Synthesis and antimicrobial activity of some 5-substituted 7-tetrazolylacetamido-3-acetoxycephalosporanic acids and their sodium salts

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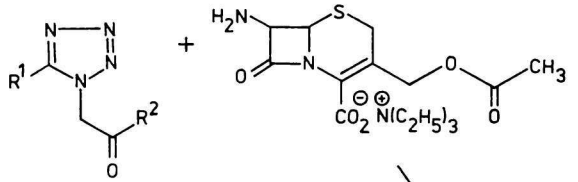
5-Substituted 7-(1- resp. 2-tetrazolylacetamido)-3-acetoxycephalosporanic acids and their sodium salts were obtained by *N*-acylation of 7-aminocephalosporanic acid with reactive derivatives of 5-substituted 1- resp. 2-tetrazolylacetic acids. The final products were tested against Gram-positive and Gram-negative strains of microorganisms.

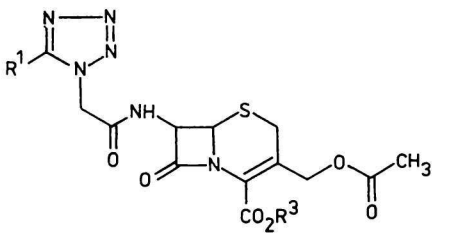
Посредством *N*-ацилирования 7-аминоцефалоспорановой кислоты реакционноспособными производными замещенных в положении 5 1- и 2-тетразолилуксусных кислот были получены замещенные в положении 5 7-(1- или 2-тетразолилацетамидо)-3-ацетоксицефалоспорановые кислоты и их натриевые соли. Конечные продукты были испытаны на активность против грам-положительных и грам-отрицательных штаммов микроорганизмов.

Our preceding paper [1] dealt with the preparation of 7-[5-(5-aryl-2-furyl)-1- resp. -2-tetrazolylacetamido]-3-acetoxycephalosporanic acids. Further original cephem derivatives were synthesized from substituted tetrazolylacetic acids, the antimicrobial activity of which was evidenced.

5-Substituted 7-(1- resp. 2-tetrazolylacetamido)-3-acetoxycephalosporanic acids and their sodium salts *IIIa—IIIi*, *Va*, *Vb* were prepared by *N*-acylation of triethylammonium salt of 7-aminocephalosporanic acid (*II*) with chlorides of the corresponding 5-substituted 1- resp. 2-tetrazolylacetic acids *Ia—Ii*, *IVa*, *IVb* in dichloromethane (Schemes 1 and 2). 7-[5-(2-Thienyl)-1-tetrazolylacetamido]--3-acetoxycephalosporanic acid (*IIIj*) was synthesized by *N*-acylation of triethylammonium salt of 7-aminocephalosporanic acid (*II*) with 5-(2-thienyl)--1-tetrazolylacetic 2,2-dimethylpropionic anhydride (*Ij*) in dichloromethane (Scheme 1). The 4-substituted 5-[5-phenylthio-2-furyl]-1-tetrazolylacetic acids [2, 3], 4-substituted 5-[5-phenylsulfo-2-furyl]-1-tetrazolylacetic acids [4], 5-(2--furyl)-1- resp. -2-tetrazolylacetic acids [5] and 5-(5-bromo-2-furyl)-1- resp. -2-tetrazolylacetic acids [3] were prepared according to procedures detailed in the quoted papers. 5-(2-Thienyl)-1-tetrazolylacetic acid has so far been predominantly obtained by alkylation of 5-(2-thienyl)-1*H*-1-tetrazole with ethyl bro-



Ia–Ij *II*



IIIa–IIIj

For *Ia–Ii* R^2 = Cl
For *Ij* R^2 = $(CH_3)_3CCO_2$

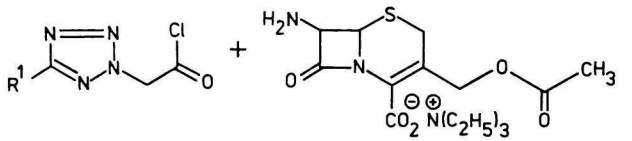
R^1

For *IIIa–IIIi* R^3 = Na
For *IIIj* R^3 = H

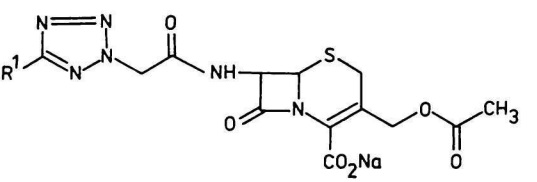
R^1

a 2-Furyl
b 5-Bromo-2-furyl
c 5-(Phenylthio)-2-furyl
d 5-(4-Tolylthio)-2-furyl
e 5-(4-Methoxyphenylthio)-2-furyl
f 5-(4-Chlorophenylthio)-2-furyl
g 5-(4-Fluorophenylthio)-2-furyl
h 5-(4-Tolylsulfo)-2-furyl
5-(4-Chlorophenylsulfo)-2-furyl
j 2-Thienyl

Scheme 1



IVa–IVb *II*



a R^1 = 2-Furyl
b R^1 = 5-Bromo-2-furyl

Scheme 2 *Va–Vb*

moacetate and hydrolysis of the separated 1- and 2-isomeric ethyl esters [5]. Since our attention was focused on 1-isomeric tetrazolylacetic acids from the antibacterial point of view, ethyl 5-(2-thienyl)-1-tetrazolylacetate was synthesized by a more convenient procedure by treatment of ethyl 2-thienylcarbamidoacetate with phosphorus pentachloride followed by a cycloaddition of the azide ion in dimethylformamide.

The IR spectra of cephalosporins *IIIa—IIIj*, *Va*, *Vb* disclosed in line with the literature [6] stretching vibrations of carbonyl groups at $\tilde{\nu} = 1542—1772\ cm^{-1}$ proving the integrity of the β-lactam ring ($\tilde{\nu} = 1760—1772\ cm^{-1}$), the presence of a secondary amide at C-7 of the cephem skeleton ($\tilde{\nu} = 1673—1681$ and $1542—1558\ cm^{-1}$), a free carboxylic acid at C-4 ($\tilde{\nu} = 1619—1623\ cm^{-1}$) and a free acetoxymethyl group at C-3 ($\tilde{\nu} = 1738—1746\ cm^{-1}$), which tends to form an unwanted γ-lactone [7]. All cephalosporins prepared showed absorption bands ascribable to stretching vibrations of N—H bonds at $\tilde{\nu} = 3291—3304\ cm^{-1}$ and C—O bonds of the ester groups at $\tilde{\nu} = 1232—1248\ cm^{-1}$

Test of *in vitro* antimicrobial activity of cephalosporins *IIIa—IIIj*, *Va*, *Vb* showed that their effects equal those of cephalosporin antibiotics of the first generation as *e.g.* Cefalotin, Cefamandol, and Cefazolin; they are effective against *Bacillus subtilis*, *B. cereus*, *B. pumilis*, *Sarcina lutea*, *S. subflava*, *Escherichia coli*, *Staphylococcus aureus*, *S. epidermis*, and *Streptococcus pyogenes*. Their common feature is a low activity against *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *P. vulgaris*.

Experimental

Melting points were determined on a Kofler micro hot-stage, the IR spectra of cephalosporins (3 mg of the substance in 200 mg KBr) were recorded in the range $\tilde{\nu} = 500—3800\ cm^{-1}$ with a Perkin—Elmer, model 457 spectrophotometer.

7-Aminocephalosporanic acid was submitted by the Drug Research Institute, Modra, 2-thienylcarboxylic acid is a commercial product of Merck—Schuchardt. 5-(2-Thienyl)--1-tetrazolylacetic acid was obtained by an alkaline hydrolysis of the appropriate ethyl ester according to [5] in methanol.

The new cephalosporin antibiotics were tested *in vitro* against twelve Gram-negative and Gram-positive microorganisms (The Czechoslovak Collection of Microorganisms, the J. E. Purkyně University, Brno) by a dilution method according to *Barry* [8]. Their antimicrobial activity was expressed as minimal inhibitory concentrations in μg of the compound tested in 1 cm^3 of the culture medium (MIC, $\mu g\,cm^{-3}$) and the effect was compared with that of the reference cephalosporin antibiotics (Cefalotin, Cefamandol, Cefazolin — Eli Lilly, USA) (Table 1).

Table 1

In vitro antibacterial activities of cephalosporin antibiotics Cefalotin (CEL), Cefamandol (CEM), and Cefazolin (CEZ)

Microorganism	MIC/(μg cm^{-3})		
	CEL	CEM	CEZ
Bacillus subtilis Bs 5/58	0.125	0.25	0.125
Bacillus cereus Bcm 4/58	0.125	0.125	0.125
Bacillus pumilis Bac 2/65	0.125	0.125	0.125
Sarcina lutea Sar 5/58	0.125	0.125	2
Sarcina subflava Sar 6/58	0.25	0.125	1
Escherichia coli Eck 67/59	0.25	0.25	0.5
Staphylococcus aureus Mau 78/71	32	32	0.125
Staphylococcus epidermis M 12/63	0.25	0.5	16
Streptococcus pyogenes A 1/49	0.5	0.5	128
Pseudomonas aeruginosa Ps 133/72	128	128	128
Proteus mirabilis Prmi 7/44	128	1	4
Proteus vulgaris PrH 4/42	128	16	32

5-Substituted 1- resp. 2-tetrazolylacetyl chlorides Ia—Ii, IVa, IVb

A mixture of 5-substituted 1- or 2-tetrazolylacetic acid (10 mmol) and phosphorus pentachloride (2.10 g; 10 mmol) was heated in a water bath tempered to 60 °C and stirred for 15 min. The solution was evaporated to dryness, benzene (10 cm^3) was added and the evaporation was repeated to remove the residues of phosphorus oxychloride. The evaporation residue was extracted into dichloromethane (20 cm^3), the solution was filtered, the solvent was removed and the crude chloride was crystallized from a convenient solvent.

The characteristic data of chlorides *Ia*, *Ib*, *IVa*, *IVb* are listed in Table 2. Chlorides *Ic—Ii* are crystalline products, which were not purified, but directly used for *N*-acylation of 7-aminocephalosporanic acid.

5-Substituted sodium 7-(1- resp. 2-tetrazolylacetamido)-3-acetoxycephalosporanates IIIa—IIIi, Va, Vb

To a cooled (0 °C) suspension of 7-aminocephalosporanic acid (1.36 g; 5 mmol) in dichloromethane (25 cm^3) triethylamine (0.84 cm^3; 6 mmol) was added with stirring till the acid dissolved (30—50 min). Triethylamine (0.73 cm^3; 5.25 mmol) was once more added to a filtered and to −5 °C cooled solution to which the respective substituted tetrazolylacetyl chloride (5 mmol) in dichloromethane (10 to 25 cm^3) was added at −5 —0 °C. The mixture was stirred at 0 °C for 1 h and at an ambient temperature for

Table 2

Characteristic data of 5-substituted 1- resp. 2-tetrazolylacetyl chlorides *Ia*, *Ib*, *IVa*, *IVb*

Compound	R^1	Formula	M_r	w_i(calc.)/% / w_i(found)/%			Yield	M.p. °C	$\tilde{\nu}$(ν(C=O)$_{carbonyl}$)
				C	H	N	%	Solvent	cm^{-1}
Ia	2-Furyl	$C_7H_5ClN_4O_2$	212.6	39.55	2.37	26.35	91	103—104	1755
				39.61	2.30	26.48		Hexane	
IVa	2-Furyl	$C_7H_5ClN_4O_2$	212.6	39.55	2.37	26.35	86	84—86	1756
				39.69	2.46	26.31		Hexane	
Ib	5-Bromo-2-furyl	$C_7H_4BrClN_4O_2$	291.5	28.84	1.38	19.22	90	131—132	1755
				28.75	1.44	19.10		Heptane	
IVb	5-Bromo-2-furyl	$C_7H_4BrClN_4O_2$	291.5	28.84	1.38	19.22	82	102—103	1754
				28.68	1.33	19.30		Hexane	

Table 3

Characteristics of cephalosporins *IIIa*—*IIIj*, *Va*, *Vb*

Compound	Formula	M_r	w_i(calc.)/% w_i(found)/%			Yield	M.p.	$\tilde{\nu}$(ν(C=O)$_{lactam}$)
			C	H	N	%	°C	cm^{-1}
IIIa	$C_{17}H_{15}N_6NaO_7S$	470.4	43.40	3.20	17.86	67	195—197	1760
			43.58	3.18	17.94			
IIIb	$C_{17}H_{14}BrN_6NaO_7S$	549.3	37.17	2.56	15.29	64	231—232	1765
			36.91	2.69	14.93			
IIIc	$C_{23}H_{19}N_6NaO_7S_2$	578.6	47.74	3.31	14.52	65	182—184	1760
			47.97	3.50	14.71			
IIId	$C_{24}H_{21}N_6NaO_7S_2$	592.6	48.64	3.57	14.18	63	167—170	1762
			48.47	3.58	14.01			
IIIe	$C_{24}H_{21}N_6NaO_8S_2$	608.6	47.36	3.47	13.80	59	215—218	1759
			47.04	3.51	13.67			
IIIf	$C_{23}H_{18}ClN_6NaO_7S_2$	613.0	45.06	2.96	13.70	63	177—179	1756
			44.78	3.03	13.43			
IIIg	$C_{23}H_{18}FN_6NaO_7S_2$	596.6	46.30	3.04	14.08	61	213—216	1757
			46.64	2.87	13.84			
IIIh	$C_{24}H_{21}N_6NaO_9S_2$	624.6	46.15	3.39	13.46	59	190—193	1765
			45.89	3.45	13.21			
IIIi	$C_{23}H_{18}ClN_6NaO_9S_2$	645.0	42.83	2.81	13.03	60	230—232	1761
			42.68	2.89	13.23			
IIIj	$C_{17}H_{16}N_6O_6S_2$	464.5	43.96	3.47	18.09	62	166—168	1772
			44.06	3.38	17.98			
Va	$C_{17}H_{15}N_6NaO_7S$	470.4	43.40	3.20	17.86	65	181—183	1767
			43.61	3.12	17.86			
Vb	$C_{17}H_{14}BrN_6NaO_7S$	549.3	37.17	2.56	15.29	63	223 224	1764
			36.98	2.51	15.17			

another 1 h. Dichloromethane was distilled off, the residue was dissolved in water (30 cm^3) overlayed with ethyl acetate (100 cm^3) and acidified with HCl (c = 4 mol dm^{-3}) to pH = 2. The acetate layer was separated, dried with sodium sulfate and evaporated to dryness. The crude *N*-substituted amides were dissolved in a mixture of methanol (20 cm^3) and acetone (10 cm^3) at 40 °C; sodium acetate (0.41 g; 5 mmol) in methanol (5 cm^3) was added to the solution, which was then concentrated to one fourth of its original volume. Ethanol (25 cm^3) was added to the solution with partly separated crystals and the sodium salt of 5-substituted 7-(1- resp. 2-tetrazolylacetamido)-3--acetoxycephalosporanic acids thus precipitated was filtered off and dried. Characterization of these products and their *in vitro* antibacterial activities are presented in Tables 3 and 4.

Ethyl 2-thienylcarbamidoacetate

Triethylamine (15.29 cm^3; 0.11 mol) was added to a cooled solution (0 °C) of 2-thienylcarboxylic acid (12.81 g; 0.1 mol) in acetone (350 cm^3). 2,2-Dimethylpropionyl chloride (13.26 cm^3; 0.11 mol) in acetone (50 cm^3) was added to the previous solution cooled to −10 °C; at this temperature the mixture was stirred for 0.5 h, the separated triethylammonium chloride was filtered off and the filtrate was evaporated to dryness at a temperature not exceeding 30 °C. The oily residue of the mixed anhydride was dissolved in chloroform (200 cm^3), cooled to −10 °C and stored at this temperature.

Triethylamine (17.95 cm^3; 0.13 mol) was added to a cooled (−5 °C) suspension of ethoxycarbonylmethylammonium chloride (13.95 g; 0.1 mol) in chloroform (200 cm^3) at

Table 4

In vitro antibacterial activities of cephalosporins *IIIa—IIIj*, *Va*, *Vb*

Microorganism	MIC/(μg cm^{-3})			
	IIIa	*IIIb*	*IIIc*	*IIId*
Bacillus subtilis Bs 5/58	0.125[a]	0.125[b]	0.125[c]	0.5[d]
Bacillus cereus Bcm 4/58	0.125	0.125	0.5	0.25
Bacillus pumilis Bac 2/65	0.125	0.125	0.125	0.25
Sarcina lutea Sar 5/58	0.125	0.125	0.25	0.25
Sarcina subflava Sar 6/58	0.25	0.125	0.125	0.25
Escherichia coli Eck 67/59	0.125	0.125	16	0.25
Staphylococcus aureus Mau 78/71	0.5	0.125	32	32
Staphylococcus epidermis M 12/63	16	128	128	128
Streptococcus pyogenes A 1/49	0.25	0.125	1	1
Pseudomonas aeruginosa Ps 133/72	128	128	128	128
Proteus mirabilis Prmi 7/44	128	128	128	>128
Proteus vulgaris PrH 4/42	>128	128	128	>128

a) 2.6×10^{-1} μmol dm^{-3}; *b*) 2.3×10^{-1} μmol dm^{-3}; *c*) 2.1×10^{-1} μmol dm^{-3}; *d*) 8.4×10^{-1} μmol dm^{-3}

Table 4 (Continued)

Microorganism	MIC/(μg cm^{-3})			
	IIIe	*IIIf*	*IIIg*	*IIIh*
Bacillus subtilis Bs 5/58	0.125	0.125	0.125	0.125
Bacillus cereus Bcm 4/58	0.125	0.125	0.125	0.125
Bacillus pumilis Bac 2/65	0.125	0.125	0.125	0.125
Sarcina lutea Sar 5/58	0.125	0.125	0.125	0.25
Sarcina subflava Sar 6/58	0.125	0.125	0.125	0.25
Escherichia coli Eck 67/59	0.125	64	64	2
Staphylococcus aureus Mau 78/71	0.125	0.5	0.25	0.25
Staphylococcus epidermis M 12/63	128	128	>128	128
Streptococcus pyogenes A 1/49	0.125	0.25	0.125	0.25
Pseudomonas aeruginosa Ps 133/72	128	128	128	128
Proteus mirabilis Prmi 7/44	128	>128	>128	128
Proteus vulgaris PrH 4/42	128	>128	>128	128

Table 4 (Continued)

Microorganism	MIC/(μg cm^{-3})			
	IIIi	*IIIj*	*Va*	*Vb*
Bacillus subtilis Bs 5/58	0.125	0.125	0.125	0.125
Bacillus cereus Bcm 4/58	0.125	0.125	0.25	0.125
Bacillus pumilis Bac 2/65	0.125	0.125	0.5	0.5
Sarcina lutea Sar 5/58	0.5	0.125	0.125	0.125
Sarcina subflava Sar 6/58	0.5	0.125	0.125	0.125
Escherichia coli Eck 67/59	2	0.125	0.5	0.5
Staphylococcus aureus Mau 78/71	1	0.125	0.125	0.125
Staphylococcus epidermis M 12/63	128	32	>128	128
Streptococcus pyogenes A 1/49	0.125	0.125	0.25	0.25
Pseudomonas aeruginosa Ps 133/72	128	128	64	128
Proteus mirabilis Prmi 7/44	128	128	128	128
Proteus vulgaris PrH 4/42	128	64	128	128

a temperature not exceeding 0 °C. The clear solution of the liberated base was added to the solution of mixed anhydride at −10 °C and stirred at this temperature for 30 min and at room temperature for 3 h. The chloroform solution was washed with an aqueous solution of hydrochloric acid ($c = 1.4$ mol dm^{-3}), water, sodium carbonate ($c = 0.5$ mol dm^{-3}) and water to neutral reaction. The chloroform layer was dried with sodium sulfate, the solvent was removed and the residue was crystallized from ethyl acetate with addition of petroleum ether to the first turbulence. Yield = 18.98 g (89 %), m.p. = 87—88 °C (Ref. [5] gives m.p. = 88—89 °C).

Ethyl 5-(2-thienyl)-1-tetrazolylacetate

Powdered phosphorus pentachloride (14.56 g; 70 mmol) was added to a solution of ethyl 2-thienylcarbamidoacetate (14.92 g; 70 mmol) in benzene (140 cm^3) with stirring. The temperature was adjusted to 75 °C during 2 min and was kept constant for 10 to 15 min. The mixture was evaporated to dryness, the crude imidoyl chloride was dissolved in dimethylformamide (70 cm^3) and this solution was added to a suspension of sodium azide (8.12 g; 126 mmol) in dimethylformamide (70 cm^3) during 45 min. The suspension was stirred for additional 30 min and the solvent was distilled off. Acetone (140 cm^3) was added to the hot residue, the inorganic salts were filtered off, the filtrate was evaporated to dryness and the oily residue was crystallized from ethyl acetate with addition of petroleum ether. Yield = 10.0 g (60 %), m.p. = 67—68 °C (Ref. [5] gives m.p. = 68—69 °C).

7-[5-(2-Thienyl)-1-tetrazolylacetamido]-3-acetoxycephalosporanic acid (IIIj)

Triethylamine (1.53 cm^3; 11 mmol) was added to a cooled (0 °C) solution of 5-(2-thienyl)-1-tetrazolylacetic acid (2.28 g; 10 mmol) in acetone (40 cm^3). A solution of 2,2-dimethylpropionyl chloride (1.33 cm^3; 11 mmol) in acetone (10 cm^3) was added to the previous solution cooled to −10 °C and stirred at this temperature for 30 min. The separated triethylammonium salt was filtered off, the solvent was distilled off at a bath temperature not exceeding 30 °C. The oily residue of the mixed anhydride was dissolved in dichloromethane (30 cm^3), cooled to −10 °C and stored at this temperature.

Triethylamine (1.7 cm^3; 12 mmol) was added to a suspension of 7-aminocephalosporanic acid (2.72 g; 10 mmol) in dichloromethane (50 cm^3) cooled to −5 °C and the mixture was stirred till all 7-aminocephalosporanic acid dissolved (30—50 min). Its filtrate was added to the 5-(2-thienyl)-1-tetrazolylacetic 2,2-dimethylpropionic anhydride at −10 °C. The mixture was stirred at −10 °C for 30 min and at room temperature for 2 h. Dichloromethane was distilled off, the residue was dissolved in water (40 cm^3), the aqueous layer was washed with ethyl acetate (2 × 20 cm^3), which removes the liberated 2,2-dimethylpropionic and the unreacted 5-(2-thienyl)-1-tetrazolylacetic acids. The separated aqueous layer was overlayed with ethyl acetate (50 cm^3) and acidified with hydrochloric acid ($c = 4$ mol dm^{-3}) to pH = 2 with stirring. The acetate portion was separated, dried with sodium sulfate, the solvent was removed and the residue was crystallized from ethanol. Yield = 2.88 g (62 %), m.p. = 166—168 °C.

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