SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF 1-ALKOXYPHENYLCYCLOALKYL-1-DEACETOXYCEPHALOSPORINS

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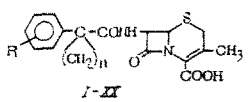
In previous papers we have described the synthesis and biological properties of a large series of 1-alkoxyphenylcycloalkyl-1-penicillins, amongst which we have found compounds with distinct antibacterial activity [2-4].

During investigations in the field of β-lactam antibiotics it was of interest to look into the change in antibacterial properties of cephalosporin derivatives having at the 7 position of the cephem nucleus analogous cycloaliphatic substituents in which the number of methylene groups varied from two to five.

Therefore, we have synthesized 1-alkoxyphenylcycloalkyl-1-deacetoxycephalosporanic acids (I-XX) by acylation of 7-aminodeacetoxycephalosporanic acid (XXV) with the earlier described cycloaliphatic acids by means of the mixed anhydride method. Desired acids I-XX were isolated as the sodium salts.

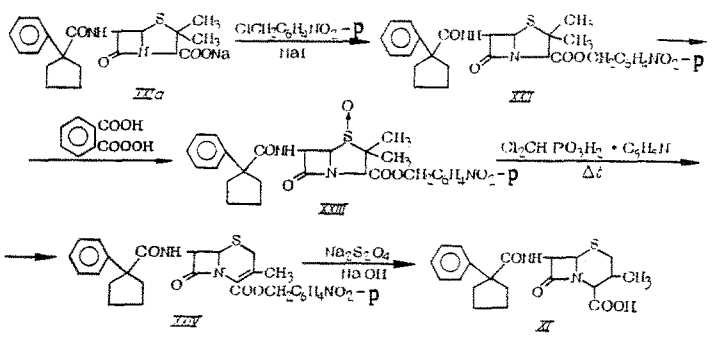


I-XX

I $n=2$, R = H; II $n=2$, R = m-OCH_3; III $n=2$, R = p-OCH_3; IV $n=2$, R = o-OC_2H_5; V $n=2$, R = o-OC_3H_7; VI $n=2$, R = p-OC_3H_7; VII $n=2$, R = o-OC_4H_9; VIII $n=3$, R = p-OC_3H_7, IX $n=3$, R = o-OC_4H_9; X $n=3$, R = p-OC_4H_9; XI $n=4$, R = H; XII $n=4$, R = p-OCH_3; XIII $n=4$, R = p-OC_2H_5; XIV $n=4$, R = p-OC_3H_7; XV $n=4$, R = p-OC_3H_7; XVI $n=4$, R = p-OC_3H_7; XVII $n=4$, R = p-OC_4H_9; XVIII $n=4$, R = m-OC_4H_3; XIX $n=4$, R = p-OC_4H_9; XX $n=5$, R = p-OC_2H_5.

We have also studied the possibility of chemical transformation of 1-phenylcyclopentyl-1-penicillin (XXI) [4], with the result that another synthesis of XI has been realized. That method consists of thermal rearrangement of an ester of the penicillin sulfoxide to the deacetoxycephalosporin [10] and finds wide application in the production of cephalosporin antibiotics [8, 9].

As starting compound for the transformation, which proceeds as depicted in the scheme below, we used the p-nitrobenzyl ester of the penicillin (XXII), which was prepared by reaction of sodium salt XXI with p-nitrobenzyl chloride in the presence of a catalytic amount of sodium iodide.



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Oxidation of XXII with an ethereal solution of monoperphthalic acid yields the p-nitrobenzyl ester of 1-phenylcyclopentyl-1-penicillin sulfoxide (XXIII). Transformation of the latter to the p-nitrobenzyl ester of the deacetoxycephalosporin (XXIV) was carried out under various conditions described in the literature [7, 8], but the best results are obtained by using the monopyridinium salt of dichloromethylphosphonic acid as catalyst and absolute dioxane as solvent [5].

Formation of the six-membered dihydrothiazine ring was proved by the PMR spectrum, in which the signals of the protons of the CH_3 group at $C_{(2)}$ and the CH group at $C_{(3)}$ of XXIII are absent, and in which a singlet at 2.1 ppm (3H) and a multiplet having its center at 3.25 ppm (2H) appear, which correspond with the methyl group at the double bond and the cyclic 2-methylene group (AB spin system).

Removal of the protective p-nitrobenzyl group by reduction of XXIV with sodium dithionite [6] leads to 1-phenylcyclopentyl-1-deacetoxycephalosporin (XI), the physicochemical constants, IR and PMR spectra of which correspond with the data of XI prepared by acylation of XXV.

EXPERIMENTAL CHEMICAL

IR spectra were recorded on a UR-20 (GDR) spectrophotometer from suspensions in paraffin oil. PMR spectra were taken on a Varian T-60 (Switzerland), using TMS as internal standard. Control of the course of the reaction and analysis of the individual compounds was carried out by means of TLC on Silufol UV-254 plates (Czechoslovakia) in the systems tert-butanol—ether—acetone 10:9:3 (A), benzene—ethyl acetate 2:1 (B), and propanol—water 7:3 (C).

1-Alkoxyphenylcycloalkyl-1-deacetoxycephalosporins (I-XX). To 0.02 mole of 1-alkoxyphenylcycloalkane-1-carboxylic acid [2-4] in 50 ml of absolute acetone is added at 0-2°C 2.4 g (0.024 mole) of triethylamine in 25 ml of absolute acetone and 3 g (0.028 mole) of ethyl chloroformate in 20 ml of absolute acetone. The mixture is stirred at 20°C for 2 h, the precipitate is filtered off, and the filtrate is added with stirring to 2.4 g (0.02 mole) of XXV in 100 ml of acetone and 200 ml of a 3% sodium bicarbonate solution. The mixture is stirred for 3-4 h and extracted with ethyl acetate. The extract is discarded, and the aqueous layer is covered with ethyl acetate and acidified at 5-7°C with 1 N hydrochloric acid to pH 2-2.5. The ethyl acetate layer is extracted with ice water dried over anhydrous sodium sulfate, and treated with an 8% solution of sodium bicarbonate to pH 7-7.5. The aqueous layer is extracted with ethyl acetate and freeze dried. For determination of the physicochemical constants a small part of the sodium salt was converted to the acid (Table 1). III, IR spectrum, γ_{max}, cm^{-1}: 1760 (CO β-lactam), 1710 (CO acid), 1660 (CO amide). PMR spectrum (D_2O), δ, ppm: 1.0-1.7 (4H, m, CH_2-CH_2); 2.0 (3H, s, 3-CH_3); 3.3 (2H, m, AB system, J_{AB} 17.5 Hz, 2-CH_2); 3.82 (3H, s, OCH_3); 4.95 (1H, d, J 5Hz, 6-H); 5.55 (1H, d, J 5Hz, 7-H); 7.1 and 7.45 (each 2H, AABB system, C_6H_4). XI, IR spectrum, γ_{max}, cm^{-1}: 1775 (CO β-lactam), 1725 (CO acid), 1640 (CO amide), 3340 (NH). PMR spectrum (C_5D_5N), δ, ppm: 1.45-3.15 [8H, m, $(CH_2)_4$]; 2.1 (3H, s, CH_3); 3.3 (2H, m, AB system, J_{AB} 18 Hz, 2-CH_2); 5.15 (1H, d, J 5 Hz, 6-H); 6.1 (1H, dxd, $J_{5,6}$ 5Hz, $J_{5,NH}$ 9 Hz, 7-H); 7.2-7.6 (5H, m, Ph); 10.7 and 10.9 (each 1 H, m.s., NH and OH).

p-Nitrobenzyl ester of 1-Phenylcyclopentyl-1-penicillin (XXII). A mixtute of 4.1 g (0.01 mole) of the sodium salt of XXI (XXIa), 0.37 g of dry sodium iodide, and 1.7 g (0.01 mole) of p-nitrobenzyl chloride [1] in 80 ml of absolute dimethylformamide is stirred at 20°C for 30-36 h, poured out into 250 ml of ethyl acetate, and extracted in succession with cooled water, 3% sodium bicarbonate solution, and water. The ethyl acetate layer is separated, dried over anhydrous sodium sulfate, and evaporated to dryness under vacuum. The oily residue obtained crystallizes on trituration with petroleum ether and is dried in a vacuum desiccator. Yield: 4.8 g (92.3%) of XXII, mp 105-106°C; R_f 0.75 (system A). Found %: N 8.30, S 6.21. M^+ (mass spectrophotometrically) 523. $C_{27}H_{29}N_3O_6S$. Calculated, %: N 8.06, S 6.12. IR spectrum, γ_{max}, cm^{-1}: 1778 (CO β-lactam), 1742 (CO ester), 1672 (CO amide), 1350 (NO_2). PMR spectrum ($CDCl_3$), δ, ppm: 1.37 [6H, s, 2-$(CH_3)_2$], 1.5-2.8 [8H, m, $(CH_2)_4$], 4.4 (1H, s, 3-H), 5.23 (2 H, s, $COOCH_2$), 5.4-6.0 (3 H, m, 5-H, 6-H, and NH), 7.3 (5 H, s, Ph , 7.5 and 8.18 (each 2 H, m, AABB system, C_6H_4).

p-Nitrobenzyl Ester of 1 Phenylcyclopentyl-1-penicillin Sulfoxide (XXIII). To 4.8 g (0.009 mole) of XXII in 40 ml of absolute chloroform is added dropwise at a temperature not exceeding 10°C a freshly prepared solution of monoperphthalic acid [11]. The end of the reaction is determined by means of chromatography in system A; the total oxidation time is 1-1.5 h.

TABLE 1. 1-Alkoxyphenylcycloalkyl-1-deacetoxycephalosporins

Compound	Yield: %	Mp, °C (with dec.)	R_f^*	Found, %		Empirical formula	Calculated, %	
				N	S		N	S
I	75,5	78—79	0,68	7,64	8,72	$C_{18}H_{18}N_2O_4S$	7,84	8,95
II	72,4	90—95	0,76	7,07	8,30	$C_{19}H_{20}N_2O_5S$	7,21	8,25
III	75,0	104—105	0,65	7,02	7,92	$C_{19}H_{20}N_2O_5S$	7,21	8,25
IV	62,8	64—66	0,65	6,68	7,88	$C_{20}H_{22}N_2O_5S$	6,96	7,96
V	60,5	128—130	0,70	6,82	7,74	$C_{21}H_{24}N_2O_5S$	6,72	7,69
VI	73,5	100—101	0,60	6,61	7,38	$C_{21}H_{24}N_2O_5S$	6,72	7,69
VII	70,4	102—103	0,72	6,80	7,08	$C_{22}H_{26}N_2O_5S$	6,50	7,44
VIII	68,2	125—126	0,67	7,05	7,17	$C_{22}H_{26}N_2O_5S$	6,50	7,44
IX	63,5	119—120	0,60	6,56	7,00	$C_{23}H_{28}N_2O_5S$	6,30	7,21
X	68,8	178—180	0,68	6,06	7,50	$C_{23}H_{28}N_2O_5S$	6,30	7,21
XI	78,5	199—200	0,60	7,33	8,59	$C_{20}H_{22}N_2O_4S$	7,24	8,29
XII	74,5	102—103	0,58	6,46	8,16	$C_{21}H_{24}N_2O_5S$	6,72	7,69
XIII	69,8	72—73	0,54	6,85	7,41	$C_{22}H_{26}N_2O_5S$	6,50	7,44
XIV	67,5	178—180	0,60	6,50	7,17	$C_{23}H_{28}N_2O_5S$	6,30	7,21
XV	70,4	165—166	0,62	6,15	7,55	$C_{23}H_{28}N_2O_5S$	6,30	7,21
XVI	72,8	104—105	0,62	6,68	7,22	$C_{23}H_{28}N_2O_5S$	6,30	7,21
XVII	67,8	79—80	0,58	5,78	6,70	$C_{24}H_{30}N_2O_5S$	6,10	6,99
XVIII	75,5	62—63	0,60	5,83	6,63	$C_{24}H_{30}N_2O_5S$	6,10	6,99
XIX	70,2	120—121	0,62	6,18	7,08	$C_{24}H_{30}N_2O_5S$	6,10	6,99
XX	63,5	175—176	0,65	6,13	7,15	$C_{23}H_{28}N_2O_5S$	6,30	7,21

Note. *TLC of the sodium salts of I-XX in system C.

The precipitated phthalic acid is filtered off, and the filtrate is extracted with a cooled 3% sodium bicarbonate solution and with water. The organic layer is separated, dried over anhydrous sodium sulfate, and vacuum evaporated. The oily residue crystallizes on trituration with absolute ether; the crystals are reprecipitated from an ethyl acetate solution with petroleum ether to obtain 3.5 g (71.4%) of XXIII, mp 149-150°C, R_f 0.78 (system B). Found, %: N 8.06, S 5.92. $C_{27}H_{29}N_3O_7S$. Calculated, %: N 7.78, S 5.93. IR spectrum, ν_{max}, cm^{-1}: 1790 (CO β-lactam), 1760 (CO ester), 1690 (CO amide), 1355 (NO_2), 1030-1040 (S → O). PMR spectrum ($CDCl_3$), δ, ppm: 1.1 and 1.6 [each 3H, s, 2-$(CH_3)_2$], 1.5-2.8 [8 H, m, $(CH_2)_4$], 4.6 (1 H, 3, 3-H), 4.94 (1 H, d, $J_{5,6}$ 5 Hz, 5-H), 5.25 (2 H, s, $COOCH_2$), 5.9 (1 H, d × d, $J_{6,5}$ 5 Hz, $J_{6,NH}$ 10 Hz, 6-H , 6.95 (1 H, d, $J_{NH,6H}$ 10 Hz, NH), 7.28 (5 H, s, Bh , 7.5 and 8.17 (each 2 H, AABB system, C_6H_4).

p-Nitrobenzyl Ester of 1 Phneylcyclopentyl-1-deacetoxycephalosporin (XXIV). A mixture of 2.7 g (0.005 mole) of XXIII, 0.035 g of catalyst prepared by the method of [5], and 0.015 ml of dry pyridine in 10 ml of absolute dioxane is refluxed for 11-12 h. The end of the reaction is determined by chromatography in system B. The solution is evaporated under vacuum, the oily residue is dissolved in 20-30 ml hot ethanol, and filtered. On cooling crystals separate from the solution; these are filtered off, washed with cold ethanol, and recrystallized from acetonitrile. Yield 1.8 g (70.5%) of XXIV, mp 127-128°C, R_f 0.81 (system B). Found, %: N 8.40, S 5.98. M^+ 521 (mass spectrophotometrically). $C_{27}H_{27}N_3O_6S$. Calculated, %: N 8.05, S 6.14. IR spectrum, ν_{max}, cm^{-1}: 1760 (CO β-lactam), 1710 (CO ester), 1660 (CO amide), 1350 (NO_2). PMR spectrum ($CDCl_3$), δ, ppm: 1.5-2.8 [8 H, m, $(CH_2)_4$], 2.1 (3 H, s, CH_3), 3.25 (2 H, m, AB system, J_{AB} 18 Hz, 2-CH_2), 4.85 (1 H, d, $J_{5,6}$ 4.5 Hz, 6-H), 5.25 (2 H, s, $COOCH_2$), 5.6 (1 H, d × d, $J_{6,5}$ 4.5 Hz, $J_{6,NH}$ 9 Hz, 7-H), 5.9 (1 H, d, $J_{NH,6}$ 9 Hz, NH), 7.3 (5 H, s, Ph), 7.5 and 8.15 (each 2 H, m, AABB system, C_6H_4).

1-Phenylcyclopentyl-1-deacetoxycephalosporin (XI). To 1.5 g (0.0028 mole) of XXIV in a 1:1 mixture of acetonitrile and water is added a solution of sodium hydroxide to pH 7.5-8.0, then is added dropwise 1.2 g (0.007 mole) of sodium dithionite in 10 ml of 1 N NaOH in such a way that the temperature does not rise above 35-38°C. The reaction mixture is stirred at 20°C for 30 min, cooled to 5°C, filtered, and the filtrate is acidified to pH 4 with concentrated hydrochloric acid. After stirring for 15-20 min the precipitated crystals are filtered off, washed with a 4:1 mixture of acetonitrile and water, and dried in a vacuum desiccator. Yield: 0.8 g (72.7%) of XI, mp 197-199°C (dec.). Mixing the sample with a sample of XI prepared by the second method does not give melting point depression.

EXPERIMENTAL BIOLOGICAL

The antibacterial activity of substituted deacetoxycephalosporins I-XX was studied by the method of twofold dilution in beef-extract broth (pH 7.2-7.4) at a microbial load of $2 \cdot 10^6$ microbial cells per ml medium. In the experiments we used Gram-positive microorganisms: sen-

TABLE 2. Antibacterial Activity and Tolerance of Deacetoxycephalosporins I-XX

Compound	MIC, μg/ml				MTD mg/kg
	staphylococci			gram-negative bacilli*	
	sensitive strains		resistant strain S		
	209p	Smith			
I	7,8	15,6	62,5	>1000	>2000
II	1,9	1,9	125	>1000	2000
III	31,2	31,2	125	>1000	1500
IV	7,8	15,6	250	>1000	750
V	0,24	0,24	125	31,2—125	1500
VI	0,9	0,9	125	>1000	1000
VII	1,9	3,9	15,6	>1000	1000
VIII	15,6	31,2	31,2	>1000	>2500
IX	7,8	15,6	31,2	>1000	>2500
X	250	250	>500	>1000	>2000
XI	62,5	125	>500	>1000	>2500
XII	62,5	62,5	>500	>1000	>2000
XIII	125	125	>5000	>1000	>2000
XIV	250	250	250	from 1000 to > 1000	>2500
XV	62,5	62,5	250	from 1000 to > 1000	>2000
XVI	125	125	>500	>1000	>2000
XVII	250	250	>500	>1000	>2000
XVIII	125	125	>500	>1000	>2000
XIX	125	125	500	>1000	>2500
XX	125	250	250	from 1000 to > 1000	>2500
Cephalexin	7,8	7,8	15,6	7,8—31,2	...

*The following microorganisms were used: *E. coli Sh. dysenteriae, Sh. flexneri* 6858, *Prot. vulgaris*, E. *typhy* 79.

sitive international strains of staphylococcus 209p and Smith, and penicillinase producing strain 5, and Gram-negative microorganisms: *E. coli* 0.55, *Sh. dysenteriae*, *Sh. flexneri* 6858, *Prot. vulgaris*, and *E. typhi* 79. For comparison and control, in each experiment we determined the minimal inhibitory concentration (MIC) of cephalexin together with those of the compounds under investigation. The maximal tolerance dose (MTD) was determined in experiments on white mongrel mice of both sexes weighing 19-20 g on single-dose intravenous administration. In total, 220 mice were used in these experiments.

DISCUSSION

Phenylcyclopropyldeacetoxycephalosporins I-VII show distinct antibacterial activity with regard to the sensitive staphylococci 209p and Smith (Table 2). The MICs of these compounds regardless of the size and position of the alkoxy group at the phenyl ring, range from 0.24 to 31.2 μg/ml. The highest activity is shown by the o-propoxy- and p-isopropoxyphenyl derivatives (V, VI), for which the MICs are 0.24 and 0.9 μg/ml, respectively. The values mentioned for the MICs of these cephalosporins are considerably larger than the MICs (0.012-0.048 μg/ml [3]) of cephalosporins studied earlier. Yet, compounds V and VI are more active than cephalexin. The other compounds of this group, with the exception of III, inhibit the growth of sensitive staphylococci at the same (I, IV) or lower (II, VII) concentrations than cephalexin does. Phenylcyclopropylcephalosporins I-VII do not inhibit the growth of the resistant staphylococcus, with the exclusion of o-butoxy derivative VII, which, just like cephalexin, inhibits the growth at a concentration of 15.6 μg/ml. Compound V, which is the most active one against sensitive staphylococci, also stands out in its activity against Gram-negative microorganisms, inhibiting their growth at concentrations of 31.2-125 μg/ml.

The MTDs of I-VII range from 750 to >2000 mg/kg. The least toxic is unsubstituted compound I. Introduction of an alkoxy group into the phenyl ring leads to an increase of toxic properties. It should be noted that in comparison with the penicillin analogs [3] compounds I-VII are four times less toxic.

Enlargement of the ring with one methylene group (VIII-X) is accompanied by lowering of the antibacterial action against sensitive staphylococci. MICs of the alkoxyphenylcyclobutylcephalosporins range from 7.8 to 250 μg/ml. The activity of the p-propoxy- and o-butoxyphenyl substituted compounds (VIII, IX) is close to that of cephalexin with regard to sensitive staphylococci. The same compounds have distinct activity against the resistant staphylococcus; the MIC is 31.2 μg/ml (a similar activity is shown by o-butoxyphenylcyclopropyl deriva-

tive VII). Changing over from cyclopropyl to cyclobutyl derivatives of cephalosporin results in lower toxicity of the latter, of which the MTD is >2500 mg/kg, which more than ten times surpasses the tolerance of the corresponding penicillin analogs [3].

Further enlargement of the ring with one methylene group (XI-XIX) is accompanied by lowering of the antibacterial activity and increase in tolerance (MTD 2500-3500 mg/kg). The toxicity of the phenylcyclopentylcephalosporins, just as those of the cyclopropyl and cyclobutyl derivatives mentioned above, is considerably lower than that of their penicillin analogs [4]. The cephalosporin with a six-membered ring (XX) is an inactive and nontoxic compound. All the deacetoxycephalosporins studied do not widen the antibacterial spectrum of activity.

As a result of the investigation of the 1-alkoxyphenylcycloalkyl-1-deacetoxycephalosporins it was established that an increase in the number of methylene groups in the ring is accompanied by lowering of the antibacterial activity against sensitive staphylococcus strains and increase of their tolerance. In comparison with the penicillin analogs, the cephalosporins are considerably less toxic.

Among the compounds investigated we have detected o-propoxy- and p-isopropoxyphenylcyclocephalosporin, which surpass cephalexin in their activity against sensitive strains.

Thus, the data obtained demonstrate the advisability of further studying other representatives of this series of cephalosporin derivatives.

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