DIASTEREOMERIC 7-UREIDOACETYL CEPHALOSPORINS. I

SUPERIORITY OF 7α-H-L-ISOMERS OVER D-ISOMERS[1]

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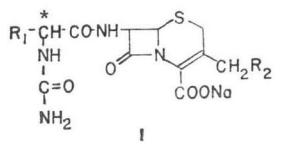
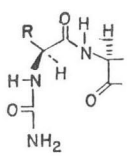
The synthesis and *in vitro* structure-activity relationship of 7-ureidoacetyl cephalosporins carrying various substituents in the 3-position, compounds that showed an enhanced broad spectrum of antibacterial activity, has been outlined. Contrary to most of the previous observations with diastereomeric isomers of cephalosporins, it has been found that the L-side chain isomers also are very potent antibiotics and are even more active inhibitors of certain β-lactamase-producing Gram-negative bacteria than the corresponding D-side chain isomers. SQ 69,613, 7β-[[L-[(aminocarbonyl)amino]-2-furanylacetyl]amino]-3-[[(1-methyl-1H-tetrazol-5-yl) thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, sodium salt, the most active compound tested, except for activity against staphylococci, was as active *in vitro* as cefamandole.

Many clinically important bacteria like *e.g. Enterobacter, Proteus, Pseudomonas* and *Serratia* are more or less resistant to commercially available penicillins and cephalosporins. The extensive use of β-lactam antibiotics has resulted in the selection of resistant mutants of bacteria that were originally sensitive. It is believed that in most cases[2] the resistance is due to the fact that the bacteria are producing β-lactamases, enzymes that are capable of hydrolysing β-lactam antibiotics and thus inactivating them. Therefore, it is an important goal to develop new β-lactam antibiotics that are less sensitive towards hydrolysis by β-lactamases than the commercially available drugs.

During the course of a program directed towards the development of new broad spectrum β-lactam antibiotics, 7-ureidoacetyl cephalosporins were prepared. It was found that particularly 3-heterothiomethyl substituted ureidoacetyl cephalosporins are potent broad spectrum antibiotics which exhibit an excellent antimicrobial activity *in vitro* against a broad variety of Gram-positive and Gram-negative bacteria. The new compounds are represented by the formula (I). Surprisingly, the L-diastereomers (with the absolute configuration depicted in I) were more active than the corresponding D-diastereomers of absolute configuration II. This finding is contrary to previous observations with cephalospor-



I



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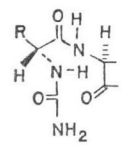
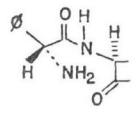
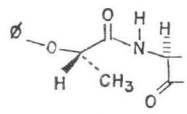


II

R = Phenyl, 2- and 3-thienyl, 2-furyl



III



IV

ins[3~5] and also penicillins[6~10] which demonstrate that the most active diastereomer always corresponds to absolute configuration **II**. This is true for the familiar penicillins and cephalosporins derived from D-phenylglycine (absolute configuration **III**) as well as for the phenoxypropyl penicillins in which the most active diastereomer is derived from L-α-phenoxypropionic acid (absolute configuration **IV**).

This paper describes the preparation of the new compounds and their antimicrobial activity *in vitro*.

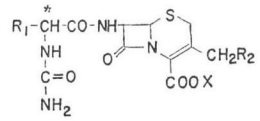
Chemistry

The amino acids used as starting materials in this study were either commercially available (D-phenylglycine, D-thienylglycine, DL-thienylglycine, D-*p*-hydroxyphenylglycine) or prepared by literature procedures which were modified in some cases. DL-2-Furylglycine[11~13] was prepared by a modified STRECKER synthesis from furan-2-aldehyde. The preparation of DL-3-thienylglycine started with α-bromo-3-thiophene acetic acid[15] *via* the α-azido acid. D-3-Thienylglycine was made from the racemate *via* the camphor-10-sulfonate. The L-isomers were obtained from the DL-chloroacetyl amino acids by treatment with hog kidney acylase[14]. D-Furylglycine was made from D-chloroacetylfurylglycine by deacylation with thiourea[16]. The α-ureido acetic acids **5** were prepared from the amino acids by treatment with alkali-cyanate in aqueous solution.

The 7-ureidoacetyl cephalosporins **1** used in this study are listed in Table 1. The 7-aminoacetyl cephalosporins **2** used as intermediates and for comparison studies are compiled in Table 2.

Schemes 1~5 illustrate the various routes that were used for the preparation of the ureidoacetyl

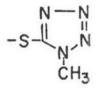
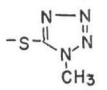
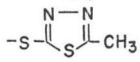
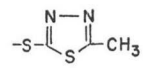
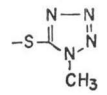
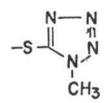
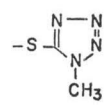
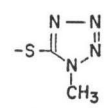
Table 1. 7-Ureidoacetyl cephalosporins **1**



Compound	R_1	R_2	X	*	Mp °C(dec)[a]	Formula	Analyses[b]
Ia		$-OCOCH_3$	H	D	133~134	$C_{19}H_{20}N_4O_7S \cdot H_2O$	C,H,N,S
Ib		$-OCOCH_3$	H	L	116~118	$C_{19}H_{20}N_4O_7S$	[d]
Ic			H	D	164~165	$C_{19}H_{20}N_8O_5S_2 \cdot H_2O$	C,H,N,S
Id			K	L	173~174	$C_{19}H_{19}KN_8O_5S_2 \cdot H_2O$	C,H,N,S,K
Ie			(−)	D	180~183	$C_{23}H_{22}N_6O_6S \cdot 3H_2O$	C,H,N,S
If			(−)	L	187~188	$C_{23}H_{22}N_6O_6S \cdot 3H_2O$	C,H,N,S
Ig			Na	D	214~215	$C_{19}H_{19}N_8NaO_6S_2 \cdot H_2O$	C,H,N,Na,S
Ih			Na	L	128~130	$C_{19}H_{19}N_8NaO_6S_2 \cdot 2H_2O$	C,H,N,Na

(to be continued)

Table 1. (continued)

Compound	R_1	R_2	X	*	Mp °C(dec)[a]	Formula	Analyses[b]
1j	2-Thienyl		H	D	152~162	$C_{17}H_{18}N_8O_5S_3 \cdot 2H_2O$	C,H,N,S
1k	2-Thienyl		H	L	149~153	$C_{17}H_{18}N_8O_5S_3 \cdot 1.5H_2O$	C,H,N,S
1l	2-Thienyl		H	D	123~124	$C_{18}H_{18}N_6O_5S_4$	C,H,N
1m	2-Thienyl		H	L	128~129	$C_{18}H_{18}N_6O_5S_4$	C,H,N,S
1n	2-Thienyl		H	D	175~177	$C_{20}H_{19}N_5O_6S_3 \cdot H_2O$	C,H,N,S
1o	2-Thienyl		H	L	190~196	$C_{20}H_{19}N_5O_6S_3 \cdot DMF$[c]	C,H,N,S
1p	3-Thienyl		K	D	168~169	$C_{17}H_{17}KN_8O_5S_3 \cdot 3H_2O$	K
1q	3-Thienyl		K	L	173~174	$C_{17}H_{17}KN_8O_5S_3 \cdot 3H_2O$	K
1r	2-Furyl		Na	D	189~190	$C_{17}H_{17}N_8NaO_6S_2$	C,H,N,S
1s	2-Furyl		Na	L	170~174	$C_{17}H_{17}N_8NaO_6S_2 \cdot H_2O$	C,H,N,Na,S

a) The cephalosporins melt with decomposition. Therefore their melting points may not be accurately reproducible.

b) Symbols of the elements indicate that analyses are coincident with the calculated value within ±1% deviation.

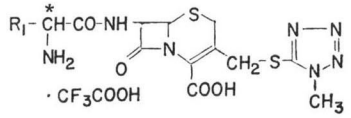
c) Dimethylformamide

d) No elemental analyses carried out. NMR- and IR-spectra are in coincidence with structure.

cephalosporins. Method B is accompanied by a certain degree of epimerisation (10~30%, verified by HPLC). Of the five methods mentioned, the 2-aminooxazolone hydrochloride method (E) has proven to be the most suitable one. It starts with easily accessible intermediates. The yields are good and no or only very little epimerisation is observed. The 2-aminooxazolone hydrochlorides **7** are new compounds that have not yet been described in the literature. This method, when extended to the acylation of 7β-amino-7-methoxycephalosporanic acid derivatives, proved to be the method of choice. It will therefore be discussed in more detail in the following paper[17] which deals with 7-methoxy derivatives of 7-ureidoacetyl cephalosporins.

The purity, especially the steric uniformity (with respect to the α-carbon atom of the acyl side chain) of the new 7-ureidoacetyl cephalosporins, could easily be determined by high performance liquid chroma-

Table 2. 7-Aminoacetyl cephalosporins **2**



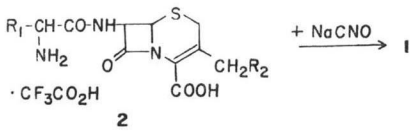
Compound	R_1	*	Mp °C(dec)[a]	Formula	Analyses[b]
2c	Phenyl	D	134~135	$C_{20}H_{20}F_3N_7O_6S_2$	C,H,N,S,F
2d	Phenyl	L	113~116	$C_{20}H_{20}F_3N_7O_6S_2$	c)
2g	*p*-Hydroxy-phenyl	D	156~157	$C_{20}H_{20}F_3N_7O_7S_2$	c)
2h	*p*-Hydroxy-phenyl	L	144~145	$C_{20}H_{20}F_3N_7O_7S_2$	c)
2j	2-Thienyl	D	127~131	$C_{18}H_{18}F_3N_7O_6S_3$	C,H,N,S,F
2k	2-Thienyl	L	135~147	$C_{18}H_{18}F_3N_7O_6S_3$	c)
2p	3-Thienyl	D	124~125	$C_{18}H_{18}F_3N_7O_6S_3$	c)
2q	3-Thienyl	L	129~130	$C_{18}H_{18}F_3N_7O_6S_3$	c)
2r	2-Furyl	D	119~120	$C_{18}H_{18}F_3N_7O_7S_2$	C,H,N,S,F
2s	2-Furyl	L	104~105	$C_{18}H_{18}F_3N_7O_7S_2$	C,H,N,S

a) The cephalosporins melt with decomposition. Therefore their melting points may not be accurately reproducible.

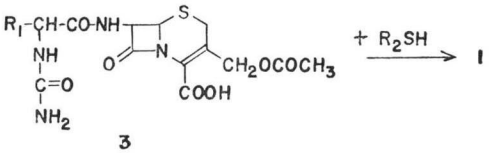
b) Symbols of the elements indicate that analyses are coincident with the calculated value within $\pm 1\%$ deviation.

c) No elemental analyses carried out. NMR- and IR-spectra are in coincidence with structure.

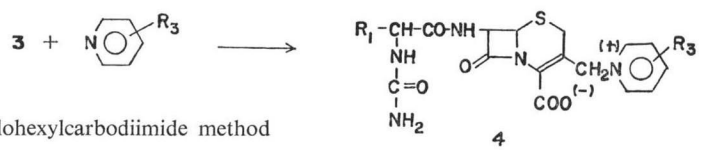
Scheme 1. Conversion of 7-aminoacetyl cephalosporins with cyanates (Method A)



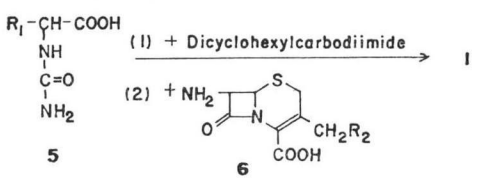
Scheme 2. Nucleophilic displacement of the acetoxy group in the 3-position with appropriate thiols (Method B)



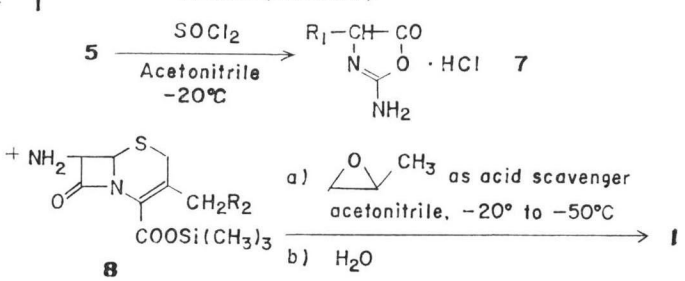
Scheme 3. Nucleophilic displacement of the acetoxy group in the 3-position with pyridines (Method C)



Scheme 4. Dicyclohexylcarbodiimide method (Method D)

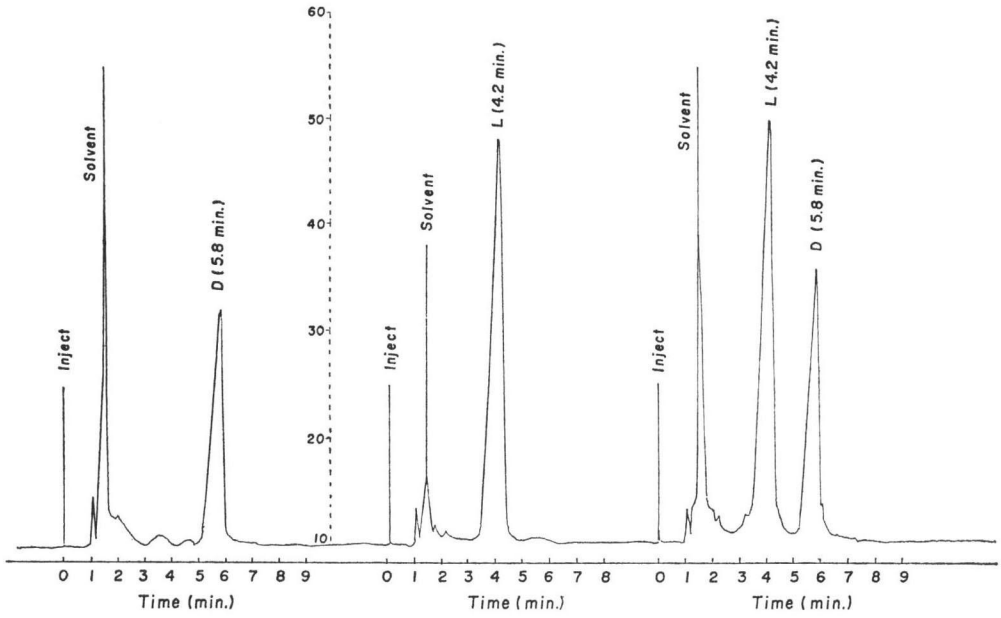


Scheme 5. 2-Aminooxazolone hydrochloride method (Method E)



tography[18]. The retention times of the diastereomers are significantly different as can be seen from Fig. 1. The chromatograms were obtained with the aid of a high performance liquid chromatograph (Waters Associates, model: ALC-GPC 244). A reversed phase column (C_{18}-μ-bondapak) was used and a mixture of methanol-water served as eluent.

Fig. 1.



Antimicrobial Activity *In Vitro*

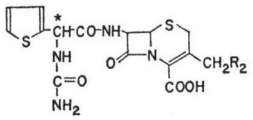
Bacterial cultures: All test organisms were recent clinical isolates obtained from human sources.

Broth-dilution susceptibility tests: MUELLER-HINTON (MH) broth was used for all organisms. For the test, a few colonies of each organism were seeded into the medium and, after overnight incubation, an amount of diluted culture was added to each antibiotic dilution series to provide an initial concentration of *ca.* 10^5 colony-forming units (CFU) per ml. Incubation was at 37°C for 18~24 hours.

Agar dilution susceptibility tests: All tests were carried out in MH agar. Stock solutions of the test substances were made in 0.05 M phosphate buffer (pH 7.0) for controls, and in sterile distilled water for all candidate compounds to an initial concentration of 1 mg/ml. Further dilutions of each antibiotic were prepared in MH broth (pH 7.4); 1 ml amounts added to 90-mm Petri dishes, mixed with 9.0 ml of MH agar and allowed to harden. Test cultures grown in MH broth for *ca* 18~24 hours at 37°C were deposited on the solidified surface of the agar using an automated multiple-pronged replicator designed to deposit 10^4 CFU on the agar surface. All plates were incubated aerobically at 37°C. The MIC in both broth and agar tests was defined as the lowest concentration that suppressed visible growth. The results of the screening *in vitro* are shown in Tables 3~8.

Out of all substituents that were introduced in the 3-position, methyltetrazolylthio was the most potent one (Table 3). The thiadiazolylthio compound was slightly less active. However, also with this as with many others not shown in the table, the L-compound was more active than the D-compound, especially against resistant Gram-negative bacteria. With the 3-pyridine-N-oxide compounds included in the table, the D- and the L-isomers are equally active. This demonstrates that the nature of the 3-substituent affects the relative activity of the two diastereomers.

Table 3. 3-Heterothio 7-ureidoacetyl cephalosporins—Activity *in vitro*



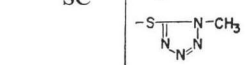
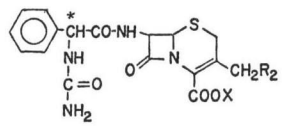
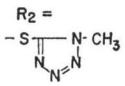
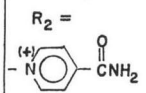
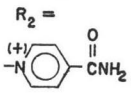
Organism	SC	MIC μg/ml					
		Ij *D R_2 = 	Ik *L R_2 =	Il *D R_2 =	Im *L R_2 =	In *D R_2 =	Io *L R_2 =
Sensitive strains							
Staph. aureus	2399	1.2	2.4	2.6	1.2	0.78	1.2
E. coli	8294	1.2	1.6	3.1	1.6	9.4	6.3
K. pneumoniae	8340	1.2	0.8	4.7	1.2	9.4	4.7
Sal. schottmuelleri	3850	≤0.19	1.6	0.8	0.6	1.2	2.4
Resistant strains							
Staph. aureus	2400	1.2	3.1	2.4	4.7	2.4	2.4
E. coli	3552	2.4	1.6	6.3	9.4	12.5	9.4
E. cloacae	8415	37.5	1.6	50	4.7	37.5	50.0
Pr. rettgeri	8217	9.4	1.6	18.7	6.3	75.0	50.0
Ps. aeruginosa	8329	25.0	>100	>50	>100	>100	>100
Serr. marcescens	9782	≥50	4.7	≥100	50	50.0	>100

Table 4. 3-Pyridinium 7-ureidoacetyl cephalosporins—Activity *in vitro*

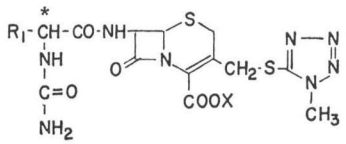


Organism	SC	MIC μg/ml			
		Ic *D R_2 =  X = Na	Id *L R_2 = X = Na	Ie *D R_2 =  X= (−)	If *L R_2 =  X= (−)
Staph. aureus	2399	0.05	3.1	0.6	3.1
E. coli	8294	3.5	1.6	1.6	37.5
K. pneumoniae	8340	2.4	0.8	1.6	18.7
Sal. schottmuelleri	3850	0.39	0.8	0.6	12.5
Staph. aureus	2400	1.4	3.1	1.2	4.7
E. coli	3552	N.T.	1.6	9.4	37.5
E. cloacae	8415	>100	4.7	>100	≥100
Pr. rettgeri	8217	12.5	6.3	50.0	18.7
Ps. aeruginosa	8329	>50	>100	12.5	>100
Serr. marcescens	9782	N.T.	18.7	75.0	75.0

N.T.=not tested

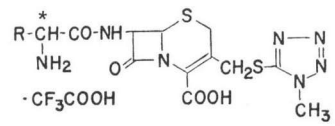
There are a few examples of ureidoacetyl cephalosporins in which the L-isomers are clearly less active than the corresponding D-isomers. The 3-pyridinium derivatives which are shown in Table 4 represent such compounds.

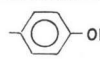
Table 5. D- and L-7-Ureidoacetyl cephalosporins—Activity *in vitro* against β-lactamase producing Gram-negative microorganisms



Compound	R_1	X	*	MIC μg/ml		
				Pr. rettgeri SC 8217	*E. cloacae* SC 8415	*Serr. liquefaciens* SC 9068
1j	2-Thienyl	Na	D	12.5	>100	>100
1k	2-Thienyl	Na	L	3.1	1.2	50
1p	3-Thienyl	K	D	25.0	>100	>100
1q	3-Thienyl	K	L	3.1	1.2	50
1r	2-Furyl	Na	D	12.5	25.0	>100
1s	2-Furyl	Na	L	0.4	0.6	6.3
1c	Phenyl	Na	D	12.5	>100	>100
1d	Phenyl	K	L	6.3	4.7	25
1g	*p*-Hydroxyphenyl	Na	D	100	>100	>100
1h	*p*-Hydroxyphenyl	Na	L	25.0	⩾100	>100

Table 6. D- and L-7-Aminoacetyl cephalosporins—Activity *in vitro*



Organism	SC	MIC μg/ml							
		R = 		R = 		R = 		R = 	
		2g * D	**2h** * L	**2j** * D	**2k** * L	**2p** * D	**2q** * L	**2r** * D	**2s** * L
Staph. aureus	2399	0.8	50	3.12	12.5	9.4	25	1.6	18.7
E. coli	8294	1.2	25	2.4	9.4	1.6	9.4	9.4	37.5
K. pneumoniae	8340	1.2	75	1.6	4.7	1.2	12.5	6.3	18.7
Sal. schottmuelleri	3850	0.3	18.7	0.6	4.7	0.2	4.7	3.1	4.7
Staph. aureus	2400	12.5	⩾100	6.3	25	25	75	6.3	37.5
E. coli	3552	1.2	75	9.4	25	31	18.7	25	12.5
E. cloacae	8415	2.4	>100	12.5	⩾100	9.4	⩾100	37.5	>100
Pr. rettgeri	8217	18.7	>100	25	⩾100	50	75	⩾50	⩾100
Ps. aeruginosa	8329	⩾50	>100	>50	>100	>100	>100	>50	⩾100
Serr. marcescens	9782	18.7	>100	>50	>100	50	>100	>50	>100
E. cloacae	9965	N.T.	>100	N.T.	N.T.	12.5	75	37.5	>100

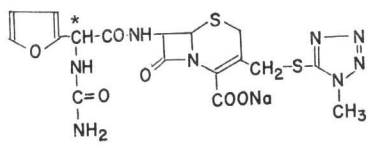
N.T.=not tested

Variation of the aromatic substituent in the acyl side chain resulted in the same picture, namely a greater activity *in vitro* of the diastereomer carrying an L-side chain. The results obtained with five pairs of diastereomers against three β-lactamase producing Gram-negative microorganisms can be seen from Table 5.

Of interest is a comparison of the L- and D-aminoacetyl cephalosporins from which the ureidoacetyl cephalosporins are derived. A great number of 3-heterothio-7-D-phenylglycyl cephalosporins have been studied[19] but, in view of earlier investigations as *e.g.* ref. 3), not compared with the corresponding L-isomers. In coincidence with the earlier investigations we have found that the L-aminoacetyl cephalosporins are less active than the corresponding D-isomers (Table 6).

The 2-furyl derivative **1s** (SQ 69,613) is the most active L-ureidoacetyl cephalosporin. A comparison of the activity *in vitro* of SQ 69,613 with that of other new cephalosporins shows that SQ 69,613 is at least equal in activity. The only exception is *Staphylococcus aureus*, against which the L-ureidoacetyl cephalosporins are generally not very active (Table 7). Over 230 strains comprising 14 genera of bacteria including both Gram-positive and Gram-negative organisms, isolated from patients representing a geographic cross section of the U. S., were collected and tested for susceptibility to SQ 69,613 and cefamandole. The results of the conventional tube dilution assay show that SQ 69,613 is generally at least as active as cefamandole, with the exception of *Staph. aureus*. These results are compiled in Table 8.

Table 7. SQ 69,613 (**1s**) and reference compounds—Activity *in vitro*



Organism	SC	MIC μg/ml				
		1s SQ 69,613 *L	Cefamandole	Cefuroxime	Cefoxitin	Cefazolin
Staph. aureus	2399	2.4	0.12	0.6	1.2	0.12
E. coli	8294	0.16	0.6	3.12	3.12	1.2
K. pneumoniae	8340	0.32	0.6	1.2	1.2	1.2
Sal. schottmuelleri	3850	0.12	0.39	0.78	1.2	1.2
Staph. aureus	2400	6.3	0.39	0.78	1.56	0.78
E. coli	3552	0.47	0.6	1.56	1.56	1.2
E. cloacae	8415	0.6	4.7	6.25	>50	>50
E. cloacae	9965	0.6	3.12	6.25	>50	>50
Pr. rettgeri	8217	0.4	1.56	0.78	12.5	>50
Ps. aeruginosa	8329	>100	>50	>50	>50	>50
Serr. marcescens	9782	1.6	9.4	12.5	4.7	>50

In conclusion, 7-ureidoacetyl cephalosporins are potent antibiotics that deserve further investigation. The finding that L-ureidoacetyl cephalosporins are more active against resistant Gram-negative microorganisms than the corresponding D-isomers deserves special attention and further studies will attempt to elucidate this unusual behavior[20].

Table 8. Cumulative susceptibility of clinical isolates (% at MIC)

Organism	Compound	No. of strains	Cumulative percentage of strains inhibited at each concentration (μg/ml)											
			<0.1	0.1	0.2	0.4	0.8	1.6	3.12	6.25	12.5	25.0	50.0	100	>100
Enterob. cloacae	69,613	20	0	0	0	10	20	50	60	60	60	60	60	65	100
	CMT*	20	0	0	0	0	15	45	45	45	55	55	55	55	100
Enterob. aerogenes	69,613	19	0	0	21	74	79	79	79	84	84	84	89	89	100
	CMT	19	0	0	0	0	58	68	79	79	79	79	84	84	100
Shigella sp.	69,613	20	0	0	20	60	80	100							
	CMT	20	5	15	55	60	75	75	80	100					
Citrobacter sp.	69,613	14	0	0	0	79	86	100							
	CMT	14	0	0	57	71	93	100							
Salmonella sp.	69,613	20	0	0	10	80	100								
	CMT	20	0	10	15	35	85	95	100						
Serratia	69,613	21	0	0	0	0	0	24	43	62	71	81	81	81	100
	CMT	21	0	0	0	0	0	0	10	14	52	62	71	71	100
Serr. liquefaciens	69,613	4	0	0	0	0	0	0	25	50	100				
	CMT	4	0	0	0	0	0	0	0	25	50	100			
Staph. Pen.-resistant	69,613	21	0	0	0	0	0	0	0	52	90	100			
	CMT	21	0	0	0	24	67	90	100						
Staph. Methicillin-r.	69,613	20	0	0	0	0	0	0	0	0	25	90	100		
	CMT	20	0	0	0	0	5	5	85	90	100				
E. coli	69,613	21	0	52	90	95	100								
	CMT	21	0	0	57	81	95	100							
Proteus indole (+)	69,613	20	10	10	15	25	30	40	65	80	85	85	90	90	100
	CMT	20	0	0	10	20	25	25	35	45	60	75	85	90	100
Proteus indole (−)	69,613	21	0	0	0	62	86	90	95	100					
	CMT	21	0	0	0	29	62	95	95	100					
Proteus inconstans	69,613	14	14	36	71	86	93	93	100						
	CMT	14	7	14	21	43	71	86	93	100					

* CMT=Cefamandole

Experimental

DL-α-Amino-2-furanacetic Acid

To a stirred solution of 78.5 g of sodium cyanide in 670 ml of 34% aqueous ammonia 85.5 g of ammonium chloride was added. After the solution had become clear, a solution of 76.8 g furfural in 350 ml of methanol was added at 15~18°C within 20 minutes.

The solution was then stirred at the same temperature for 5 hours and, after adding 800 ml of water and 400 ml of chloroform and stirring for half an hour, the chloroform layer was separated. The chloroform extraction of the aqueous phase was repeated with 400 ml of chloroform.

The combined chloroform extracts were washed with 200 ml of water and the solvent then removed by concentrating *in vacuo* at 35°C. The residue of approximately 95 g was slowly added to 560 ml of concentrated hydrochloric acid with stirring and cooling, the temperature not exceeding 35°C. After stirring for 1 hour at 18~20°C the dark colored precipitate was filtered off, washed with 100 ml of alcohol and dried. This yielded 88 g of a dark-grey product.

The content of the furylglycineamide hydrochloride was approximately 85% (determined by Cl^- titration), corresponding to a yield of about 55 mole %.

The crude amide (88 g) was added to a solution of 48 g sodium hydroxide in 660 ml of water and the mixture refluxed for 3 hours. After cooling to room temperature the pH was adjusted to 6.5 by adding 12% hydrochloric acid. Then a small quantity of a black by-product was filtered off by suction and washed with cold water. After treating the filtrate with 8 g of charcoal by stirring for half an hour at ambient temperature it was filtered off again and washed with cold water.

The filtrate was concentrated *in vacuo* to a volume of 150 ml and after cooling to 5~10°C the crystals were filtered off, washed with 20 ml of cold water and dried. Further concentration of the mother liquor yielded a second crop of 27.0 g containing 42% NaCl and a third crop of 13.8 g containing 67% NaCl. The total yield was 57.6 g of DL-α-amino-2-furanacetic acid, corresponding to 51.1% of the theoretical amount.

DL-α-Amino-3-thiopheneacetic Acid

(a) DL-α-Bromo-3-thiopheneacetic acid: 3-Thienylbromide was treated with butyl lithium and chloral to yield 3-[(1-hydroxy-2,2,2,-trichloro)ethyl] thiophene which was then treated with sodium methoxide to obtain α-methoxy-3-thienylacetic acid[15].

One hundred and fifty ml of 30% hydrogen bromide in acetic acid was added to a solution of 16 g (100 mmol) of (α-methoxy-3-thienyl) acetic acid in 50 ml of glacial acetic acid. The mixture was left to stand at room temperature for 24 hours and then poured into ice water. The solution was extracted three times with 60 ml of ether. The ether phase was washed with water, dried over magnesium sulfate and evaporated. The residue, 18 g of crude DL-α-bromo-3-thiopheneacetic acid, was recrystallized from cyclohexane; yield 14 g; m.p. 80~82°C.

(b) DL-α-Azido-3-thiopheneacetic acid: Four g (62 mmol) of sodium azide and 3.5 g (33 mmol) of sodium carbonate were added to a solution of 12 g (54 mmol) of DL-α-bromo-3-thiopheneacetic acid in 75 ml of acetone (96%). The mixture was stirred at room temperature for 12 hours in darkness and after this time the solvent was evaporated and the residue dissolved in 75 ml of water. Fifty ml of ether was added, the water phase was acidified with 2 N sulfuric acid and extracted quickly twice more with 50 ml of ether. After washing with water and drying over sodium sulfate, the combined ether phases were evaporated. Crystallization of the residue from cyclohexane yielded 7.4 g of white crystalline DL-α-azido-3-thiopheneacetic acid; m.p. 58~59°C.

(c) DL-α-Amino-3-thiopheneacetic acid: Palladium/barium sulfate catalyst, 0.3 g, as added to a solution of 6 g of DL-α-azido-3-thiopheneacetic acid in 40 ml of ethanol and 40 ml of 0.5 N hydrochloric acid. Hydrogenation took place at about 60 psi after 2 hours. After filtration, the volume was concentrated to about 30 ml. When the pH was brought to 6.5 with ammonia, the amino acid separated as a white powder. After washing with ethanol-water and drying, 3.5 g of the product, DL-α-amino-3-thiopheneacetic acid, were obtained; m.p. 283~285°C.

D-α-Ureido-2-thiopheneacetic Acid

D-2-Thienylglycine, 15.8 g (0.1 mol), was heated together with 8.2 g (0.1 mol) of potassium cyanate in 100 ml of water. After 30 minutes, the mixture was cooled and acidified with dilute hydrochloric acid. The precipitated product, D-α-ureido-2-thiopheneacetic acid, was filtered, washed with ice water and a small amount of ethanol. Recrystallization from methanol yielded 17 g of white crystals, of D-α-ureido-2-thiopheneacetic acid; m.p. 191.5~193.5°C (dec).

D-2-Amino-4-(2-thienyl)-5(4H)-oxazolone Hydrochloride[**7**, R_1=2-thienyl]

Two g of D-α-ureido-2-thiopheneacetic acid (**5**, R_1=2-thienyl) were suspended in 50 ml absolute acetonitrile. The suspension was cooled to −10°C and stirred. Then, 1.25 g of thionylchloride were added and stirring was continued for 15 minutes. The 2-aminooxazolone hydrochloride was isolated by filtration. The yield was nearly quantitative.

Preparation of 7-Aminoacetyl Cephalosporins

(a) L-[[[(4-Methoxyphenyl)methoxy]carbonyl]amino]-2-thiopheneacetic acid

L-(2-Thienyl) glycine (14.2 g; prepared by the method of NISHIMURA *et al.*, Nippon Kagaku Zasshi, Vol. 82, pp. 1688~1691, 1961; Chem. Abst., Vol. 58, p. 11464f) was suspended in 142 ml of water and brought into solution by the addition of 37.9 ml of triethylamine. A solution of 20.6 g of (*p*-methoxyphenyl) methoxycarbonylazide in 142 ml of dioxane was added with stirring. The mixture which was turbid at first became clear after 30 minutes. This was stirred for an additional hour at room temperature. The dioxane was then evaporated in vacuum. Flakes formed in the aqueous phase which were extracted by shaking with ether. The aqueous phase was cooled to 0°C, layered over with ethyl acetate and acidified with 2 N hydrochloric acid to pH 2.5. The aqueous phase was extracted twice more with ethyl acetate, the combined ethyl acetate extracts were dried with magnesium sulfate and concentrated in vacuum to yield 23.5 g of L-[[[(4-methoxyphenyl) methoxy] carbonyl] amino]-2-thiopheneacetic acid; m.p. 100~102°C; $[\alpha]_D^{20}$ +68.3° (*c* 1, tetrahydrofuran).

(b) 7β-[[L-[[[(4-Methoxyphenyl) methoxy] carbonyl] amino]-2-thienylacetyl] amino]-3-[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, diphenylmethyl ester

3-[(1-Methyl-1H-tetrazol-5-yl)thio]-7-amino-cephalosporanic acid diphenylmethyl ester, 14.9 g, was dissolved in 300 ml of methylene chloride and 300 ml of anhydrous tetrahydrofuran were added. Then 11.62 g of L-[[[(4-methoxyphenyl)methoxy]carbonyl]amino]-2-thiopheneacetic acid from part (**a**) were added, the mixture was cooled to 0°C, and a solution of 6.79 g of dicyclohexylcarbodiimide in 100 ml of anhydrous tetrahydrofuran was added dropwise with stirring over 30 minutes. The reaction mixture was stirred for 90 minutes at 0~5°C and 90 minutes at room temperature. The precipitated dicyclohexylurea was then filtered off and the filtrate was concentrated in vacuum. The residue was taken up with ethyl acetate, filtered, washed with a sodium bicarbonate solution and with water. The ethyl acetate solution was dried with magnesium sulfate, treated with activated charcoal, filtered and concentrated in vacuum to a small volume. On stirring in excess petroleum ether, 24 g of 7β-[[L-[[[(4-methoxyphenyl)methoxy]carbonyl]amino]-2-thienylacetyl]amino]-3-[[(1-methyl-1H-tetrazol-5-yl)-thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, diphenylmethyl ester, m.p. 110°C, were obtained as a precipitate.

(c) 7β-[[L-Amino-2-thienylacetyl]amino]-3-[[(1-methyl-1H-tetrazol-5-yl) thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, trifluoroacetic acid salt **2k**

Twenty-four g of the diphenylmethyl ester product from part (**b**) were stirred in 100 ml of anisole and 300 ml of trifluoroacetic acid were added dropwise at 0°C. After 10 minutes, this mixture was evaporated under vacuum. The residue was treated with ether and filtered to yield 17.8 g of 7β-[[L-amino-2-thienylacetyl)amino]-3-[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, trifluoroacetic acid salt.

Preparation of 7-Ureidoacetyl Cephalosporins

Method A

7β-[[L-[(Aminocarbonyl)amino]-2-thienylacetyl]amino]-3-[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid **1k**

Twelve g of **2k** were added to a solution of 3.4 g of potassium cyanate in 85 ml of water and stirred for 3 hours at room temperature. This mixture was filtered and the filtrate acidified to pH 1.5 with 2 N hydrochloric acid while cooling. The precipitate was isolated and yielded 6.8 g of **1k**; m.p. 149~153°C (dec.).

An aqueous equimolar solution of this acid and sodium bicarbonate was lyophilized to yield 7β-[[L-[(aminocarbonyl)amino]-2-thienylacetyl]amino]-3-[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, sodium salt; m.p. 187~188°C (dec.).

Method B

7β-[[D-[(Aminocarbonyl)amino]phenylacetyl]amino]-3-[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, sodium salt **1c**

1-Methyl-1H-tetrazol-5-thiol (0.42 g; 0.0036 mole) was suspended in 15 ml of H_2O and brought into solution by the addition of 2 N NaOH. Then 1.4 g (0.003 mole) of **3** (R_1 = phenyl, D-form) were added and the pH of the solution adjusted to 6.5. The mixture was kept at this pH at 60°C for 8 hours, cooled, filtered and the filtrate acidified with 2 N hydrochloric acid to pH 1.5~2. The precipitated acid was filtered, dried, dissolved with an equivalent amount of sodium hydrogen carbonate and lyophilized to yield 0.9 g of **1c**; m.p. 164~165°C.

Method C

7β-[[D-[(Aminocarbonyl)amino]-2-thienylacetyl]amino]-3-[[4-(aminocarbonyl)pyridino]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid **1n**

(a) D-[[[(4-Methoxyphenyl)methoxy]carbonyl]amino]-2-thiopheneacetic acid

D-2-Thienylglycine, 74 g, were dissolved in 940 ml of water; 37.8 g of magnesium oxide were added and to this resulting suspension a solution of 107.5 g of *p*-methoxybenzyloxycarbonylazide in 940 ml of dioxane was added with stirring. The mixture was stirred at room temperature for 24 hours. It was then filtered and the filtrate extracted with 600 ml of ether. The extract was discarded. The water-dioxane phase was layered over with 600 ml of ethyl acetate, cooled to 5°C and brought to pH 2 with 2 N hydrochloric acid. The layers were separated and the aqueous layer was again extracted with 300 ml of ethyl acetate. The combined ethyl acetate extracts were washed with water, dried with magnesium sulfate, filtered and concentrated. The oily residue crystallized upon trituration with petroleum ether to yield 118 g of D-[[[(4-methoxyphenyl)methoxy]carbonyl]amino]-2-thiopheneacetic acid; m.p. 84~94°C; $[\alpha]_D^{20} - 69°$ (*c* 1, tetrahydrofuran).

(b) 3-[(Acetyloxy)methyl]-7β-[[D-[[[(4-methoxyphenyl)methoxy]carbonyl]amino]-2-thienylacetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid

D-2-[[[(4-Methoxyphenyl)-methoxy]carbonyl]amino]-2-thiopheneacetic acid (3.2 g, 0.01 mole) from part (a) were brought into solution in 40 ml of methylene chloride with 1.1 ml of N-methylmorpholine. The solution was cooled to −15°C, 1.39 ml of isobutylchloroformate were added, and the mixture stirred for 10 minutes. To this was added a solution of 3.26 g (0.1012 mol.) of 7-aminocephalosporanic acid and 3.1 ml of triethylamine in 40 ml of methylene chloride. The mixture was stirred for 1 hour at −5°C and 1 hour at 5°C. This mixture was then evaporated to dryness on a rotary evaporator. The solid residue was triturated with ether and filtered under suction. The substance was then dissolved in ice water, layered over with ethyl acetate and acidified to pH 2.5. The layers were separated, the aqueous layer was extracted once more with ethyl acetate, the combined ethyl acetate extracts were washed with water, dried with magnesium sulfate and concentrated. The residue (4.9 g) was dissolved in 200 ml of ethyl acetate and the solution treated with activated charcoal. After filtration, 2 g of 3-[(acetyloxy)methyl]-7β-[[D-[[[(4-methoxyphenyl)methoxy]carbonyl]amino]-2-thienylacetyl]amino]-8-oxo-5-thia-1-

azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid crystallized; m.p. 142~143°C (dec.).

(c) 3-[(Acetyloxy)methyl]-7β-[[D-amino-2-thienylacetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid

Two g of the product from part (b) were added at −5°C to a mixture of 10 ml of trifluoroacetic acid and 4 ml of anisole. The mixture was stirred for 15 minutes and then concentrated on a rotary evaporator. The residue was treated with ether and filtered under suction. The crude 3-[(acetyloxy)methyl]-7β-[[D-amino-2-thienylacetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, trifluoroacetic acid salt was dissolved in 50 ml of water and added to a suspension (20 ml) of the acetate form of the ion-exchange resin Amberlite LA 1 in isobutylmethyl ketone. The mixture was stirred for 2 hours at room temperature. The layers were separated, the aqueous phase was washed several times with ether and freeze-dried to yield 3-[(acetyloxy)methyl]-7β-[[D-amino-2-thienylacetyl]amino]-8-oxo-5-thia-1-azabicyclo-[4.2.0]oct-2-ene-2-carboxylic acid.

(d) 3-[(Acetyloxy)methyl]-7β-[[D-[(aminocarbonyl)amino]-2-thienylacetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxyclic acid, sodium salt

A mixture of 1 g of the product from part (c) and 0.194 g of potassium cyanate in 7.5 ml of water were quickly heated in a preheated bath at 80°C. The mixture was then immediately cooled to room temperature and permitted to stand overnight. The reaction mixture was concentrated to about 4 ml and the pH adjusted to 1.5 with 2 N hydrochloric acid. The precipitate was filtered under suction to obtain 3-[(acetyloxy)methyl]-7β-[[D-[(aminocarbonyl)amino]-2-thienylacetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

An aqueous equimolar mixture of this acid and sodium bicarbonate was lyophilized to yield 3-[(acetyloxy)methyl]-7β-[[D-[(aminocarbonyl)amino]-2-thienylacetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, sodium salt.

(e) 7β-[[D-[(Aminocarbonyl)amino]-2-thienylacetyl]amino]-3-[[4-(aminocarbonyl)pyridino]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid

A mixture of 2.38 g (0.005 mole) of the sodium salt product from part (d), 0.915 g (0.0075 mole) of 4-pyridinecarboxamide, 12 g of potassium thiocyanate and 7.5 ml of water was heated at 50°C for 24 hours. A clear solution was obtained.

A chromatography column was filled with 150 g of ion-exchange resin Amberlite XAD-2. An additional 150 g were made into a paste with a little water and added to the above reaction solution. This solution was stirred for 30 minutes and then added to the chromatography column containing the 150 g of resin. The column was first eluted with water. After 120 fractions of 30 ml each which were discarded, the column was eluted with a mixture of water and methanol (80: 20) and fractions of 10 ml each were collected. Fractions 47~120 was concentrated to about 150 ml and freeze-dried. The residue was triturated with ether to obtain 0.7 g of 7β-[[D-[(aminocarbonyl)amino]-2-thienylacetyl]amino]-3-[[4-(aminocarbonyl)pyridino]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid; m.p. 160~165°C (dec.).

Method D

3-[(Acetyloxy)methyl]-7β-[[D-[(aminocarbonyl)amino]-2-thienylacetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid **1a**

In 40 ml of absolute dimethylformamide, were dissolved 9.2 g (50 mmol.) of D-α-ureido-2-thiopheneacetic acid, 10.3 g (50 mmol.) of dicyclohexylcarbodiimide dissolved in 10 ml of methylene chloride were added dropwise at 0°C. After stirring for 30 minutes, a solution of 13.5 g (50 mmol.) of 7-aminocephalosporanic acid and 10 g (100 mmol.) of triethylamine was added. This mixture was stirred for 24 hours at 5°C. After filtering, the filtrate was concentrated under vacuum, the oily residue was taken up in water, filtered and after treating with activated charcoal at 5°C it was layered over with ethyl acetate and acidified with 2 N hydrochloric acid. The ethyl acetate solution was washed with water, dried and concentrated to obtain 8.1 g of a viscid residue.

The product, 3-[(acetyloxy)methyl]-7β-[[D-[aminocarbonyl)amino]-2-thienylacetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, was recrystallized twice from isopropanol to yield

2.1 g **1a**; m.p. 133~143°C (dec.).

Method E

7β-[[L-[(Aminocarbonyl)amino]-2-thienylacetyl]amino]-3-[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid **1k**

In 50 ml of absolute acetonitrile were suspended 3.4 g of **6** (R_2=1-methyl-1H-tetrazol-5-ylthio). Then 4.5 g of BSA (bis-trimethylsilylacetamide) were added to form a solution of **8** (R_2=1-methyl-1H-tetrazol-5-ylthio). To this 2 g of propyleneoxide were added and, after cooling the solution to 0°C, followed by the addition of a suspension of 2.4 g of L-**7** (R_1=2-thienyl). The mixture was stirred for 30 minutes and then poured into a mixture of 250 ml of water and 50 ml of tetrahydrofuran, filtered and the solution concentrated to about 1/3 of the original volume. 3.2 g of **1k** were obtained. Another crop of 0.4 g of **1k** crystallized from the mother liquor on standing overnight.

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