamidophenylacetic acid, respectively, produces	
15	7 - (D - α - 2 - thienylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - ceph - 3 - em - 4 - oic acid,	15
	7 - (D - α - 3 - thienylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - ceph - 3 - em - 4 - oic acid,	
	7 - (D - α - m - nitrophenylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthio - methyl) - ceph - 3 - em - 4 - oic acid.	
20	7 - (D - α - p - methylphenylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthio - methyl) - ceph - 3 - em - 4 - oic acid.	20
	7 - (D - α - m - methylphenylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthio - methyl) - ceph - 3 - em - 4 - oic acid.	
	7 - (D - α - p - chlorophenylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthio - methyl) - ceph - 3 - em - 4 - oic acid.	
25	7 - (D - α - m - chlorophenylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthio - methyl) - ceph - 3 - em - 4 - oic acid.	25
	7 - (D - α - p - fluorophenylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthio - methyl) - ceph - 3 - em - 4 - oic acid.	
30	7 - (D - α - m - fluorophenylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthio - methyl) - ceph - 3 - em - 4 - oic acid.	30
	7 - (D - α - p - aminophenylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthio - methyl) - ceph - 3 - em - 4 - oic acid.	
	7 - (D - α - p - dimethylaminophenylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - ceph - 3 - em - 4 - oic acid,	
35	7 - (D - α - m - p - dimethoxyphenylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - ceph - 3 - em - 4 - oic acid,	35
	7 - (D - α - m - iodophenylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthio - methyl) - ceph - 3 - em - 4 - oic acid.	
40	7 - (D - α - m - chloro - p - hydroxyphenylacetamido) - 3 - (1 - carboxymethyltetra - zol - 5 - ylthiomethyl) - ceph - 3 - em - 4 - oic acid,	40
	7 - (D - α - p - methoxyphenylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - ceph - 3 - em - 4 - oic acid,	
	7 - (D - α - m - methoxyphenylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - ceph - 3 - em - 4 - oic acid,	
45	7 - (D - α - m - hydroxyphenylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - ceph - 3 - em - 4 - oic acid,	45
	7 - (D - α - p - acetamidophenylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - ceph - 3 - em - 4 - oic acid,	
50	7 - (D - α - m - aminophenylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - ceph - 3 - em - 4 - oic acid, and	50
	7 - (D - α - m - acetamidophenylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - ceph - 3 - em - 4 - oic acid, respectively.	

Example 9.

55	7 - Phenoxyacetamido - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem-4-carboxylic acid by thiolation of 7-phenoxyacetamidocephalosporanic acid. Sodium 7-phenoxyacetamidoccephalosporanate (0.27 mole) is suspended in 1000 ml. of 0.1 M phosphate buffer pH 6.4 to which is added 0.31 mole disodium 1-carboxy-methyl-5-mercaptopotetrazole. The solution is heated at 55° C. under a nitrogen atmo- sphere for 5 hr. After 1 hr. the pH is adjusted to 6.4 by addition of a small amount of 40% H ₃ PO ₄ . At the end of the 5 hr. heating period, the solution is cooled to 23° C. and the pH adjusted to 2 by 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. It is then filtered through a pad of diatom-	55
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aceous earth ("Celite") and the ethyl acetate removed under vacuum to leave an oil which is triturated to a solid with diethyl ether, collected by filtration and dried over P_2O_5 under vacuum to yield solid 7-phenoxyacetamido-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid.

Example 10.

- 5 Replacement of the sodium 7-phenoxyacetamidocephalosporanate in the procedure of Example 9 with an equimolar weight of
- 10 sodium 7-phenylacetamido-cephalosporanate,
sodium 7-(2-thienylacetamido)-cephalosporanate,
sodium 7-(3-thienylacetamido)-cephalosporanate,
sodium 7-(4-pyridylthioacetamido)-cephalosporanate,
sodium 7-cyanoacetamido-cephalosporanate,
sodium 7-(1-tetrazolylacetamido)-cephalosporanate,
sodium 7-trifluoromethylthioacetamido-cephalosporanate,
sodium 7-(α -azidophenylacetamido)-cephalosporanate,
15 sodium 7-(sydnone-3-acetamido)-cephalosporanate,
sodium 7-chloroacetamido-cephalosporanate, and
sodium 7-bromoacetamido-cephalosporanate, respectively, produces
- 20 7-phenylacetamido-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(2-thienylacetamido)-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(3-thienylacetamido)-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid,
25 7-(4-pyridylthioacetamido)-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-cyanoacetamido-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(1-tetrazolylacetamido)-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid,
30 7-trifluoromethylthioacetamido-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-(α -azidophenylacetamido)-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid,
35 7-(sydnone-3-acetamido)-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid,
7-chloroacetamido-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid, and
7-bromoacetamido-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid, respectively.

Example 11.

- 40 7-[D- α -Amino- α -(p-hydroxyphenyl)acetamido]-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid by thiolation of 7-[D- α -t-butoxycarbonylamino- α -(p-hydroxyphenyl)acetamido]-cephalosporanic acid followed by deblocking.
- 45 A. 7-[α -(4-Hydroxyphenyl)- α -D-(t-butoxycarbonylamino)-acetamido]-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid.
- Sodium 7-[α -(4-hydroxyphenyl)- α -D-(t-butoxycarbonylamino)-acetamido]cephalosporanate (0.27 mole) is suspended in 1000 ml. of 0.1 M phosphate buffer pH 6.4 to which is added 0.31 mole disodium 1-carboxymethyl-5-mercaptopotrazole. The solution is heated at 55° C. under a nitrogen atmosphere for 5 hr. After 1 hr. the pH is adjusted to 6.4 by addition of a small amount of 40% H_3PO_4 . At the end of the 5 hr. heating period, the solution is cooled to 23° C. and the pH adjusted to 2 by addition of 3N HCl under a layer of ethyl acetate. The product is extracted into acetate and stirred for 15 min. at 23° C. with 2 g. of ("Darco KB") decolorizing charcoal. It is then filtered through a pad of diatomaceous earth ("Celite"—registered Trade Mark) and the ethyl acetate removed under vacuum to leave an oil which is triturated to a solid with diethyl ether, collected by filtration and dried over P_2O_5 under vacuum to yield solid 7-[α -(4-hydroxyphenyl)- α -D-(t-butoxycarbonylamino)-acetamido]-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid.

B. 7 - [D - α - Amino - α - (p - hydroxyphenyl)acetamido] - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid.

7 - [α - (4 - Hydroxyphenyl) - α - D - (t - butoxycarbonylamino) - acetamido] - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid (0.1 mole) is dissolved in 30 ml. of trifluoroacetic acid at 5° C. It is allowed to come to 23° C. and stirred for an additional 30 min. This solution is slowly poured into 1000 ml. of anhydrous ethyl ether with vigorous stirring. The precipitate is collected by filtration, washed with 100 ml. ethyl ether and dried over P₂O₅ under vacuum for 1 hr. It is then dissolved in 75 ml. H₂O and after stirring 30 min. at 23° C. the solids are filtered off. 2 g. of ("Darco KB") decolorizing charcoal is added to the filtrate and after stirring 10 min. at 23° C. the slurry is filtered through a "Celite" pad. The pH of the filtrate is adjusted to 4 by addition of triethylamine and the solids filtered off. The filtrate is evaporated under high vacuum to an oil and triturated with acetonitrile. The product, 7-[D- α -amino- α -(p-hydroxyphenyl)acetamido]-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid, is collected by filtration and air dried.

Example 12.

Replacement of the sodium 7-[α -(4-hydroxyphenyl)- α -D-(t-butoxycarbonylamino)acetamido]-cephalosporanate in the procedure of Example 11 with an equimolar weight of the cephalo. poranic acid produced, for example, by acylation of 7-ACA in the usual manner with

2-(tert.-butoxycarbonylaminomethyl)-1,4-cyclohexadienylacetic acid,
D-(—)- α -tert.-butoxycarboxamidophenylacetic acid,
2-(tert.-butoxycarbonylaminomethyl)-1,2-cyclohexenylacetic acid,
2-t-butoxycarbonylaminomethyl-4-hydroxyphenylacetic acid,
o-(tert.-butoxycarbonylaminomethyl)phenylthioacetic acid,
D-[o-(tert.-butoxycarbonylaminomethyl)phenyl]propionic acid,
D-(—)-N-t-butoxycarbonyl-2-(3'-methyl-4'-hydroxyphenyl)glycine,
D-(—)-N-t-butoxycarbonyl-2-(3'-methoxy-4'-hydroxyphenyl)glycine,
D-(—)-N-t-butoxycarbonyl-2-(3-acetoxyphenyl)glycine,
D-(—)-N-t-butoxycarbonyl-2-(1'-cyclohexenyl)glycine,
D-(—)-N-t-butoxycarbonyl-2-(3'-chloro-4'-hydroxyphenyl)glycine,
D-(—)-N-t-butoxycarbonyl-2-(1',4'-cyclohexadienyl)glycine,
D-(—)-2-tert.-butoxycarboxamido-3-(1',4'-cyclohexadienyl)propionic acid,
D-(—)-2-tert.-butoxycarboxamido-3-(4'-methoxy-1',4'-cyclohexadienyl)propionic acid,
2-t-butoxycarbonylaminomethylphenyl-acetic acid,
N-tert.-butoxycarbonyl-1-aminocyclohexanecarboxylic acid,
D-(—)- α -tert.-butoxycarboxamido-3-thienylacetic acid,
D-(—)- α -tert.-butoxycarboxamido-2-thienylacetic acid,
 β -(2-tert.-butoxycarbonylaminomethyl-1,4-cyclohexadienyl)propionic acid,
 β -(2-t-butoxycarbonylaminomethyl-1-cyclohexenyl)propionic acid, and
D-(—)-N-tert.-butoxycarbonyl-2-(4'-hydroxymethylphenyl)glycine,
respectively, produces

7 - (2 - aminomethyl - 1,4 - cyclohexadienylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid.

7 - (D - α - aminophenylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid,

7 - (2 - aminomethyl - 1,2 - cyclohexenylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid.

7 - (2 - aminomethyl - 4 - hydroxyphenylacetamido) - 3 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid,

7 - (o - aminomethylphenylthioacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid,

7 - (β - o - aminomethylphenylpropionamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid,

7 - (D - α - amino - α - (3' - methyl - 4' - hydroxyphenyl)acetamido] - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid,

7 - [D - α - amino - α - (3' - methoxy - 4' - hydroxyphenyl)acetamido] - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid,

7 - [D - α - amino - α - (3 - acetoxyphenyl)acetamido] - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid,

7 - [D - α - amino - α - (1' - cyclohexenyl)acetamido] - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid,

- 7 - [D - α - amino - α - (3' - chloro - 4' - hydroxyphenyl)acetamido] - 3 - (1-carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid,
 7 - [D - α - amino - α - (1',4' - cyclohexadienyl)acetamido] - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid,
 5 7 - [D - 2 - amino - 3 - (1',4' - cyclohexadienyl)propionamido] - 3 - (1 - carboxymethyltetrazol - 5 - ylthio ethyl) - 3 - cephem - 4 - carboxylic acid, 5
 7 - [D - 2 - amino - 3 - (4' - methoxy - 1',4' - cyclohexadienyl)propionamido] - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid,
 10 7 - (2 - aminomethylphenylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid, 10
 7 - (1 - aminocyclohexanecarboxamido - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid,
 7 - (D - α - amino - 3 - thienylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid,
 15 7 - (D - α - amino - 2 - thienylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid, 15
 7 - (2 - aminomethyl - 1,4 - cyclohexadienyl - propionamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid,
 20 7 - [β - (aminomethyl - 1 - cyclohexenyl)propionamido] - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid and 20
 7 - [D - α - amino - α - (4' - hydroxymethylphenyl)acetamido] - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid, respectively.

Example 13.

- Replacement of the D-(—)-N-tert.-butoxycarbonyl-p-hydroxyphenylglycine in the procedure of Example 5 with an equimolar weight of 25
 2-(tert.-butoxycarbonylaminoethyl)-1,4-cyclohexadienylacetic acid, 25
 D-(—)- α -tert.-butoxycarboxamidophenylacetic acid,
 2-(tert.-butoxycarbonylaminoethyl)-1,2-cyclohexenylacetic acid,
 2-t-butoxycarbonylaminoethyl-4-hydroxyphenylacetic acid,
 30 o-(tert.-butoxycarbonylaminoethyl)phenylthioacetic acid, 30
 β -[o-(tert.-butoxycarbonylaminoethyl)phenyl]propionic acid,
 D-(—)-N-t-butoxycarbonyl-2-(3'-methyl-4'-hydroxyphenyl)glycine,
 D-(—)-N-t-butoxycarbonyl-2-(3'-methoxy-4'-hydroxyphenyl)glycine,
 D-(—)-N-t-butoxycarbonyl-2-(3-acetoxyphenyl)glycine,
 35 D-(—)-N-t-butoxycarbonyl-2-(1'-cyclohexenyl)glycine, 35
 D-(—)-N-t-butoxycarbonyl-2-(3'-chloro-4'-hydroxyphenyl)glycine,
 D-(—)-N-t-butoxycarbonyl-2-(1',4'-cyclohexadienyl)glycine,
 D-(—)-2-tert.-butoxycarboxamido-3-(1',4'-cyclohexadienyl)propionic acid,
 D-(—)-2-tert.-butoxycarboxamido-3-(4'-methoxy-1',4'-cyclohexadienyl)propionic acid,
 40 2-t-butoxycarbonylaminoethylphenyl-acetic acid, 40
 N-tert.-butoxycarbonyl-1-aminocyclohexanecarboxylic acid,
 D-(—)- α -tert.-butoxycarboxamido-3-thienylacetic acid,
 D-(—)- α -tert.-butoxycarboxamido-2-thienylacetic acid,
 β -(2-tert.-butoxycarbonylaminoethyl-1,4-cyclohexadienyl)propionic acid,
 45 β -(2-t-butoxycarbonylaminoethyl-1-cyclohexenyl)-propionic acid, and 45
 D-(—)-N-tert.-butoxycarbonyl-2-(4'-hydroxymethylphenyl)glycine, respectively, produces
 7 - (2 - aminomethyl - 1,4 - cyclohexadienylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid,
 50 7 - (D - α - aminophenylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid, 50
 7 - (2 - aminomethyl - 1,2 - cyclohexenylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid,
 7 - (2 - aminomethyl - 4 - hydroxyphenylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid,
 55 7 - (o - aminomethylphenylthioacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid, 55
 7 - (β - o - aminomethylphenylpropionamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid,
 60 7 - (D - α - amino - α - (3' - methyl - 4' - hydroxyphenyl)acetamido] - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid, 60
 7 - [D - α - amino - α - (3' - methoxy - 4' - hydroxyphenyl)acetamido] - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid,

- 7 - [D - α - amino - α - (3 - acetoxyphenyl)acetamido] - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid,
- 7 - [D - α - amino - α - (1' - cyclohexenyl)acetamido] - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid,
- 5 7 - [D - α - amino - α - (3' - chloro - 4' - hydroxyphenyl)acetamido] - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid, 5
- 7 - [D - α - amino - α - (1',4' - cyclohexadienyl)acetamido] - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid,
- 10 7 - [D - 2 - amino - 3 - (1',4' - cyclohexadienyl)propionamidol] - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid, 10
- 7 - [D - 2 - amino - 3 - (4' - methoxy - 1',4' - cyclohexadienyl)propionamidol] - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid,
- 7 - (2 - aminomethylphenylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid,
- 15 7 - (1 - aminocyclohexanecarboxamido - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid, 15
- 7 - (D - α - amino - 3 - thienylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid,
- 20 7 - (D - α - amino - 2 - thienylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid, 20
- 7 - (2 - aminomethyl 1,4 - cyclohexadienyl - propionamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid,
- 7 - [β - (aminomethyl - 1 - cyclohexenyl)propionamido] - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid and
- 25 7 - [D - α - amino - α - (4' - hydroxymethylphenyl)acetamido] - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid, respectively. 25

Example 14.

Sodium 7 - (D - α - [3 - (2 - furoyl)ureido] - 4 - hydroxyphenylacetamido) cephalosporanate.

- 30 Triethylamine (6.4 ml., 0.0454 mole) and 12.7 g. (0.1039 mole) of 2-furoyl isocyanate was added to an ice cooled suspension of 20.8 g. (0.0413 mole) of 7-(D- α -amino-4-hydroxyphenylacetamido)cephalosporanic acid (as a dimethylformamide-water solvate) in 175 ml. of dry dimethylformamide. After about 1 minute the ice bath was removed and the mixture stirred for 1 hr. The dark solution was diluted with 600 ml. of water, layered with ethyl acetate and the aqueous phase acidified to pH 2.5 with 42% phosphoric acid. The phases were separated and the aqueous dimethylformamide phase was extracted four times more with ethyl acetate. The combined organic extracts were washed five times with water, carbon treated, filtered and concentrated at reduced pressure to remove residual water. Fresh ethyl acetate was added and the stirred solution was treated slowly with 15.1 ml. (0.0408 mole) of sodium 2-ethylhexanoate in 1-butanol causing the product to crystallize; 18.1 g., 75.5%. The IR spectrum was consistent for the desired product.

- 45 7 - (D - α - [3 - (2 - Furoyl)ureido] - 4 - hydroxyphenylacetamido) - 3 - (1 - carboxymethyltetrazol - 5 - ylthiomethyl) - cephem - 4 - carboxylic acid disodium salt. 45

- 50 A mixture of 4.0 g. (0.0069 mole) of sodium 7-{D- α -[3-(2-furoyl)ureido]-4-hydroxyphenylacetamido}-cephalosporanate, 1.83 g. (0.00895 mole) of 5-mercapto-1-tetrazole acetic acid disodium salt and 200 ml. of pH 6.4, 0.1 M phosphate buffer was heated at 75° for 6 hours under a nitrogen atmosphere. The reaction mixture which had been stored in the refrigerator overnight was filtered to remove a small amount of precipitate. The filtrate was adjusted to pH 2 with 6 N HCl and the mixture extracted three times with ethyl acetate. The mixture was filtered during the first extraction to remove a small amount of insoluble material. The combined ethyl acetate extracts (volume=500 ml.) were washed twice with water, dried (sodium sulfate), filtered and treated with 5.1 ml. (0.0138 mole, 2 eqv.) of sodium 2-ethylhexanoate in 1-butanol causing an oil to separate. The solvent was concentrated somewhat to remove water and the mixture stored in the refrigerator. The solvent was decanted and the gummy product was triturated with acetone giving a filterable solid; yield 2.0 g. The product was dried *in vacuo* over phosphorus pentoxide. The IR spectrum was consistent for the desired product.

Samples of the compounds prepared in Examples 1, 2, 3, 4, 5 and 14 after solution in water and dilution with Nutrient Broth were found to exhibit the following Minimum Inhibitory Concentrations (M.I.C.) in mcg./ml. versus the indicated microorganisms as determined by overnight incubation at 37° C. by Tube Dilution.

In Vitro Antibacterial Activity
M.I.C. ($\mu\text{g./ml.}$)

Organisms	Ex. 1 (soluble at ≥ 250 mg/ml as Na^+ salt)	Ex. 2	Ex. 3	Ex. 4	Ex. 14	Ex. 5
<i>Str. pneumoniae</i> * (10^{-5})**	A9585 0.13	0.6	0.6	0.06	0.13	0.25
<i>Str. pyogenes</i> * (10^{-5})	A9604 0.13	0.6	0.6	0.13	0.13	1
<i>S. aureus</i> Smith (10^{-4})	A9537 1	1.3	2.5	0.5	>1	>1
<i>S. aureus</i> -50% serum (10^{-4})	A9537 4	16	32	>0.5	>63	16
<i>S. aureus</i> BX1633 (10^{-5})	A9606 1	2.5	>2.5	1.3	2	8
<i>S. aureus</i> BX1633 (10^{-5})	A9606 2	4	16	2.5	8	8
<i>S. aureus</i> Meth-Res (10^{-5})	A15097 4	8	16	4	63	32
<i>Sal. enteritidis</i> (10^{-4})	A9531 0.06	0.08	0.3	0.16	0.5	0.5
<i>E. coli</i> Juhl (10^{-4})	A15119 0.5	4	8	1.3	16	8
<i>E. coli</i> (10^{-4})	A9675 16	32	16	16	16	32
<i>K. pneumoniae</i> (10^{-4})	A9977 0.13	1	1	0.3	1	1
<i>K. pneumoniae</i> (10^{-4})	A15130 2	32	8	2	125	8
<i>Pr. mirabilis</i> (10^{-4})	A9900 0.13	0.5	1	0.3	0.5	1

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4. The carbon is removed by filtration and washed with 10 ml. of water. The wash is added to the filtrate.

5. The pH 7.4—7.7 solution of step 4 is made sterile and pyrogen-free by suitable aseptic filtration and techniques.

The combined time required for completion of steps 3, 4 and 5 should not exceed 5 hours at ambient room temperature.

6. Using sterile technique, an approximate equal volume (65 ml.) of sterile, pyrogen-free acetone is added to the rapidly stirring sterile solution of step 5 over a 5 minute period.

7. Based upon the approximate original aqueous volume, an additional 2 volumes (120 ml.) of sterile, pyrogen-free acetone is added with rapid stirring over 15—20 minutes. Crystals form.

8. The mixture is slurried for 10 minutes.

9. An additional 3 volumes (180 ml.) of sterile acetone is added over a 15 minute interval. The mixture is slurried for 0.5 hour.

10. The crystals are collected by filtration, washed with 75 ml. of sterile acetone and vacuum dried at 45—50° C. or air dried at 50—56° C. for 24 hours. Yield: Approximately 8.8 grams.

Properties of trihydrate of monosodium salt of 7-(2-aminomethylphenylacetamido)-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid:

% Water (KF) = 9.2 (theory = 9.05%)

% Sodium (flame photometer) = 4.0 (theory = 3.86%)

Solubility in water = > 500 mg./ml.

Stability in water = Stable for at least 24 hours at room temperature at 250 mg./ml.

Formulation of Injectable Products.

In situ preparation of monosodium salt of 7-(2-aminomethylphenylacetamido)-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid:

A) 2.5 grams of 7-(2-aminomethylphenylacetamido)-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid (zwitterion) is suspended in 8.5 ml. of water with rapid stirring, sodium citrate or Na_2HPO_4 or Na_3PO_4 or other suitable "bases" are added until a solution is obtained (the pH should not be over 7.8). The amount of added "base" is noted.

B) A physical mixture of 2.5 grams of 7-(2-aminomethylphenylacetamido)-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid and the solid "base" in proportions determined in "A" above is made. The later addition of water to obtain various concentrations of 7-(2-aminomethylphenylacetamido)-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid gives a solution of *in situ* prepared monosodium salt of 7-(2-aminomethylphenylacetamido)-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid.

This procedure may be desirable as trihydrate of monosodium salt of 7-(2-aminomethylphenylacetamido)-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid is not as stable at elevated temperatures as is the free-acid 7-(2-aminomethylphenylacetamido)-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid (zwitterion) monohydrate.

With regard to the stability of 7-(2-aminomethylphenylacetamido)-3-(1-methyl-1,2,3,4-tetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid solutions at room temperature and at pH 10.3 (the lowest pH able to dissolve 150 mg./ml.) an almost instantaneous 50% loss of bioactivity is noted. An additional 21% activity is lost in the next 30 minutes.

By contrast, a solution containing 125.0 mg./ml. of the trihydrate of monosodium salt of 7-(2-aminomethylphenylacetamido)-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid at pH 7.0 showed no significant loss at room temperature for at least 24 hours.

The compound entitled 7-(2-aminomethylphenylacetamido)-3-(1-methyl-1,2,3,4-tetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid of example 1 and claim 2 of U.S. 3,766,175 is a very potent cephalosporin exhibiting a highly desirable spectrum of activity particularly against certain Gram-negative organisms. Unfortunately, this zwitterion exhibits quite a low solubility in water and particularly in the blood stream which means at about pH 7.2 or thereabouts. To be more specific, attempts to measure this solubility gave results in the range of about 1.0—3.0 mg./ml. in both buffered aqueous media and in dog urine at room temperature. The pH of fresh beagle dog urine is 7.6. This raises a question as to the possible toxic effect in man of the administration of this zwitterion because of the fact that it is assumed that it will precipitate in crystalline form in the kidneys as it is concentrated herein during excretion. This,

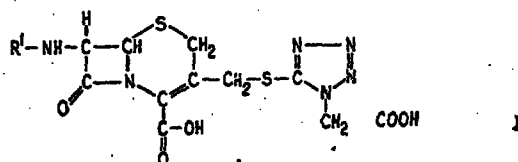
in man, would be highly undesirable. Conventional attempts to solve this prospective problem by the use of ordinary water soluble forms and derivatives of 7-(o-aminomethylphenylacetamido) - 3 - (1 - methyl - 1,2,3,4 - tetrazol - 5 - ylthiomethyl) - 3 - cephem-4-carboxylic acid have proven unsuccessful because of conversion in the body of the salt or derivative to the zwitterion which then exhibits its natural low solubility in aqueous media and the blood. It was an objective of the present invention to solve this problem without loss of the valuable biological activity of 7-(o-aminomethylphenylacetamido) - 3 - (1 - methyl - 1,2,3,4 - tetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid. After various failures the problem was solved by the provision according to the present invention of 7-(2-aminomethylphenylacetamido)-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid which has the desired properties. To be more specific a sample of 7-(2-aminomethylphenylacetamido)-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid in pH 7.0 phosphate buffer at 25° C. exhibited a solubility in mg./ml. greater than 15.3 and less than 13.6; in this instance the capacity of the buffer was not sufficient and the pH dropped to 6.48. Thus the 7-(2-aminomethylphenylacetamido)-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid provided by the present invention even in its zwitterionic form is too soluble in the blood stream to crystallize in the kidneys and thereby cause fear of toxic results in at least some patients.

In addition, as set forth above the combination of the lack of aqueous solubility presented a problem which was solved by the preparation of the trihydrate of monosodium salt of 7 - (2 - aminomethylphenylacetamido) - 3 - (1 - carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid of the present invention which exhibits such solubility at pH's suitable for injection in man such as about pH 7 and still exhibits satisfactory solubility in the blood stream and the fluids in the kidney even if converted in the body to the zwitterionic form.

In accordance with the above, the advantage of the compounds of the instant invention is their improved water solubility as compared to many of the other compounds known in the art, and in addition, the generally superior blood levels the compounds achieve upon parenteral administration by the intramuscular route.

WHAT WE CLAIM IS:—

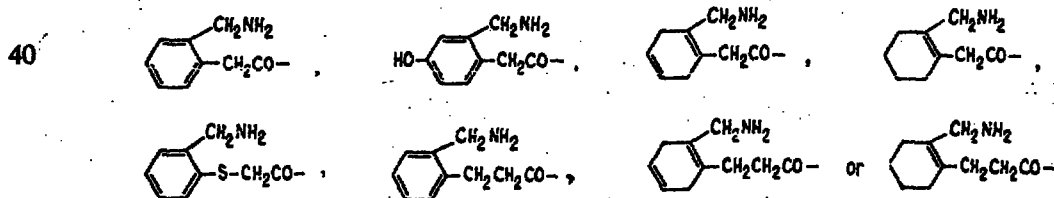
1. A compound having the formula



wherein R¹ is acyl or hydrogen or an ester or non-toxic pharmaceutically acceptable salt thereof.

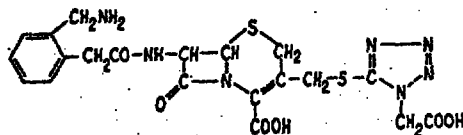
2. The pivaloyloxymethyl-, acetoxymethyl-, methoxymethyl-, acetonyl-, phenacyl-, *p*-nitrobenzyl-, β,β,β -trichloroethyl-, 3-phthalidyl- or 5-indanyl ester of a compound as claimed in Claim 1.

3. A compound as claimed in Claim 1 or 2, wherein R¹ is of the formula



or the benzaldehyde or salicylaldehyde derivative of the above defined R¹ groups.

4. The compound as claimed in any one of Claims 1 to 3 having the formula





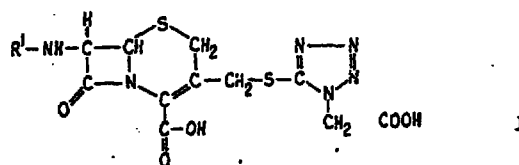
in man, would be highly undesirable. Conventional attempts to solve this prospective problem by the use of ordinary water soluble forms and derivatives of 7-(o-aminomethylphenylacetamido) - 3 - (1 - methyl - 1,2,3,4 - tetrazol - 5 - ylthiomethyl) - 3 - cephem-4-carboxylic acid have proven unsuccessful because of conversion in the body of the salt or derivative to the zwitterion which then exhibits its natural low solubility in aqueous media and the blood. It was an objective of the present invention to solve this problem without loss of the valuable biological activity of 7-(o-aminomethylphenylacetamido) - 3 - (1 - methyl - 1,2,3,4 - tetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid. After various failures the problem was solved by the provision according to the present invention of 7-(2-aminomethylphenylacetamido)-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid which has the desired properties. To be more specific a sample of 7-(2-aminomethylphenylacetamido)-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid in pH 7.0 phosphate buffer at 25° C. exhibited a solubility in mg./ml. greater than 15.3 and less than 13.6; in this instance the capacity of the buffer was not sufficient and the pH dropped to 6.48. Thus the 7-(2-aminomethylphenylacetamido)-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid provided by the present invention even in its zwitterionic form is too soluble in the blood stream to crystallize in the kidneys and thereby cause fear of toxic results in at least some patients.

In addition, as set forth above the combination of the lack of aqueous solubility presented a problem which was solved by the preparation of the trihydrate of monosodium salt of 7 - (2 - aminomethylphenylacetamido) - 3 - (1 - carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid of the present invention which exhibits such solubility at pH's suitable for injection in man such as about pH 7 and still exhibits satisfactory solubility in the blood stream and the fluids in the kidney even if converted in the body to the zwitterionic form.

In accordance with the above, the advantage of the compounds of the instant invention is their improved water solubility as compared to many of the other compounds known in the art, and in addition, the generally superior blood levels the compounds achieve upon parenteral administration by the intramuscular route.

WHAT WE CLAIM IS:—

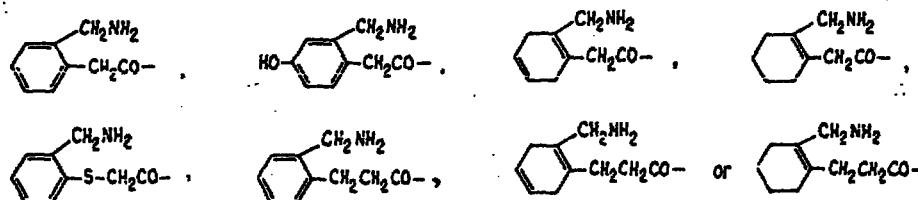
1. A compound having the formula



wherein R¹ is acyl or hydrogen or an ester or non-toxic pharmaceutically acceptable salt thereof.

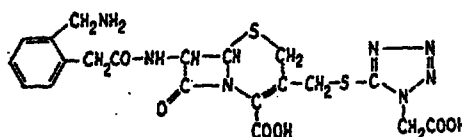
2. The pivaloyloxymethyl-, acetoxymethyl-, methoxymethyl-, acetonyl-, phenacyl-, *p*-nitrobenzyl-, β,β,β -trichloroethyl-, 3-phthalidyl- or 5-indanyl ester of a compound as claimed in Claim 1.

3. A compound as claimed in Claim 1 or 2, wherein R¹ is of the formula



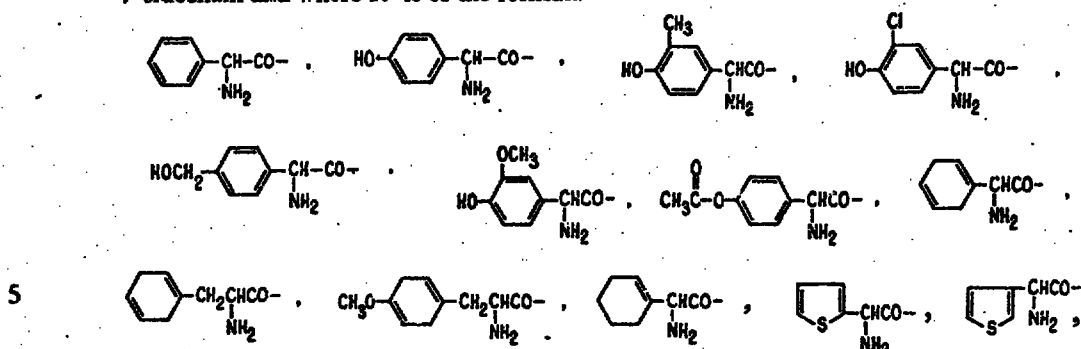
or the benzaldehyde or salicylaldehyde derivative of the above defined R¹ groups.

4. The compound as claimed in any one of Claims 1 to 3 having the formula

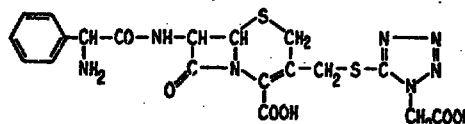




5. A compound as claimed in Claim 1 or 2 having the D configuration in the 7-sidechain and where R^1 is of the formula

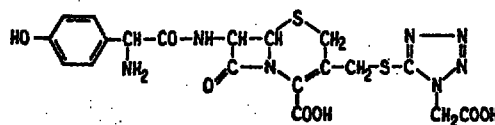


6. The compound of claim 5 having the D configuration in the 7-sidechain and the formula



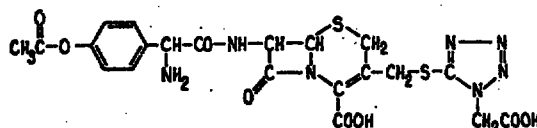
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7. The compound of claim 5 having the D configuration in the 7-sidechain and the formula



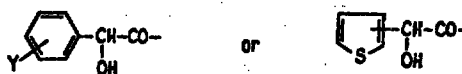
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8. The compound of claim 5 having the D configuration in the 7-sidechain and the formula



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9. A compound as claimed in claim 1 or 2, having the D configuration in the 7-sidechain, wherein R^1 is of the formula

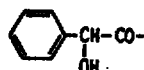


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in which Y is hydrogen, chlorine, bromine, fluorine, trifluoromethyl, amino, nitro, hydroxy, lower alkyl of 1-4 carbon atoms or lower alkoxy of 1-4 carbon atoms.

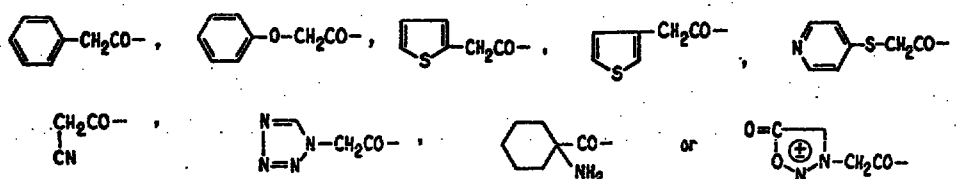
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10. A compound as claimed in claim 9, having the D configuration in the 7-sidechain, wherein R^1 has the structure

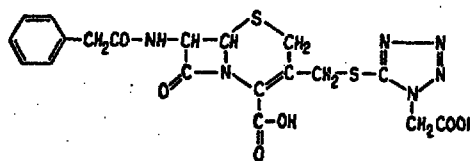


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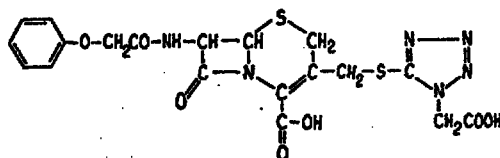
11. A compound as claimed in Claim 1 or 2, wherein R^1 has the structure



12. The compound of claim 11 having the formula



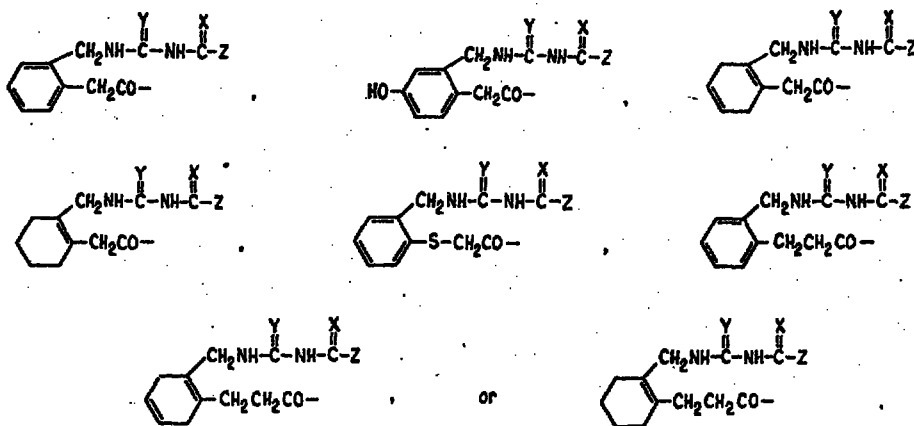
13. The compound of claim 11 having the formula



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14. A compound as claimed in claim 1 or 2, wherein R¹ has the structure

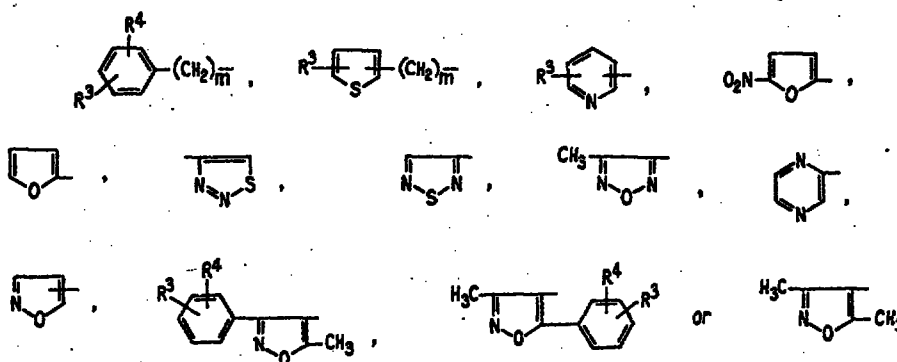
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in which X represents oxygen or imino, Y represents oxygen or sulfur and Z represents amino, (lower)alkyl, cycloalkyl having 4, 5, 6 or 7 carbon atoms, monohalo(lower)alkyl, dichloromethyl, trichloromethyl, (lower)alkenyl of 2 to 6 carbon atoms,

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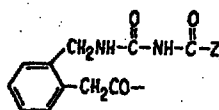
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m is an integer from 0 to 3 inclusive and each of R³ and R⁴ is hydrogen, nitro, di-(lower)alkylamino, (lower)alkanoylamino, (lower)alkanoyloxy, (lower)alkyl, (lower)-alkoxy, sulfamyl, chloro, iodo, bromo, fluoro or trifluoromethyl.

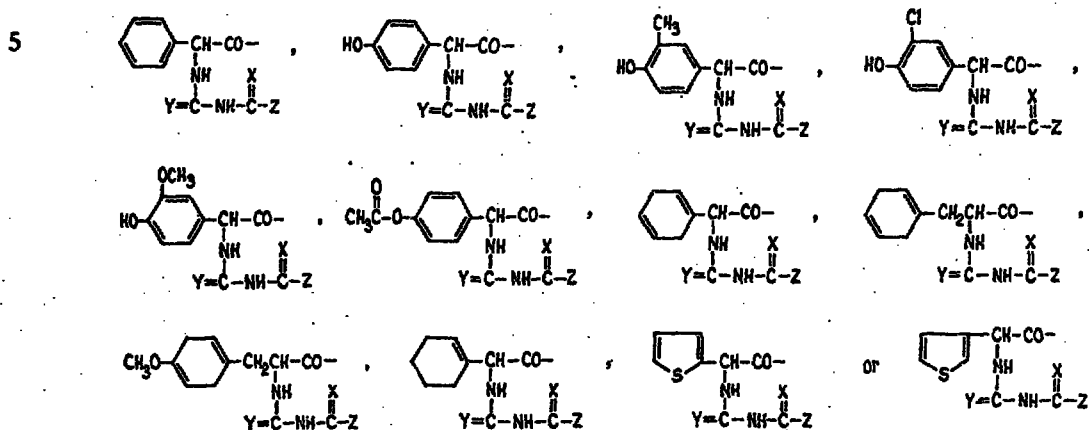
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15. A compound as claimed in claim 14, wherein Z is phenyl, 2-thienyl, 3-thienyl, 2-furyl or 5-nitro-2-furyl.

16. A compound as claimed in claim 14 or 15, wherein R^1 has the structure



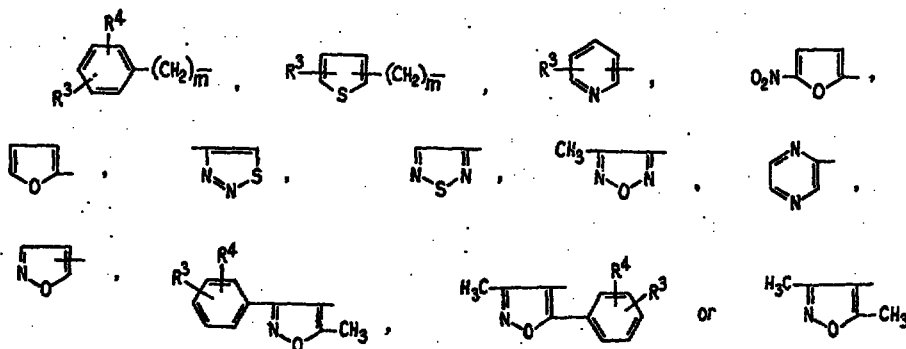
17. A compound as claimed in claim 1 or 2, having the D configuration in the 7-sidechain, wherein R^1 has the structure



in which X represents oxygen or imino, Y represents oxygen or sulfur and Z represents amino, (lower)alkyl, cycloalkyl having 4, 5, 6 or 7 carbon atoms, monohalo(lower)-alkyl, dichloromethyl, trichloromethyl, (lower)alkenyl of 2 to 6 carbon atoms,

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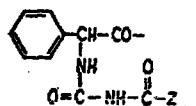
m is an integer from 0 to 3 inclusive and each of R^3 and R^4 is hydrogen, nitro, di(lower)alkylamino, (lower)alkanoylamino, (lower)alkanoyloxy, (lower)alkyl, (lower)alkoxy, sulfamyl, chloro, iodo, bromo, fluoro or trifluoromethyl.

18. A compound as claimed in claim 17, wherein Z is phenyl, 2-thienyl, 3-thienyl, 2-furyl or 5-nitro-2-furyl.

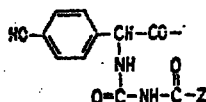
19. A compound as claimed in claim 17 or 18, wherein R^1 has the structure

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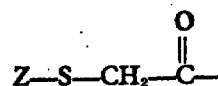


20. A compound as claimed in claim 17 or 18, wherein R^1 has the structure

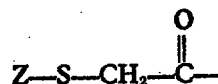




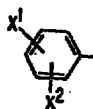
5	4-cyano-1,2,3-triazol-1-ylacetyl, imidazol-(1)-acetyl, m- and p-aminomethylphenylacetyl, α -amino-2,4,6-cycloheptatrienyl-acetyl, lower alkoxyacetyl, hexanoyl, heptanoyl, octanoyl, butylthioacetyl, trifluoromethylthioacetyl, allylthioacetyl, phenoxyacetyl, 2-phenoxy-2-phenylacetyl, 2-phenoxypropionyl, 2-phenoxybutyryl, benzyloxycarbonyl, 2-methyl-2-phenoxypropionyl, p-cresoxyacetyl, p-methylthiophenoxyacetyl, S-phenylthioacetyl, S-chlorophenylthioacet, l, S-fluorophenylthioacetyl, pyridylthioacetyl, S-benzylthioacetyl, p-aminomethylphenylthioacetyl, cyanomethylthioacetyl, 4-pyridylthioacetyl, S-benzylthiopropionyl, benzoyl, aminobenzoyl, 4-isoxazolyl-carbonyl, cyclopentane-carbonyl, sydnone-carbonyl, naphthoyl, 2-ethoxynaphthoyl, quinoxaliny-carbonyl, 3-carboxy-2-quinoxaliny-carbonyl, 2,6-dimethoxybenzoyl, 2-biphenylcarbonyl, 2-methylaminobenzoyl, 2-carboxybenzoyl, 3-phenyl-5-methylisoxazol-4-yl-carbonyl, 3-o-chlorophenyl-5-methyl-isoxazol-4-yl-carbonyl or 3-2',6'-dichlorophenyl-5-methylisoxazol-4-ylcarbonyl.	5 10 15 20 25 30 35 40 45
27.	A compound as claimed in claim 1 or 2, in which R' is	



wherein Z is amino(lower)alkyl, N-(lower)alkylamino(lower)alkyl, or N,N-di(lower)-alkylamino(lower)alkyl or R' is

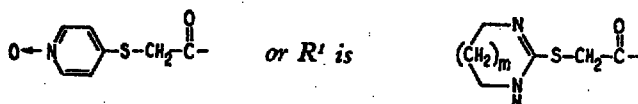
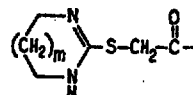


50	wherein Z is aminophenyl, aminophenyl(lower)alkyl, N-(lower)alkylaminophenyl, N,N-di(lower)alkylaminophenyl, N-(lower)alkylaminophenyl(lower)alkyl, N,N-di- (lower)alkylaminophenyl(lower)alkyl, phenylamino(lower)alkyl, phenyl(lower)alkyl- amino(lower)alkyl, substituted phenylamino(lower)alkyl or substituted phenyl(lower)- alkylamino(lower)alkyl, wherein substituted phenyl is defined as a group having the formula	50 55
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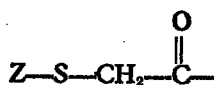
in which X^1 and X^2 are alike or different and are selected from the group consisting of hydrogen, fluoro, chloro, bromo, (lower)alkyl, (lower)alkoxy, nitro, amino, trifluoromethyl and phenyl or R' is

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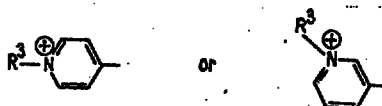
or R' is

5

wherein m is 1 or 2 or R' is



wherein Z is a radical of the formula



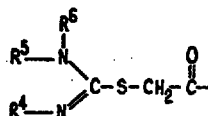
or



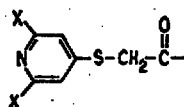
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in which R^3 is (lower)alkyl, (lower)alkenyl or (lower)alkynyl or R' is

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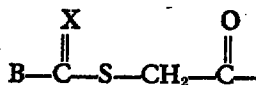
wherein formula R^4 , R^5 and R^6 are each (lower)alkyl, (lower)alkynyl, (lower)alkenyl or (lower)cycloalkyl and R^5 may also be hydrogen; or R' is



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in which X is fluoro or chloro or R' is

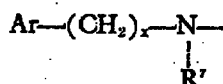
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wherein X is oxygen or sulfur; B represents di(lower)alkylamino wherein the alkyl group may be alike or different, piperidino, methylpiperidino, dimethylpiperidino, pyrrolidino, methylpyrrolidino, dimethylpyrrolidino, morpholino, methylmorpholino, dimethylmorpholino, N' -(lower)alkylpiperazino, N' -(lower)alkyl-methylpiperazino, N' -(lower)alkyl-dimethylpiperazino, trimethyleneimino, hexamethyleneimino or a radical of the formula

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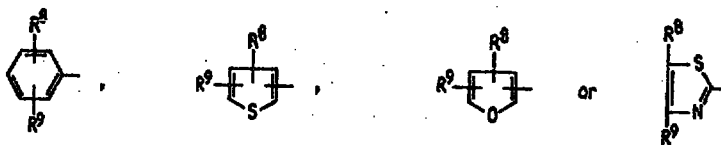
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wherein x is zero, one, two or three, R' is (lower)alkyl and Ar is a radical of the formula

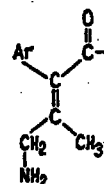
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wherein R^8 and R^9 are each hydrogen, fluoro, chloro, bromo, (lower)alkyl or (lower)-alkoxy.

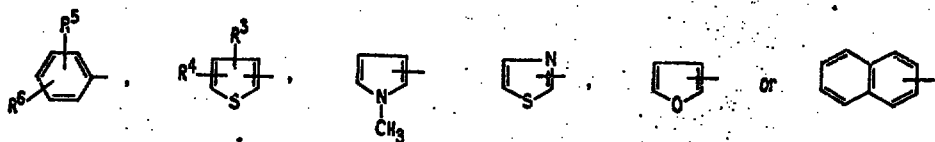
28. A compound as claimed in claim 1 or 2, wherein R^1 has the formula

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wherein Ar is

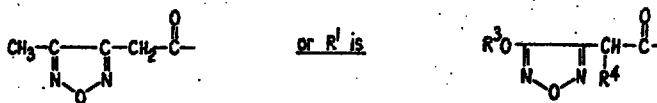


in which R^5 and R^6 are each hydrogen, fluoro, chloro, methoxy or methyl and R^3 and R^4 are each hydrogen, bromo or chloro.

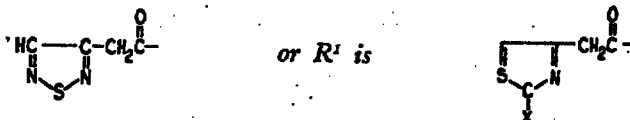
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29. A compound as claimed in claim 1 or 2, in which R^1 is

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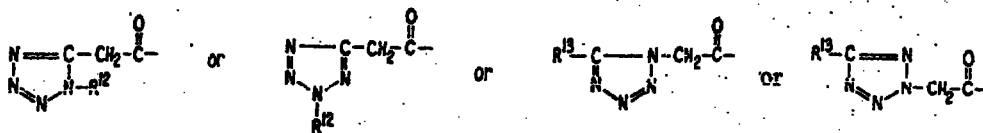
wherein R^3 and R^4 are each a member selected from the group consisting of hydrogen and (lower)alkyl or R^1 is



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wherein X represents a member selected from the group consisting of hydrogen and (lower)alkyl or R^1 is

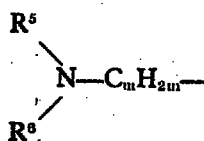
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wherein R^{12} is hydrogen or (lower)alkyl; R^{13} is hydrogen, chloro, bromo, iodo, fluoro, trifluoromethyl, (lower)alkyl, (lower)alkoxy, (lower)alkylthio, carboxymethyl, (lower)-alkylsulfonyl, phenyl, benzyl, phenoxy, benzyloxy, and radicals of the formula

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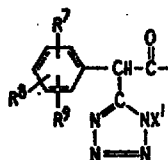


wherein R^5 and R^6 each represent hydrogen, (lower)alkyl, phenyl, benzyl, cycloalkyl

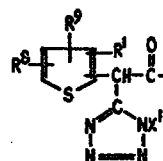


having from three to seven carbon atoms, inclusive, and when taken together with $>N-$, constitute a heterocyclic ring selected from the group consisting of pyrrolidino, piperidino, morpholino, 1,2,5,6-tetrahydropyridino, N-(lower)alkylpiperazino and hexamethyleneimino and m is an integer from 0 to 3 inclusive; or R^1 is

5



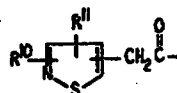
or



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wherein R^7 , R^8 and R^9 each represents a member selected from the group consisting of hydrogen, nitro, di(lower)alkylamino, (lower)alkanoylamino, (lower)alkanoyloxy, (lower)alkyl, (lower)alkoxy, sulfamyl, chloro, iodo, bromo, fluoro and trifluoromethyl; X and X' are alike or different and each represents hydrogen or R^1 is

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wherein R^{10} and R^{11} each represent a member selected from the group consisting of hydrogen, chloro, bromo, iodo and methyl.

30. A compound as claimed in claim 1 or 2, in which R^1 is

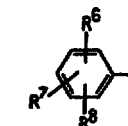
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wherein X is a divalent radical selected from the group consisting of



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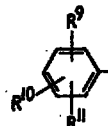
wherein R^3 and R^4 each represent a member selected from the group consisting of (lower)alkyl, chloro, bromo and the radical having the formula



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wherein R^6 , R^7 and R^8 each represent a member selected from the group consisting of hydrogen, fluoro, bromo and chloro, and R^5 represents a member selected from the group consisting of hydrogen, (lower)alkyl and the radical having the formula

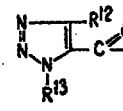
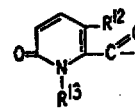
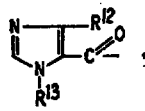
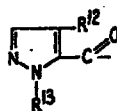
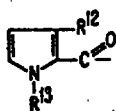
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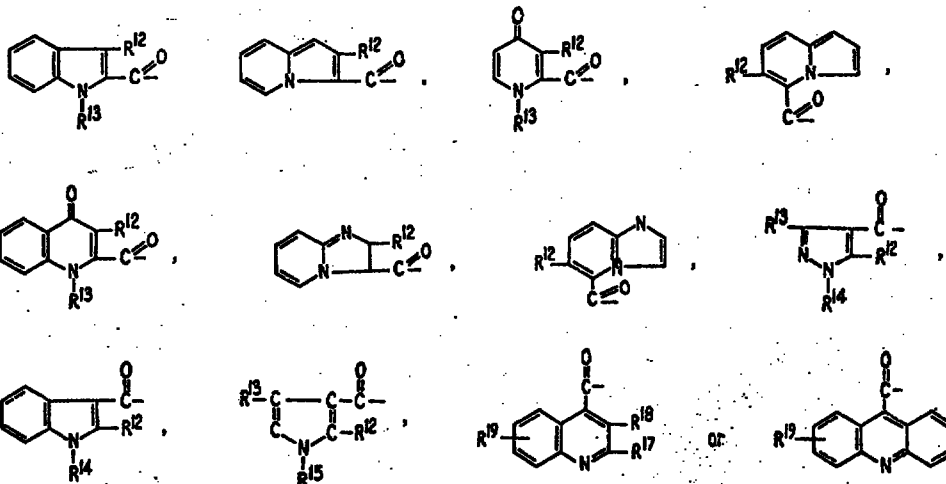


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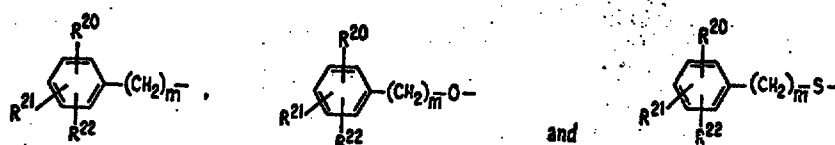
wherein R^9 , R^{10} and R^{11} each represent a member selected from the group consisting of hydrogen, fluoro, bromo and chloro and R^4 and R^5 bonded together to form a benz group, or R^1 is

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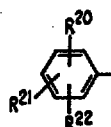




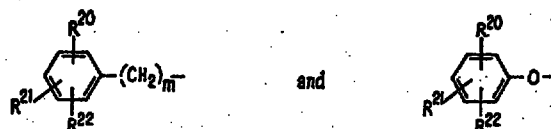
5 wherein R^{12} and R^{13} each represent a member selected from the group consisting of nitro, (lower)alkyl, (lower)alkoxy, (lower)alkanoylamino, chloro, bromo, iodo, fluoro, hydroxy, (lower)alkylthio, cyclohexyl, cyclopentyl, cycloheptyl, (lower)alkoxycarbonyl, mercapto, (lower)alkylsulfonyl, (lower)alkanoyl, (lower)alkanoyloxy and the three radicals having the formulae



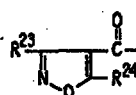
10 wherein m is an integer from zero to three inclusive and R^{20} , R^{21} and R^{22} each represent a member selected from the group consisting of hydrogen, fluoro, bromo, chloro, iodo, trifluoromethyl, (lower)alkyl, (lower)alkoxy, hydroxy, nitro and amino; R^{14} represents a member selected from the group consisting of hydrogen, (lower)alkyl and the radical having the formula



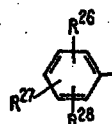
15 wherein R^{20} , R^{21} and R^{22} have the meaning defined above; R^{15} represents a member selected from the group consisting of hydrogen and (lower)alkyl; R^{16} represents a member selected from the group consisting of hydrogen and R^{12} as defined above; R^{17} and R^{18} each represent a member selected from the group consisting of (lower)alkyl, (lower)alkoxy, chloro, bromo, iodo, fluoro, cyclohexyl, cyclopentyl, cycloheptyl and the two radicals having the formulae



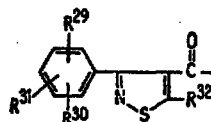
20 wherein m is an integer from zero to three inclusive and R^{20} , R^{21} and R^{22} have the meaning defined above; and R^{19} represents a member selected from the group consisting of hydrogen and R^{17} as defined above; or R^{19} is



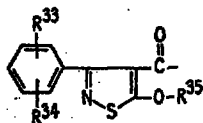
wherein R^{23} and R^{24} each represent a member selected from the group consisting of (lower)alkyl, (lower)alkylthio, benzylthio, cyclohexyl, cyclopentyl, cycloheptyl, benzyl, styryl, phenylethyl, phenylpropyl, furyl, thienyl, naphthyl and a member selected from the group consisting of a radical having the formula



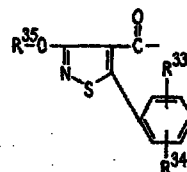
wherein R^{26} , R^{27} and R^{28} each represent a member selected from the group consisting of hydrogen, fluoro, chloro, bromo, iodo, trifluoromethyl, (lower)alkyl, (lower)alkoxy, nitro, methylsulfonyl, cyano, di(lower)alkylamino and methylmercapto; or R^1 is



wherein R^{29} , R^{30} and R^{31} represent hydrogen, chloro, bromo, iodo, trifluoromethyl, fluoro, methylsulfonyl, nitro, (lower)alkyl or (lower)alkoxy, and R^{32} represents (lower)-alkyl or R^1 is

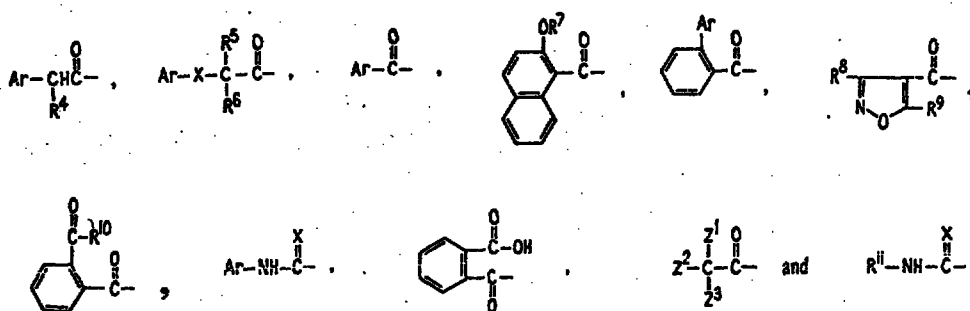


or



wherein R^{33} and R^{34} are each hydrogen, chloro, bromo, iodo, (lower)alkyl, (lower)-alkoxy or trifluoromethyl and R^{35} represents (lower) alkyl.

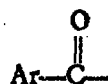
31. A compound as claimed in claim 1 or 2, wherein R^1 has the formula



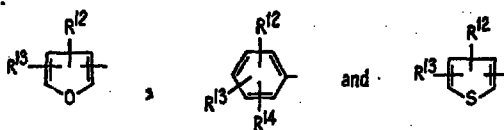
wherein R^4 represents a member selected from the group consisting of hydrogen, amino, carbobenzoxyamino, phenyl, fluoro, chloro, bromo, iodo, hydroxy, (lower)-alkanoyloxy and (lower)alkoxy; X represents a member selected from the group consisting of oxygen and sulfur; R^5 and R^6 each represent a member selected from the group consisting of hydrogen, phenyl, benzyl, phenethyl and (lower)alkyl; R^7 represents (lower)alkyl; R^8 and R^9 each represent a member selected from the group consisting of (lower)alkyl, (lower)alkylthio, benzylthio, cyclohexyl, cyclopentyl, cycloheptyl, benzyl, phenethyl, phenylpropyl, furyl, thienyl, naphthyl and Ar—; R^{10} represents a member selected from the group consisting of (lower)alkylamino, di(lower)alkylamino, cycloalkylamino having from 3 to 7 carbon atoms inclusive, allyl-



amino, diallylamino, phenyl(lower)alkylamino, morpholino, (lower)alkylamino, pyrrolidino, (lower)alkylpyrrolidino, di(lower)alkylpyrrolidino, N,N-hexamethyleneimino, piperidino, (lower)alkylpiperidino, di(lower)alkylpiperidino, 1,2,5,6-tetrahydropyridino, N-(lower)alkylpiperazino, N-(lower)alkylpiperazino, N-phenylpiperazino, N-(lower)alkyl(lower)alkylpiperazino, N-(lower)alkyl-di-(lower)alkylpiperazino, furfurylamino, tetrahydrofurfurylamino, N-(lower)alkyl-N-furfurylamino, N-alkyl-N-anilino and (lower)alkoxyanilino; Z¹, Z² and Z³ each represent a member selected from the group consisting of (lower)alkyl, and Ar—; R¹¹ represents a member selected from the group consisting of (lower)alkyl, (lower)-cycloalkyl, naphthyl, benzyl, phenethyl and

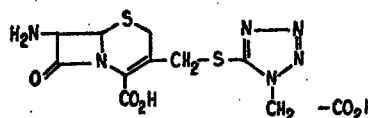


and Ar represent a monovalent radical having one of the formulae

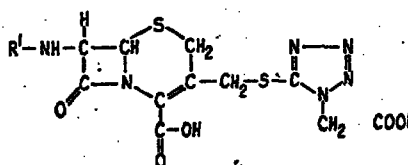


wherein R¹², R¹³ and R¹⁴ are each a member selected from the group consisting of hydrogen, chloro, bromo, iodo, trifluoromethyl, phenyl, (lower)-alkyl and (lower)-alkoxy, but only one R group may represent phenyl.

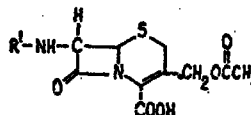
33. A compound of the formula



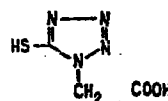
34. A process for the preparation of a compound of the formula



wherein R¹ is acyl or an ester or a non-toxic pharmaceutically acceptable salt thereof characterized by reacting a compound of the formula



in which R¹ is H or acyl or a salt or easily hydrolyzable ester thereof with a compound of the formula



and, when R¹ is H, treating the resulting compound with an acylating agent of the formula

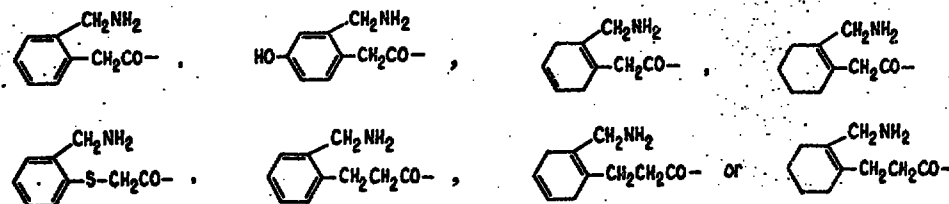




in which X is halide or a functional equivalent thereof and R¹ is acyl, which when the acyl group contains free amino or hydroxyl, said groups are blocked by conventional protective groups and subsequently removed to afford a compound of the Formula I, and, if desired, converting in a resulting free acid, salt or easily hydrolyzable ester of a compound of the Formula I said resulting compound to the corresponding ester or non-toxic pharmaceutically acceptable salt thereof, and, if desired, converting in 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.

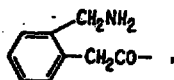
35. The process according to claim 34, wherein a resulting free acid of the formula I is converted to an ester selected from the group consisting of the pivaloyloxymethyl-, acetoxymethyl-, methoxymethyl-, acetonyl-, phenacyl-, *p*-nitrobenzyl-, β,β,β -trichloroethyl-, 3-phthalidyl- or 5-indanyl-oxy.

36. The process according to claims 34 or 35, wherein R¹ is of the formula

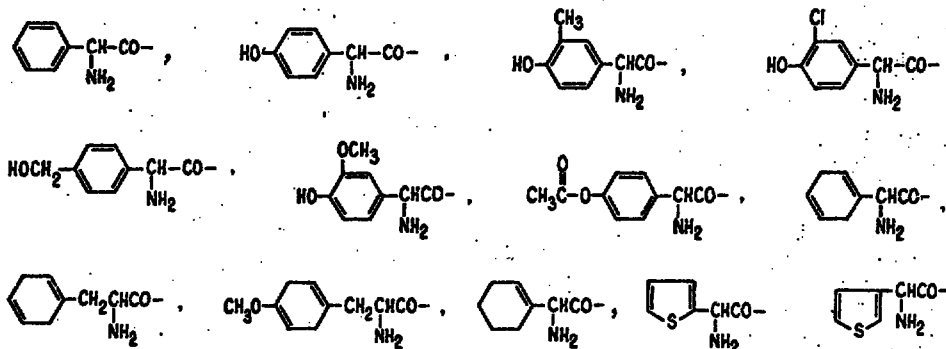


and, if desired, converting the resulting compound to the corresponding benzaldehyde or salicylaldehyde Schiff base.

37. The process according to any of claims 34—36, wherein R¹ is



38. The process according to claim 34 or 35, wherein R¹ has the D-(—) configuration and is of the formula

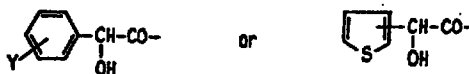


39. The process according to claim 38, wherein R² is D-(—)- α -aminophenylacetyl.

40. The process according to claim 38, wherein R¹ is D-(—)- α -amino-*p*-hydroxyphenylacetyl.

41. The process of claim 38, wherein R¹ is D-(—)- α -amino-*p*-acetoxyphenylacetyl.

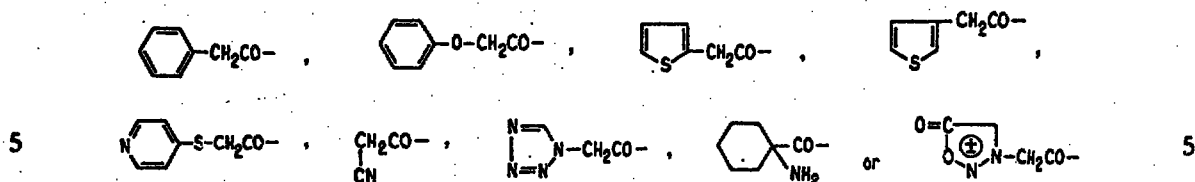
42. The process according to claim 34 or 35, wherein R¹ has the D-(—) configuration and is of the formulae



in which 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.

43. The process according to claim 42, wherein R¹ is D-(-)- α -hydroxyphenylacetyl.

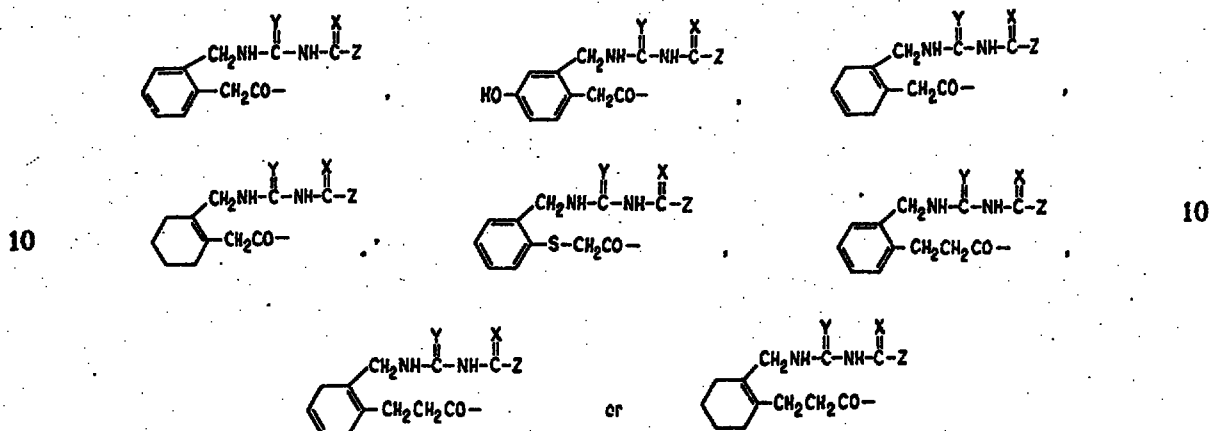
44. The process according to claims 34 or 35, wherein R¹ has the structure



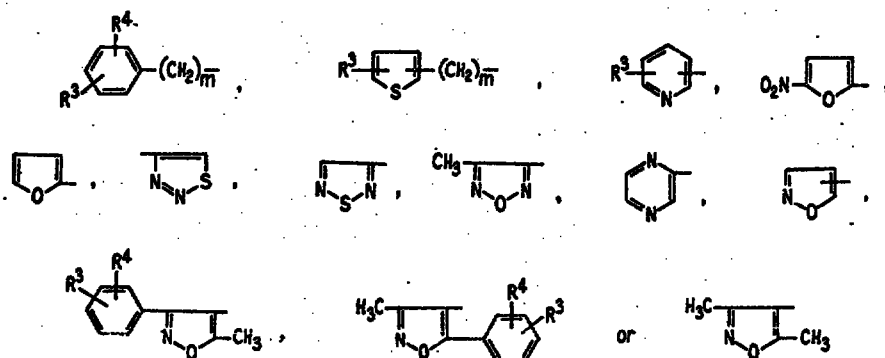
45. The process of claim 44, wherein R¹ is phenylacetyl.

46. The process of claim 44, wherein R¹ is phenoxyacetyl.

47. The process of claim 34 or 35, wherein R¹ has the structure



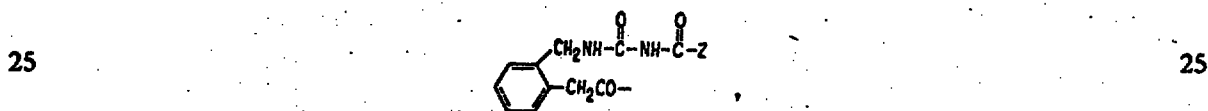
in which X represents oxygen or amino, Y represents oxygen or sulfur and Z represents amino, (lower)alkyl, cycloalkyl having 4, 5, 6 or 7 carbon atoms, mono-halo(lower)alkyl, dichloromethyl, trichloromethyl, (lower)alkenyl of 2 to 6 carbon atoms.



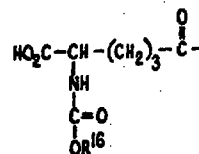
20 in is an integer from 0 to 5 inclusive and each of R and R' is hydrogen, halo, di(lower)alkylamino, (lower)alkanoylamino, (lower)alkanoyloxy, (lower)alkyl, (lower)alkoxy, sulfamyl, chloro, iodo, bromo, fluoro or trifluoromethyl. 20

48. The process according to claim 47, wherein Z is phenyl, 2-thienyl, 3-thienyl, 2-furyl or 5-nitro-2-furyl.

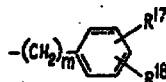
49. The process according to claim 47 or 48, wherein R¹ has the structure



54. The process as claimed in claim 34 or 35, wherein R¹ has the formula

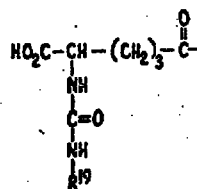


wherein R¹⁶ is (lower)alkyl or an aralkyl group of the formula

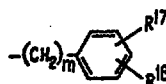


5 wherein m is an integer of 1 to 6 and R¹⁷ and R¹⁸ are alike or different and each is H, Cl, Br, F, NO₂, (lower)alkyl or (lower)alkoxy.

55. The process as claimed in claim 34 or 35, wherein R¹ has the formula

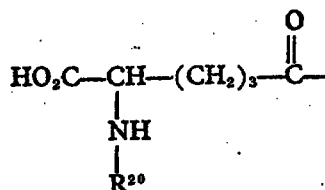


wherein R¹⁹ is (lower)alkyl or a group of the formula

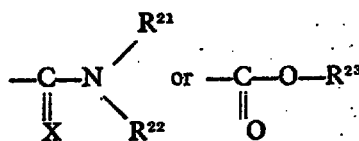


wherein m is an integer of 0 to 6 and R¹⁷ and R¹⁸ are alike or different and each is H, Cl, Br, F, NO₂, (lower)alkyl or (lower)alkoxy.

56. The process as claimed in claim 34 or 35, wherein R¹ has the formula



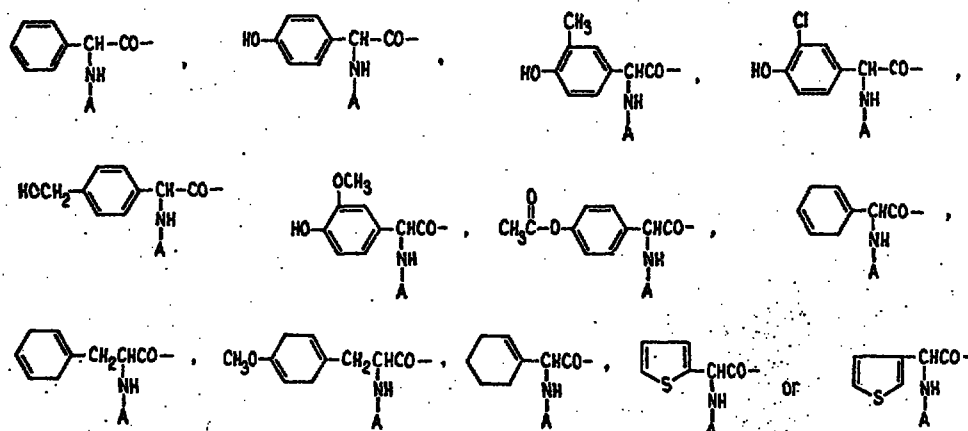
wherein R²⁰ is an α -halo or α,α -dihalo C₂—C₄ alkanoyl or R²⁰ is N-isobornyloxy-carbonyl or R²⁰ is the group of the formula



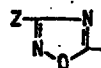
in which R²¹ and R²² are each hydrogen, lower alkyl, phenyl, halophenyl, tolyl, lower alkoxyphenyl, or naphthyl, X is oxygen or sulfur, and R²³ is lower alkyl or R²⁰ is hydrogen.



57. The process as claimed in claim 34 or 35, wherein R^1 has the D configuration at the alpha carbon atom and has the formula

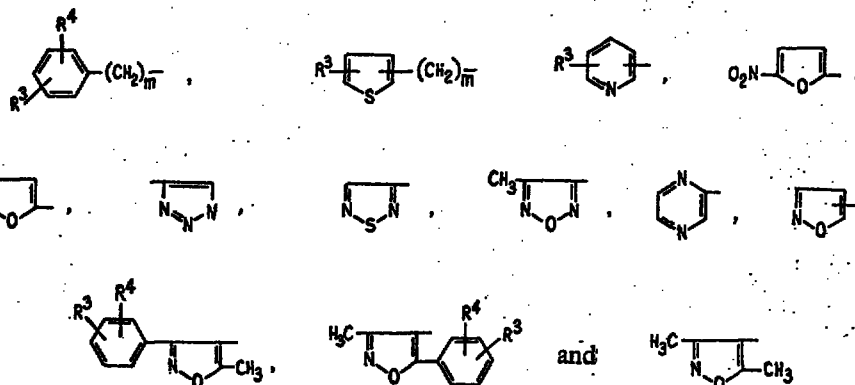


5 wherein A represents



in which Z represents (lower)alkyl, cycloalkyl having four, five, six or seven carbon atoms, monohalo(lower)alkyl, dichloromethyl, trichloromethyl, (lower)alkenyl of two to six carbon atoms,

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m is an integer from 0 to 3 inclusive and each of R^3 and R^4 is hydrogen, nitro, di(lower)alkylamine, (lower)alkanoylamino, (lower)alkanoyloxy, (lower)-alkyl (comprising straight and branched chain saturated aliphatic groups having from one to six carbon atoms inclusive), (lower)-alkoxy, sulfamyl, chloro, iodo, bromo, fluoro or trifluoromethyl and n is an integer of 1 to 4 inclusive.

15

20

58. The process as claimed in claim 34 or 35, wherein R^1 represents phenylacetyl,

20

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o-, m- and p-fluorophenylacetyl,
o-, m- and p-nitrophenylacetyl,
o-, m- and p-guanidino-phenylacetyl,
o-, m- and p-acetoxyphenylacetyl,
o-, m- and p-methoxyphenylacetyl,
o-, m- and p-methylphenylacetyl,
o-, m- and p-hydroxyphenylacetyl,
N,N'-bis-(2-chloroethyl)aminophenylpropionyl,
thien-2-acetyl,
thien-3-acetyl,
4-isoxazolylacetyl,
1-cyclohexenylacetyl,

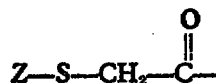
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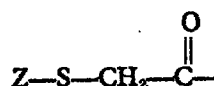
	1,4-cyclohexadienylacetyl,	
	pyridylacetyl,	
	sydnoneacetyl,	
5	3-o-chlorophenyl-5-methyl-isoxazol-4-yl-acetyl,	5
	cyanoacetyl,	
	3,5,7-triaza-1-azonia-1-adamantyl)acetyl,	
	m-aminopyridiniumacetyl,	
	o-, m- and p-(2'-aminoethoxy)phenylacetyl,	
10	4,5-dimethoxycarbonyl-1,2,3-triazol-1-ylacetyl,	10
	4-cyano-1,2,3-triazol-1-ylacetyl,	
	imidazol-(1)-acetyl,	
	m- and p-aminomethylphenylacetyl,	
	α -amino-2,4,6-cycloheptatrienyl-acetyl,	
15	lower alkoxycarbonylacetyl,	15
	cyanoacetyl,	
	hexanoyl,	
	heptanoyl,	
	octanoyl,	
20	butylthioacetyl,	20
	trifluoromethylthioacetyl,	
	allylthioacetyl,	
	phenoxyacetyl,	
	2-phenoxy-2-phenylacetyl,	
25	2-phenoxypropionyl,	25
	2-phenoxybutyryl,	
	benzyloxycarbonyl,	
	2-methyl-2-phenoxypropionyl,	
	p-cresoxyacetyl,	
30	p-methylthiophenoxyacetyl,	30
	S-phenylthioacetyl,	
	S-chlorophenylthioacetyl,	
	S-fluorophenylthioacetyl,	
	pyridylthioacetyl,	
	S-benzylthioacetyl,	
35	p-aminomethylphenylthioacetyl,	35
	cyanomethylthioacetyl,	
	4-pyridylthioacetyl,	
	S-benzylthiopropionyl,	
	benzoyl,	
40	aminobenzoyl,	40
	4-isoxazolyl-carbonyl,	
	cyclopentane-carbonyl,	
	sydnone-carbonyl,	
45	naphthoyl,	45
	2-ethoxynaphthoyl,	
	quinoxaliny-carbonyl,	
	3-carboxy-2-quinoxaliny-carbonyl,	
	2,6-dimethoxybenzoyl,	
50	2-biphenylcarbonyl,	50
	2-methylaminobenzoyl,	
	2-carboxybenzoyl,	
	3-phenyl-5-methylisoxazol-4-yl-carbonyl,	
	3-o-chlorophenyl-5-methyl-isoxazol-4-yl-carbonyl or	
55	3-2',6'-dichlorophenyl-5-methylisoxazol-4-ylcarbonyl.	55
	59. The process as claimed in claim 34 or 35, in which R ¹ is	



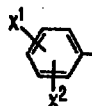
wherein Z is amino(lower)alkyl, N-(lower)alkylamino(lower)alkyl, or N,N-di(lower)-alkylamino(lower)alkyl or R¹ is



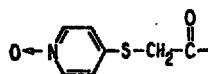
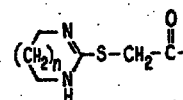
* C L M S *



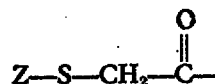
wherein Z is aminophenyl, aminophenyl(lower)alkyl, N-(lower)alkylaminophenyl, N,N-di(lower)alkylaminophenyl, N-(lower)alkylaminophenyl(lower)alkyl, N,N-di(lower)alkylaminophenyl(lower)alkyl, phenylamino(lower)alkyl, phenyl(lower)alkyl-amino(lower)alkyl, substituted phenylamino(lower)alkyl or substituted phenyl(lower)-alkylamino(lower)alkyl, wherein substituted phenyl is defined as a group having the formula



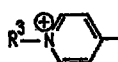
in which X¹ and X² are alike or different and are selected from the group consisting of hydrogen, fluoro, chloro, bromo (lower)alkyl, (lower)alkoxy, nitro, amino, trifluoromethyl and phenyl or R¹ is

or R¹ is

wherein m is 1 or 2 or R¹ is



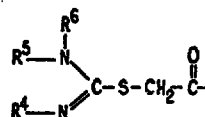
wherein Z is a radical of the formula



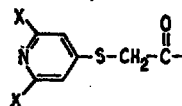
or



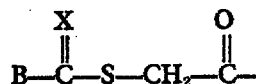
in which R³ is (lower)alkyl, (lower)alkenyl or (lower)alkynyl or R¹ is



wherein formula R⁴, R⁵ and R⁶ are each (lower)alkyl, (lower)alkynyl, (lower)alkenyl or (lower)cycloalkyl and R⁵ may also be hydrogen; or R¹ is

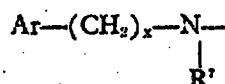


in which X is fluoro or chloro or R¹ is

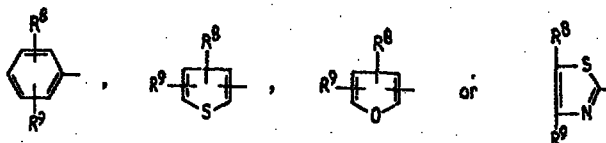


wherein X is oxygen or sulfur;

B represents di(lower)alkylamino wherein the alkyl group may be alike or different, piperidino, methylpiperidino, dimethylpiperidino, pyrrolidino, methylpyrrolidino, dimethylpyrrolidino, morpholino, methylmorpholino, dimethylmorpholino, N'-(lower)-alkylpiperazino, N'-(lower)alkyl-methylpiperazino, N'-(lower)alkyl-dimethylpiperazino, trimethyleneimino, hexamethyleneimino or a radical of the formula



wherein x is zero, one, two or three, R' is (lower)alkyl and Ar is a radical of the formula

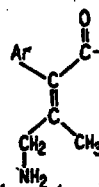


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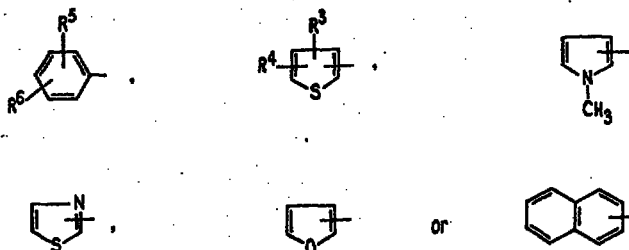
wherein R^8 and R^9 are each hydrogen, fluoro, chloro, bromo, (lower)alkyl or (lower)-alkoxy.

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60. The process as claimed in claim 34 or 35, wherein R^1 has the formula



wherein Ar is



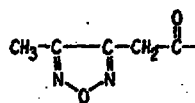
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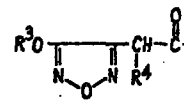
in which R^5 and R^6 are each hydrogen, fluoro, chloro, methoxy or methyl and R^3 and R^4 are each hydrogen, bromo or chloro.

61. The process as claimed in claim 34 or 35, in which R^1 is

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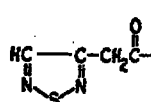


or R^1 is

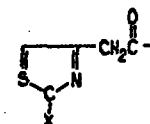


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wherein R^3 and R^4 are each a member selected from the group consisting of hydrogen and (lower)alkyl or R^1 is



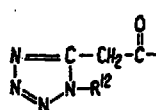
or R^1 is



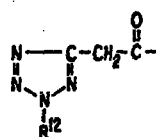
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wherein X represents a member selected from the group consisting of hydrogen and (lower)alkyl or R^1 is

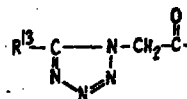
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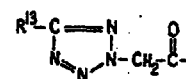
or



or



or





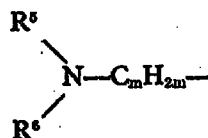
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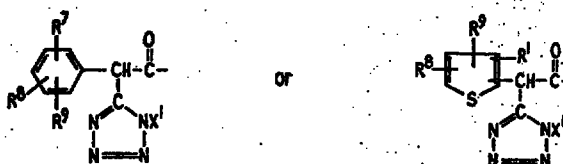
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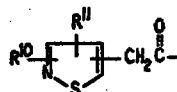
wherein R^{12} is hydrogen or (lower)alkyl; R^{13} is hydrogen, chloro, bromo, iodo, fluoro, trifluoromethyl, (lower)alkyl, (lower)alkoxy, (lower)alkylthio, carboxymethoxy, (lower)alkylsulfonyl, phenyl, benzyl, phenoxy, benzyloxy, and radicals of the formula



wherein R^5 and R^6 each represent hydrogen, (lower)alkyl, phenyl, benzyl, cycloalkyl having from three to seven carbon atoms, inclusive, and when taken together with $>N-$, constitute a heterocyclic ring selected from the group consisting of pyrrolidino, piperidino, morpholino, 1,2,5,6-tetrahydropyridino, N-(lower)alkylpiperazino and hexamethyleneimino and m is an integer from 0 to 3 inclusive; or R^1 is

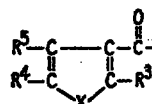


wherein R^7 , R^8 and R^9 each represents a member selected from the group consisting of hydrogen, nitro, di(lower)alkylamino, (lower)alkanoylamino, (lower)alkanoyloxy, (lower)alkyl, (lower)alkoxy, sulfamyl, chloro, iodo, bromo, fluoro and trifluoromethyl; X and X' are alike or different and each represents hydrogen or R^1 is



wherein R^{10} and R^{11} each represent a member selected from the group consisting of hydrogen, chloro, bromo, iodo and methyl.

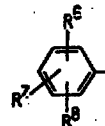
62. The process as claimed in claim 34 or 35, in which R^1 is



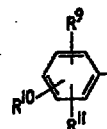
wherein X is a divalent radical selected from the group consisting of



wherein R^3 and R^4 each represent a member selected from the group consisting of (lower)alkyl, chloro, bromo and the radical having the formula



wherein R^6 , R^7 and R^8 each represent a member selected from the group consisting of hydrogen, fluoro, bromo and chloro, and R^5 represents a member selected from the group consisting of hydrogen, (lower)alkyl and the radical having the formula





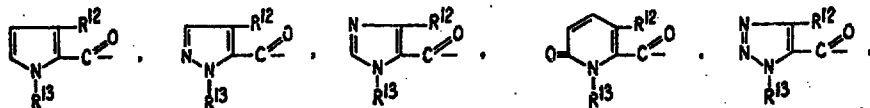
* C L M S *

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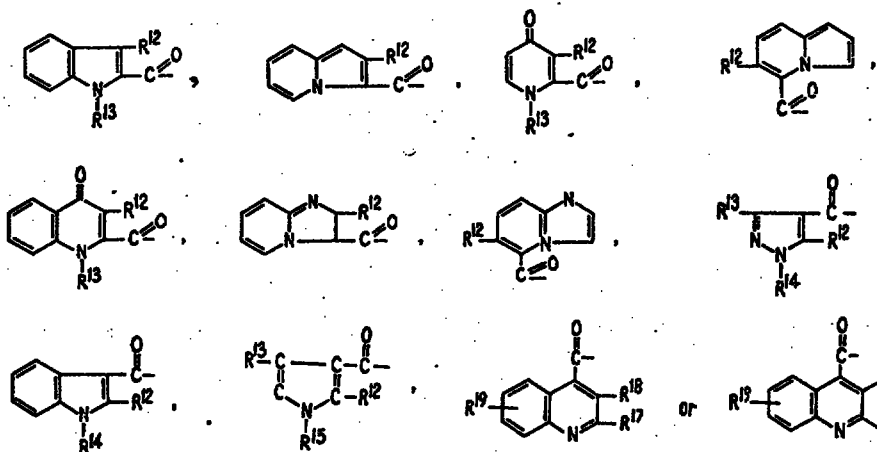
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wherein R^9 , R^{10} and R^{11} each represent a member selected from the group consisting of hydrogen, fluoro, bromo and chloro and, R^4 and R^5 bonded together to form a benz group, or R^1 is



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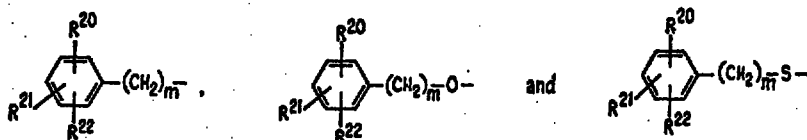


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wherein R^{12} and R^{13} each represent a member selected from the group consisting of nitro, (lower)alkyl, (lower)alkoxy, (lower)alkanoylamino, chloro, bromo, iodo, fluoro, hydroxy, (lower)alkylthio, cyclohexyl, cyclopentyl, cycloheptyl (lower)alkoxycarbonyl, mercapto, (lower)alkylsulfonyl, (lower)alkanoyl, (lower)alkanoyloxy and the three radicals having the formulae

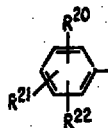
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wherein m is an integer from zero to three inclusive and R^{20} , R^{21} and R^{22} each represent a member selected from the group consisting of hydrogen, fluoro, bromo, chloro, iodo, trifluoromethyl, (lower)alkyl, (lower)alkoxy, hydroxy, nitro and amino; R^{14} represents a member selected from the group consisting of hydrogen, (lower)alkyl and the radical having the formula

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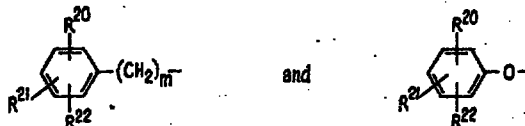


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wherein R^{20} , R^{21} and R^{22} have the meaning defined above; R^{15} represents a member selected from the group consisting of hydrogen and (lower)alkyl; R^{16} represents a member selected from the group consisting of hydrogen and R^{12} as defined above; R^{17} and R^{18} each represent a member selected from the group consisting of (lower)alkyl, (lower)alkoxy, chloro, bromo, iodo, fluoro, cyclohexyl, cyclopentyl, cycloheptyl and the two radicals having the formulae

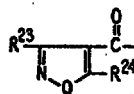
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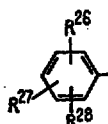
wherein m is an integer from zero to three inclusive and R^{20} , R^{21} and R^{22} have the meaning defined above; and R^{19} represents a member selected from the group consisting of hydrogen and R^{17} as defined above; or R^1 is



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wherein R^{23} and R^{24} each represent a member selected from the group consisting of (lower)alkyl, (lower)alkylthio, benzylthio, cyclohexyl, cyclopentyl, cycloheptyl, benzyl, styryl, phenylethyl, phenylpropyl, furyl, thienyl, naphthyl and a member selected from the group consisting of a radical having the formula

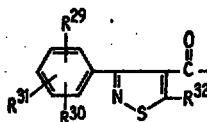
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wherein R^{26} , R^{27} and R^{28} each represent a member selected from the group consisting of hydrogen, fluoro, chloro, bromo, iodo, trifluoromethyl, (lower)alkyl, (lower)alkoxy, nitro, methylsulfonyl, cyano, di(lower)alkylamino and methylmercapto; or R^1 is

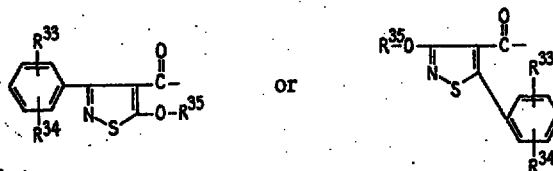
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wherein R^{29} , R^{30} and R^{31} represent hydrogen, chloro, bromo, iodo, trifluoromethyl, fluoro, methylsulfonyl, nitro, (lower)alkyl or (lower)alkoxy, and R^{32} represents (lower)alkyl or R^1 is

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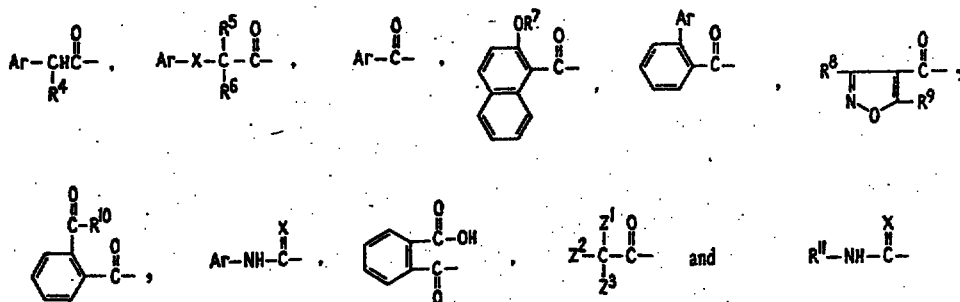


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wherein R^{33} and R^{34} are each hydrogen, chloro, bromo, iodo, (lower)alkyl, (lower)alkoxy or trifluoromethyl and R^{35} represents (lower)alkyl.

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63. The process as claimed in claim 34 or 35, wherein R^1 has the formula



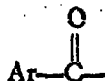
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wherein R^4 represents a member selected from the group consisting of hydrogen, amino, carbobenzoxyamino, phenyl, fluoro, chloro, bromo, iodo, hydroxy, (lower)alkoxy and (lower)alkoxy; X represents a member selected from the group consisting of oxygen and sulfur; R^5 and R^6 each represent a member selected from the group consisting of hydrogen, phenyl, benzyl, phenethyl and (lower)alkyl; R^7 represents (lower)alkyl; R^8 and R^9 each represent a member selected from the group consisting of (lower)alkyl, (lower)alkylthio, benzylthio, cyclohexyl, cyclopentyl, cyclo-

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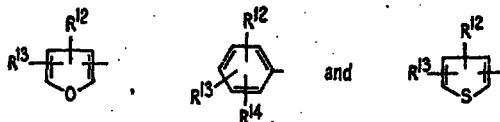
heptyl, benzyl, phenethyl, phenylpropyl, furyl, thienyl, naphthyl and Ar—; R¹⁰ represents a member selected from the group consisting of (lower)alkylamino, di-(lower)alkylamino, cycloalkylamino having from 3 to 7 carbon atoms inclusive, allylamino, diallylamino, phenyl(lower)alkylamino, morpholino, (lower)alkylamino, pyrrolidino, (lower)alkylpyrrolidino, di(lower)alkylpyrrolidino, N,N-hexamethyleneimino, piperidino, (lower)alkylpiperidino, di(lower)alkylpiperidino, 1,2,5,6-tetrahydropyridino, N-(lower)alkylpiperazino, N-(lower)alkylpiperazino, N-phenylpiperazino, N-(lower)alkyl(lower)alkylpiperazino, N-(lower)alkyl-di(lower)alkylpiperazino, furfurylamino, tetrahydrofurfurylamino, N-(lower)alkyl-N-furfurylamino, N-alkyl-N-anilino and (lower)alkoxyanilino; Z¹, Z² and Z³ each represent a member selected from the group consisting of (lower)alkyl, and Ar—; R¹¹ represents a member selected from the group consisting of (lower)alkyl, (lower)-cycloalkyl, naphthyl, benzyl, phenethyl and



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and Ar represent a monovalent radical having one of the formulae

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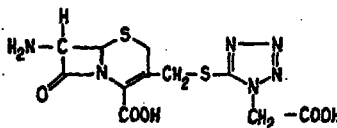


wherein R¹², R¹³ and R¹⁴ are each a member selected from the group consisting of hydrogen, chloro, bromo, iodo, trifluoromethyl, phenyl, (lower)-alkyl and (lower)-alkoxy, but only one R group may represent phenyl.

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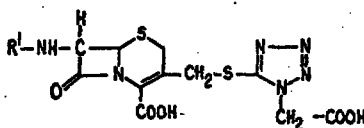
64. The process for the preparation of a compound of the formula

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characterized by the consecutive steps of

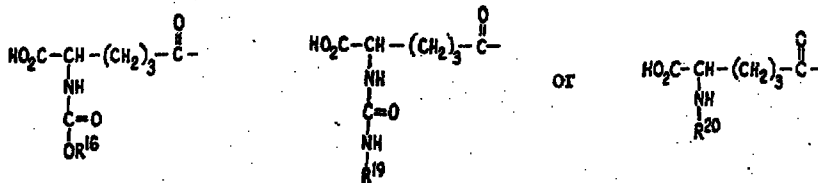
(a) treating a silyl ester of a compound of the formula



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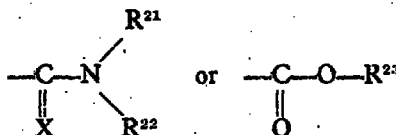
in which R¹ is of the formulae

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wherein R¹⁶ and R¹⁹ are each (lower)alkyl or an aralkyl group having 1 to 6 carbon atoms in the alkyl portion and R²⁰ is hydrogen, an α -halo, or α,α -dihalo C₂—C₄ alkanoyl, N-isobornyloxycarbonyl or a group of the formulae

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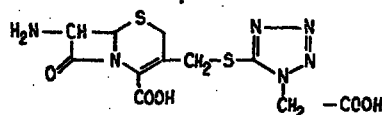
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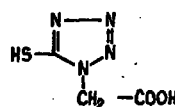
in which R^{21} and R^{22} are each H, (lower)alkyl, phenyl, halophenyl, tolyl, (lower)-alkoxyphenyl, or naphthyl, X is oxygen or sulfur, and R^{23} is (lower)alkyl with PCl_5 to afford the corresponding imino-chloride;

- 5 (b) treating said imino-chloride with methanol; and
(c) hydrolyzing the resulting imino-ether to the desired product.
65. A process for the preparation of a compound of the formula



characterized by reacting 7-amino-cephalosporanic acid or a salt or easily hydrolyzable ester or Schiff base thereof with a compound of the formula

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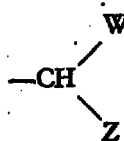
66. A process in accordance with any of claims 34 to 65 substantially as hereinbefore described.

67. A process in accordance with any one of Claims 34 to 65 substantially as hereinbefore described in any one of the Examples.

- 15 68. A compound whenever produced by the process of any one of Claims 34 to 67.
69. A compound according to Claim 1 wherein R_1 is any one of the groups (i) to (xix) as hereinbefore defined.

70. A compound according to Claim 1 which is an ester and wherein the ester has a group of the formula

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as hereinbefore defined.

71. A compound according to Claim 1 which has the D configuration in the 7-sidechain.

- 25 72. A compound according to any one of Claims 1 to 33 and 68 to 71 in unit dosage form.

73. A pharmaceutical composition comprising a compound in accordance with any one of Claims 1 to 33 and 68 to 72 in which R^1 is other than hydrogen, and a physiologically acceptable carrier or excipient.

- 30 74. A pharmaceutical composition in accordance with Claim 73 substantially as hereinbefore described.

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PATENTS ACT, 1949

SPECIFICATION NO 1525626

In pursuance of Section 8 of the Patents Act, 1949, the Specification has been amended in the following manner:—

Page 1, line 9, page 19, line 2, page 51, line 34, page 63, line 21 *after an insert* easily hydrolyzed

THE PATENT OFFICE
23 May 1979

Bas 56286/1