SYNTHESIS AND ANTIMICROBIAL PROPERTIES OF 1-(3,4-DICHLOROPHENYL)CYCLOALKYL-1-PENICILLINS AND CEPHALOSPORINS

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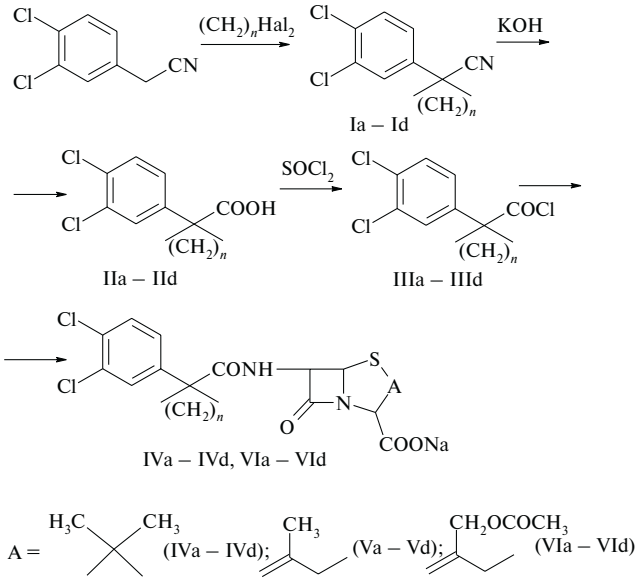
Based on the mathematical (QSAR) predictions for 1-arylcycloalkyl-1-penicillins, we may expect that introduction of substituents such as F, Cl, and OH into the aromatic nucleus of these compounds may lead to derivatives possessing lower toxicity and higher acid resistance [1, 2]. Previously [3], we synthesized semisynthetic penicillins and cephalosporins with Cl and Br substituents in *ortho*, *meta*, and *para* positions of the aromatic ring; the results of biological tests with these compounds showed that the above predictions are generally valid.

In order to continue studying the relationship between the chemical structure and biological properties of the aforementioned compounds, we modified the acyl residues of penicillins and cephalosporins by introducing two chlorine substituents into the aromatic nucleus and by varying the size of the cycloalkane ring.

The initial nitriles (Ia – Id) were synthesized by reactions of 3,4-dichlorophenylacetonitrile (i) with dibromoethane and 1,3-chlorobromopropane in 50% aqueous NaOH solution in the presence of triethylbenzylammonium chloride [4] or (ii) with 1,4-dibromobutane and 1,5-dibromopentane in the presence of powdered NaOH [5]. The subsequent alkaline hydrolysis of 1-(3,4-dichlorophenyl)cycloalkyl-1-carboxylic acid nitriles Ia – Id led to the corresponding carboxylic acids IIa – IId. Boiling carboxylic acids IIa – IId with thionyl chloride in anhydrous benzene yielded chloroanhydrides IIIa – IIId.

The related 1-(3,4-dichlorophenyl)cycloalkyl-1-penicillins (IVa – IVd) and -cephalosporins (Va – Vd, VIa – VId) were synthesized by acylating 6-aminopenicillanic (6-APA), 7-aminodeacetoxycephalosporanic (7-ADCA), and 7-aminocephalosporanic (7-ACA) acids using chloroanhydride (A) and mixed-anhydride (B) methods. The final products were isolated in the form of sodium salts.



n = 2 (a), 3 (b), 4 (c), 5 (d).

The proposed structures were confirmed by the data of elemental analyses and the results of IR and ^{1}H NMR spectroscopic measurements; purity of the reaction products was checked by TLC on Silufol plates. The IR spectra of compounds IV – VI showed the characteristic absorption bands of β-lactam carbonyl (1760 – 1780 cm^{-1}), carboxy carbonyl (1710 – 1725 cm^{-1}), amide carbonyl (1640 – 1660 cm^{-1}), and NH groups (3300 – 3340 cm^{-1}).

EXPERIMENTAL CHEMICAL PART

The IR spectra were recorded on an UR-20 spectrophotometer (Germany) using samples prepared as nujol mulls. The ^{1}H NMR spectra were measured on a Varian T-60 spectrometer using TMS as the internal standard. The course of the

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TABLE 1. The Yields and Characteristics of the Synthesized Nitriles Ia – Id and Acids IIa – IId

Compound	Yield, %	M.p.,°C; b.p.,°C/Torr	R_f	Empirical formula	^{1}H NMR spectrum in $CDCl_3$, δ, ppm
Ia	77	84 – 86	0.41	$C_{10}H_7Cl_2N$	1.16 – 2.0 (m, 4H, 2,3-CH_2), 7.0 – 7.6 (m, 3H, C_6H_3)
Ib	47	160 – 162/3	0.87	$C_{11}H_9Cl_2N$	1.0 – 3.3 (m, 6H, 2,3,4-CH_2), 7.0 – 7.7 (m, 3H, C_6H_3)
Ic	33	155 – 158/3	0.66	$C_{12}H_{11}Cl_2N$	1.7 – 2.2 (m, 8H, 2,3,4,5-CH_2), 6.9 – 7.1 (m, 3H, C_6H_3)
Id	63	186 – 187/3	0.58	$C_{13}H_{13}Cl_2N$	1.5 – 2.9 (m, 10H, 2,3,4,5,6-CH_2), 7.3 – 7.7 (m, 3H, C_6H_3)
IIa	71	144 – 145	0.61	$C_{10}H_8O_2Cl_2$	1.1 – 1.7 (m, 4H, 2,3-CH_2), 7.1 – 7.5 (m, 3H, C_6H_3), 9.5 (s, 1H, COOH)
IIb	62	84 – 86	0.76	$C_{11}H_{10}O_2Cl_2$	1.8 – 2.9 (m, 6H, 2,3,4-CH_2), 7.1 – 7.6 (m, 3H, C_6H_3), 9.2 (s, 1H, COOH)
IIc	82	118 – 120	0.72	$C_{12}H_{12}O_2Cl_2$	1.1 – 2.7 (m, 8H, 2,3,4,5-CH_2), 7.6 – 7.8 (m, 3H, C_6H_3), 9.4 (s, 1H, COOH)
IId	71	117 – 118	0.65	$C_{13}H_{14}O_2Cl_2$	1.1 – 2.3 (m, 4H, 2,3,4,5,6-CH_2), 7.2 – 7.5 (m, 3H, C_6H_3), 8.9 (s, 1H, COOH)

reactions was monitored by TLC on Silufol UV-254 plates (Czech Republic) eluted in the propanol – water, 7 : 2 (for sodium salts IV – VI), and acetone – hexane, 2 : 3 (for compounds I, II), systems and developed by treatment with iodine vapor. The data of elemental analyses coincide with the results of analytical calculations using empirical formulas.

1-(3,4-Dichlorophenyl)cycloalkane-1-carboxylic acid nitriles (Ia – Id). *Method A*. To a mixture of 0.2 mole of 3,4-dichlorophenylacetonitrile, 0.02 mole of triethylbenzylammonium chloride, and 50 ml of a 50% aqueous NaOH solution were slowly added 0.3 mole of dibromoalkane (n = 2, 3). Then the mixture was stirred for 2 h at 55 – 75°C and cooled. The precipitated oil was separated from the aqueous layer, after which the latter phase was doubly extracted with diethyl ether. The oil phase was combined with the ether extracts, washed with water, and dried over anhydrous sodium sulfate. Finally, residual ether was distilled to obtain the target compounds Ia and Ib (Table 1).

Method B. To a mixture of 0.2 mole of powdered sodium hydroxide and 0.05 mole of 3,4-dichlorophenylacetonitrile heated to 45°C were slowly added 0.05 mole of dibromoalkane (n = 4, 5) and the mixture was stirred for 12 h at 120°C. Upon cooling, the reaction mixture was diluted with water and extracted with diethyl ether. The ether extracts were washed with water and dried over anhydrous sodium sulfate. Finally, residual ether was distilled to obtain the target nitriles Ic and Id (Table 1).

1-(3,4-Dichlorophenyl)cycloalkane-1-carboxylic acids (IIa – IId). A mixture of 0.1 mole of the initial nitrile (Ia – Id) and 0.5 mole of potassium hydroxide in 120 ml of ethylene glycol was boiled for 10 h. Upon cooling, the reaction mixture was diluted with water and washed with diethyl ether. The aqueous layer was acidified (on cooling) with diluted aqueous hydrochloric acid (1 : 1) to pH 1 – 2. The precipitated crystals were filtered and washed with water (Table 1).

1-(3,4-Dichlorophenyl)cycloalkane-1-carboxylic acid chloroanhydrides (IIIa – IIId). A mixture of 0.061 mole of

TABLE 2. The Yields and Characteristics of the Synthesized Penicillins and Cephalosporins

Compound	Yield, %	M.p., °C* (with decomp.)	R_f	Empirical formula
IVa	56	135 – 137	0.70	$C_{18}H_{17}Cl_2N_2O_4SNa$
IVb	83	74 – 76	0.70	$C_{19}H_{19}Cl_2N_2O_4SNa$
IVc	70	116 – 118	0.78	$C_{20}H_{21}Cl_2N_2O_4SNa$
IVd	85	117 – 119	0.67	$C_{21}H_{23}Cl_2N_2O_4SNa$
Va	53	72 – 74	0.68	$C_{18}H_{15}Cl_2N_2O_4SNa$
Vb	42	168 – 170	0.68	$C_{19}H_{17}Cl_2N_2O_4SNa$
Vc	66	164 – 165	0.70	$C_{20}H_{19}Cl_2N_2O_4SNa$
Vd	50	188 – 189	0.76	$C_{21}H_{21}Cl_2N_2O_4SNa$
VIa	40	109 – 111	0.67	$C_{20}H_{17}Cl_2N_2O_6SNa$
VIb	36	84 – 186	0.67	$C_{21}H_{19}Cl_2N_2O_6SNa$
VIc	31	44 – 45	0.71	$C_{22}H_{21}Cl_2N_2O_6SNa$
VId	38	124 – 126	0.77	$C_{23}H_{23}Cl_2N_2O_6SNa$

* Melting points are indicated for acids.

TABLE 3. Antimicrobial Activity of the Synthesized Penicillins and Cephalosporins

Compound	MIC, μg/ml						
	St. aureus			*Sh. dysint.*		*B. typhi* 79	*P. vulg.*
	209 p	25923	Smith	Г6858	121		
IVa	0.9	0.9	1.9	125	250	62.5	250
IVb	1.9	3.9	3.9	250	125	125	250
IVc	7.8	3.9	15.6	250	250	250	250
IVd	1.9	3.9	1.9	250	250	125	250
Benzylpenicillin	0.012	0.006	0.012	3.12	7.0	0.39	3.12
Va	3.9	1.9	7.8	31.2	62.5	62.5	62.6
Vb	1.9	1.9	3.9	62.5	62.5	125	125
Vc	7.8	3.9	7.8	31.2	62.5	31.2	62.5
Vd	3.9	3.9	7.8	62.5	125	62.5	125
Cefalexin	7.8	1.56	7.8	3.9	7.8	7.8	62.5
VIa	1.9	3.9	7.8	62.5	125	7.8	31.2
VIb	0.9	3.9	1.9	125	62.5	–	125
VIc	0.9	1.9	0.9	62.5	62.5	3.9	62.5
VId	3.9	7.8	1.9	250	125	–	125
Cefamezin	0.12	0.12	0.48	15.6	31.2	7.8	31.2

acid IIa – IId and 0.125 mole thionyl chloride in anhydrous benzene was boiled for 8 h. Upon cooling, the solvent is distilled off and the residue of chloroanhydride IIIa – IIId is used in the subsequent stage without additional purification.

1-(3,4-Dichlorophenyl)cycloalkyl-1-penicillins (IVa – IVd) and 1-cephalosporins (Va – Vd, VIa – VId). *Method A*. To a mixture of 0.01 mole of 6-APA (7-ADCA, 7-ACA), 0.033 mole $NaHCO_3$, 80 ml water, and 60 ml acetone, cooled to 0 – 2°C, was added with stirring 0.01 mole of the corresponding carboxylic acid chloroanhydride IIIa – IIId in 20 ml of anhydrous acetone. Upon completely adding the chloroanhydride, the stirring was continued for 3 – 4 h at the same temperature and then 2 h at room temperature. Then the aqueous solution was washed with ethyl acetate and acidified with 1 N HCl solution to pH 2. The precipitated acid was extracted with ethyl acetate, washed with ice-cold water, and dried over anhydrous sodium sulfate. Then the ethyl acetate extract was treated with an 8% aqueous $NaHCO_3$ solution to pH 7 – 7.5. The aqueous layer was washed with ether and lyophilized to obtain the target sodium salts IV – VI.

Method B. To a solution of 0.01 mole of acid IIa – IId in 60 ml of anhydrous acetone was added, with stirring and cooling to 0 – 2°C, a solution of 0.026 mole triethylamine in 40 ml of the same solvent and 0.028 mole of ethyl chlorocarbonate. The reaction mixture was stirred for 30 min at 0°C, then 2 h at room temperature, and filtered. The filtrate was slowly added to a solution of 0.26 mole of 6-APA (7-ADCA, 7-ACA) in 100 ml of acetone and 200 ml of 3% $NaHCO_3$. The mixture was stirred for 4 h and then treated as in method A. The melting temperatures were determined for the acids (Tafle 2).

EXPERIMENTAL BIOLOGICAL PART

The antibacterial activity of the synthesized sodium salts of penicillins IVa – IVd and cephalosporins Va – Vd and VIa – VId was studied by the conventional method of double serial dilutions in a beef-infusion broth (pH 7.2 – 7.4) at a bacterial load of 2×10^6 microbial cells/ml. The tests were performed with standard strains of both Gram-positive (*Staphylococcus aureus* 209p, Smith, 25923) and Gram-negative bacteria (*Bacillus typhi*, *Shigella dysinteriae*, *Proteus vulgaris*). The reference drugs were benzylpenicillin, cefalexin, and cefamezin. Each test was repeated not less than thrice.

The acid resistance of penicillins IVa – IVd was studied in an water – ethanol mixture at pH 1.3 and a temperature of 37°C. The amount of undecomposed penicillin was determined by iodometric titration. The rate of penicillin decomposition was characterized by halflife $\tau/2$.

It was found that penicillins IVa – IVd exhibit an antistaphylococcal activity (MIC = 0.9 – 3.9 μg/ml). The most pronounced antibacterial effect was observed for compound IVa (Table 3). At the same time the penicillins showed low activity with respect to Gram-negative microbes (MIC = 62.5 – 500 μg/ml). The halflife of the penicillins studied was $\tau/2$ = 2 – 5 min (versus 2 min for benzylpenicillin), which is evidence of the instability of these compounds in acid media. A comparison of the properties of penicillins IVa – IVd to those of monochlorophenylcycloalkyl penicillins [5] indicates that introduction of the second chlorine into the aromatic ring does not increase the antibacterial properties of these compounds.

The derivatves of 7-ADCA (Va – Vd) also exhibited an antibacterial activity with respect to *St. aureus* (MIC = 1.9 – 7.8 μg/ml) that was comparable to the activity of cefalexin. The deriatves of 7-ADCA (Va – Vd), in contrast to monochlorophenylcycloalkyl cephalosporins [5], exhibited antibacterial properties relative to Gram-negative bacterial species as well. The semisynthetic cephalosporins representing the 7-ACA derivatives VIa – VId were also active with respect to the test microbes, although the antibacterial effect was less pronounced as compared to that of cefamezin.

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