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7 - HETEROMONOCYCLIC-SUBSTITUTED ACYLAMIDO DERIVATIVES OF DESACETYL CEPHALOSPORANIC ACID

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No Drawing. Continuation-in-part of applications Ser. No. 115,612, June 8, 1961, and Ser. No. 220,855, Aug. 31, 1962. This application Oct. 4, 1965, Ser. No. 492,911
The portion of the term of the patent subsequent to Nov. 16, 1982, has been disclaimed
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U.S. Cl. 260—243 2 Claims

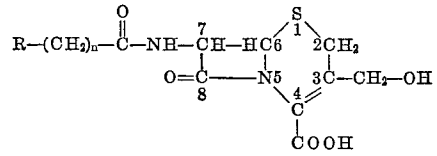
ABSTRACT OF THE DISCLOSURE

Derivatives of 7 - heteromonocyclic - substituted acylamido desacetylcephalosporanic acid are disclosed. These compounds are useful antibacterial agents.

This application is a continuation-in-part of my applications Serial No. 115,612, filed June 8, 1961, now abandoned and Serial No. 220,855 filed Aug. 31, 1962, now U.S. Patent 3,218,318 issued Nov. 16, 1965.

This invention relates to novel organic compounds and to methods for their preparation.

The novel compounds of the present invention are 7-(heteromonocyclic-substituted acylamido) derivatives of desacetylcephalosporanic acid, having the following formula:



and the salts thereof with pharmaceutically acceptable cations, in which:

R is a monocyclic ring having at least one hetero atom of the class consisting of O, S, and N; and
n is zero or 1.

Thus, R can be dioxanyl, 2-furyl, 3-furyl, imidazolyl, isoxazolyl, morpholinyl, oxazolyl, pyranyl, pyrazinyl, pyrazolyl, N-pyridyl 2-pyridyl, 3-pyridyl, pyrimidyl, N-pyrryl, 2-pyrryl, 3-pyrryl, thiazolyl, 2-thienyl, 3-thienyl, triazinyl, triazolyl and the like; the partially and completely hydrogenated derivatives thereof such as tetrahydrofuryl imidazolinyl, imidazolidyl, piperidyl, tetrahydropyrimidyl, pyrrolidyl, and the like; and the derivatives thereof having chlorine, fluorine, bromine, iodine nitro, methoxy, methyl, ethyl, n-propyl, isopropyl, n-butyl, t-butyl, or other C_1–C_8 alkyl substituent at one or more positions on the ring, e.g., the picolyls, the methylfuryls, the methylthienyls, nitrofuryl, nitropyridyl, nitrothienyl, chlorofuryl, bromopyridyl, fluoropyrryl, methoxypyrimidyl, and the like.

The novel compounds of the present invention are related to cephalosporin C insofar as they contain the 2,3-dihydro-6H-1,5-thiazine ring with a fused β-lactam ring in the 5,6 position, which is characteristic of cephalosporin C. However, unlike cephalosporin C, which contains the 5'-amino-N'-adipamyl group in the 7 position, the compounds of the present invention are characterized by a heteromonocylic-substituted acylamido group in the 7 position. Moreover, unlike cephalosporin C, which contains acetoxymethyl in the 3 position, the compounds of the present invention contain hydroxymethyl in the 3 position, and may thus be termed derivatives of "desacetylcephalosporanic acid," a term sometimes shortened to "cephalosporadesic acid."

The compounds of the present invention are highly effective antibacterial agents. They are characterized by penicillinase-resistance, acid stability, and activity against a broad range of microorganisms, including both gram-positive and gram-negative pathogens, and are considerably less subject to serum binding than are the analogous 3-acetoxymethyl compounds. They are conveniently prepared and administered in the form of the salts of the carboxyl group with pharmaceutically acceptable cations, including, for example, water-soluble salts such as the sodium, potassium, lithium, ammonium, and substituted ammonium salts, as well as the less water-soluble salts such as the calcium, barium, procaine, quinine, and dibenzylethylenediamine salts. Administration is preferably by intramuscular injection in isotonic saline at a dose (for adults) around 0.25 to 0.50 g. every four to six hours.

The following examples will illustrate the types of compounds available in accordance with the present invention:

7-β-thienylacetamidocephalosporadesic acid
7-β-thienylcarboxamidocephalosporadesic acid
7-α-furylacetamidocephalosporadesic acid
7-α-furylcarboxamidocephalosporadesic acid
7-(2'-pyranylacetamido)cephalosporadesic acid
7-(2'-morpholinylacetamido)cephalosporadesic acid
7-(3'-bromo-2'-pyridylacetamido)cephalosporadesic acid
7-(5'-methoxy-3'-pyridylacetamido)cephalosporadesic acid
7-α-thienylcarboxamidocephalosporadesic acid
7-(2'-pyrazinylacetamido)cephalosporadesic acid
7-(2'-pyridylacetamido)cephalosporadesic acid
7-(α-picolyl-3'-acetamido)cephalosporadesic acid
7-β-furylcarboxamidocephalosporadesic acid
7-(2'-triazinylacetamido)cephalosporadesic acid
7-(4'(5')-imidazolylacetamido)cephalosporadesic acid
7-(2'-piperidylacetamido)cephalosporadesic acid
7-β-(5'-pyrimidyl)acetamidocephalosporadesic acid
7-β-furylacetamidocephalosporadesic acid
7-(2'-methyl-3'-furylacetamido)cephalosporadesic acid
7-(5'-oxazolylacetamido)cephalosporadesic acid
7-(3'-methyl-2'-thienylacetamido)cephalosporadesic acid
7-(5'-nitro-2'-thienylacetamido)cephalosporadesic acid
7-β-(5'-fluoro-2'-pyridyl)acetamidocephalosporadesic acid
7-(5'-imidazolinylacetamido)cephalosporadesic acid
7-(2'-thiazolylacetamido)cephalosporadesic acid
and the like.

The source material for the compounds of the present invention is cephalosporin C, more precisely known as 7-(5'-amino-adipamido)cephalosporanic acid, which can be prepared by cultivating a cephalosporin C-producing organism in a suitable nutrient medium, as described in British patent specification 810,196, published Mar. 11, 1959.

Cephalosporin C is readily converted into the corresponding nucleus compound, 7-aminocephalosporanic acid, by cleaving the 5'-amino-N'-adipamyl side chain between its amido carbonyl group and its amido nitrogen, suitably by reacting cephalosporin C with nitrosyl chloride in formic acid, then hydrolytically cleaving, according to the method of Morin et al. described in U.S. Patent 3,188,311 (June 8, 1965).

The nucleus thus obtained is conveniently converted into a 7-acylamidocephalosporanic acid as desired by acylation. For this purpose, any of the conventional acylation procedures can be employed, utilizing any of the various types of known acylating agents having the composition which yields the desired side chain. A convenient acylating agent is the appropriate acyl chloride or bromide. The acylation is carried out in water or an appropriate organic solvent, preferably under substantially neutral conditions, and preferably at reduced temperature, i.e.,

above the freezing point of the reaction mixture and up to about 20° C. In a typical procedure, 7-aminocephalosporanic acid is dissolved in water with a sufficient quantity of sodium bicarbonate or other appropriate alkali to promote solution, the concentration of the 7-aminocephalosporanic acid being about 1 to about 4 percent by weight. The solution is cooled to around 0 to 5° C., and a solution of the acylating agent is added in about 20 percent excess, with stirring and cooling. The pH of the mixture can be maintained, if it tends to vary, around the neutral level by bubbling carbon dioxide therein. After addition of the acylating agent has been completed, stirring of the reaction mixture is continued and the mixture is allowed to warm to room temperature. The reaction product is then acidified to around pH 2 and extracted with an organic solvent such as ethyl acetate. The ethyl acetate extract is back-extracted with water at pH 5.5 to 6, employing for pH adjustment a base containing the cation of the desired final product. The water solution is separated and evaporated substantially to dryness. The residue is taken up in a minimum quantity of water and the acylation product is precipitated by adding a large excess of acetone, and if necessary ether. The crystalline product obtained thereby is filtered, washed with acetone, and dried.

The acylation can also be carried out with the corresponding carboxylic acid, employed in conjunction with an equimolar proportion of a carbodiimide such as N,N'-diisopropylcarbodiimide, N,N' - dicyclohexylcarbodiimide, N,N' - bis(p - dimethylaminophenyl)carbodiimide, N-ethyl-N'-(4''-ethylmorpholinyl)carbodiimide, or the like, and the acylation proceeds at ordinary temperatures in such cases. Alternatively, the carboxylic acid can be converted into the corresponding acid anhydride, or into the azide, or into an activated ester, and any of these derivatives can be used to effect the desired acylation. Other agents can readily be ascertained from the art.

Many of the acylating agents, together with methods for their preparation, are described in the literature, and a number of them are commercially available. All of them are readily prepared by methods well known in the art.

In many cases, the acylating agent may contain one or more asymmetric carbon atoms and thus exist in optically active forms. When prepared by ordinary chemical means, such compounds are ordinarily obtained in racemic form—i.e., an equimolar mixture of the optical isomers, having no optical rotation. When the separate optical isomers are desired, the acylating agent can be resolved in a conventional manner such as by reacting the free acid with cinchonine, strychnine, brucine, or the like, then fractionally crystallizing to separate the diasteroisomeric salts, and separately acidifying the solid phase and the liquid phase to liberate the optical isomers. The free acids thus obtained can be employed as such for the acylation, preferably in conjunction with a carbodiimide, or may be converted by conventional means into the corresponding acid halide or into a mixed anhydride, care being exercised to avoid extremes of conditions which might produce racemization.

The 7-acylamidocephalosporanic acids obtained in the foregoing manner are conveniently converted into the desired 7-acylamidocephalosporadesic acids by treatment with citrus acetylesterase for several hours in aqueous phosphate buffer at pH 6.5–7 according to the method of Jansen, Jang, and MacDonnell, Archiv. Biochem., 15 (1947), 415–31.

The invention will be more readily understood from the following operating example, which is submitted only as an illustration, and not by way of limitation.

Example

7-aminocephalosporanic acid (12 g.) was suspended in a mixture of 200 ml. of water and 160 ml. of acetone and cooled in an ice bath. To the suspension were added 7.5 g. of sodium bicarbonate with stirring, as a result of which the solids went into solution. To the cooled solution were added 6.5 g. of α-thienylacetyl chloride dissolved in 40 ml. of acetone over a period of one hour, after which the reaction mixture was stirred in the cold for two additional hours. The reaction product mixture was then stripped of acetone under vacuum, and 200 ml. of ethyl acetate were added, followed by 1 N hydrochloric acid to pH 2. The mixture was filtered and the aqueous phase was separated and discarded. The ethyl acetate phase was stirred with 100 ml. of water and adjusted to pH 6.5 with aqueous 1 N potassium hydroxide solution, 45 ml. being required. The resulting aqueous extract was separated and evaporated to dryness under vacuum. The residue was recrystallized from a mixture of methanol and isopropyl alcohol. The resulting intermediate, 7-*a*-thienylacetamidocephalosporanic acid potassium salt, weighed 9.1 g.

An orange flavedo solution which had been prepared by the method of Jansen, Jang, and MacDonnell, referred to above, was further purified in the following way. To the solution (approximately 340 ml.) were added 17.5 g. of Darco G–60 activated carbon, and the mixture was stirred slowly for 15 minutes in the cold, then centrifuged in the cold at 15,000 r.p.m. The supernatant liquid (285 ml.) was poured off, and to it were slowly added 49.9 g. of ammonium sulfate crystals with stirring, giving a solution 30 percent saturated with ammonium sulfate. The mixture was again centrifuged and the solid was discarded. To the supernatant liquid (305 ml.) were slowly added 60 g. of ammonium sulfate with cooling and stirring, giving a solution 60 percent saturated with ammonium sulfate. The mixture was again centrifuged, and the supernatant liquid was discarded. The solids were dissolved in cold water and brought to a volume of 95 ml. The result was a stable solution of purified citrus acetylesterase.

7 - α-thienylacetamidocephalosporanic acid potassium salt (2.0 g.) was dissolved in water, adjusted to pH 7 with aqueous 1 N sodium hydroxide solution, and diluted to 86 ml. with water. The resulting solution was 0.05 molar in the dissolved compound. The solution was commingled with 21.5 ml. of 0.1 M phosphate buffer at pH 7, then with 43 ml. of citrus acetylesterase which had been prepared and purified as described above and adjusted to pH 7. The solution volume was adjusted to 430 ml. with water, then held in a beaker at 37° C. with slow stirring. The pH was maintained at 7 by dropwise addition of aqueous 1 N sodium hydroxide solution. Exposure to these conditions was continued for a period of 4 hours, this being one hour past the time at which a parallel Warburg control (described below) indicated completion of the reaction.

The Warburg control was run in the following manner. Into the body of a Warburg flask were placed 0.4 ml. of the original solution of 7 - α - thienylacetamidocephalosporanic acid potassium salt and 1.3 ml. of water. Into the side arm was placed 0.2 ml. of the citrus acetylesterase preparation. An aqueous 1 M solution of sodium bicarbonate was saturated with carbon dioxide, and 0.25 ml. was added to the body of the flask, and 0.05 ml. to the side arm. The manometer, having been purged with carbon dioxide, was immediately attached. The gas was allowed to flow for 15 minutes at room tempearture, and was then turned off. The apparatus was allowed to equilibrate 15 minutes in the 37° C. constant-temperature bath, the side arm being open at first to permit venting, then closed. The contents of the side arm were then poured into the body of the flask and rinsed back and forth, thus initiating the desacetylation reaction, as evidenced by the evolution of carbon dioxide. Gas evolution ceased at the end of 3 hours, and it was determined that a total of 377 ml. (corrected) of carbon dioxide had been evolved, corresponding to 16.8 millimoles or 84 percent of theory.

The product solution (approximately 400 ml.) was salted with sodium chloride (40 g.), cooled, overlayered with an equal volume of ethyl acetate, and acidified to pH 2.0 with 1 N hydrochloric acid. The mixture was filtered to break the suspension and the layers were separated. The

organic phase was adjusted in the cold to pH 5.8 with aqueous 1 N potassium hydroxide solution and the layers were separated. The aqueous extract was evaporated to dryness at reduced pressure and mildly elevated temperature. The residue was dissolved in hot methanol, filtered, diluted with isopropyl alcohol, and concentrated. The resulting white solid was filtered off and dried. Yield, 0.95 g. The filtrate was further concentrated and chilled, and a second crop was obtained weighing 200 mg.

The product, 7 - α-thienylacetamidocephalosporadesic acid potassium salt, was found to be effective against penicillin-resistant *Staphylococcus aureus*, having the following minimum inhibitory concentrations, both in the presence and in the absence of human blood serum, against four clinical isolates, as measured by the gradient-plate technique and expressed in micrograms per milliliter:

Strain	V-30	V-32	V-41	V-84
Without serum	0.5	4	0.5	2
With serum	2	2	2	3

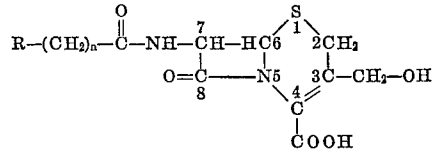
The product was also shown to be effective in varying degrees against a variety of other organisms.

Organism:	Minimum inhibitory concentration, mcg./ml.
Staphylococcus aureus, sensitive	0.4
S. albus	0.4
Bacillus subtilis	0.1
Sarcina lutea	0.4
Proteus vulgaris	3
Aerobacter aerogenes	13
Klebsiella pneumoniae	25
Salmonella enteritidis	25
Shigella paradysenteriae	25

Organism:	Minimum inhibitory concentration, mcg./ml.
Shigella sonnei	62
Escherichia coli N-10	134
E. coli N-26	89

I claim:

1. An antibiotic substance having the following formula:



wherein R is a monocyclic ring having at least one hetero atom of the class consisting of O, S, and N selected from the group consisting of β-thienyl, α-thienyl, α-furyl, 2-pyranyl, 2'-morpholinyl, 3'-bromo-2'-pyridyl, 5'-methoxy-3-pyridyl, 2'-pyrazinyl, 2'-pyridyl, α-picolyl, β-furyl, 2'-triazinyl, 4'-(5')-imidazolyl, 2'-piperidyl, 5'-pyrimidyl, 2'-methyl-3'-furyl, 5'-oxazolyl, 3'-methyl-2-thienyl, 5'-nitro-2'-pyridyl, 5'-fluoro-2'-pyridyl, 5'-imidazolinyl, and 2'-thiazolyl.

2. 7-α-thienylacetamidocephalosporadesic acid.

References Cited

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3,196,151 7/1965 Hoover et al.
3,218,318 11/1965 Flynn.

NICHOLAS S. RIZZO, Primary Examiner

U.S. Cl. X.R.

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