As can be seen from Table 2 covering minimal inhibition of microbial growth, the required concentration of the test compounds against Staph. aureus, E. coli, Ps. aeruginosa, and Bac. subtilis was within the range of 17-27 μg/ml, and 7.6-26 μg/ml against Cand. albicans. Compounds XI-XVII turned out to be somewhat more active. The range for those compounds was 19-23 μg/ml. The highest level of activity was exhibited by compounds VIII-X containing alkyl and ortho-phenyl radicals on the nitrogen heteroatom (17-19 μg/ml). Modification of the molecule is accompanied by lowered antimicrobial activity.

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7-AMINOCEPHALOSPORAMIC ACID DERIVATIVES AND THEIR ANTIBACTERIAL PROPERTIES

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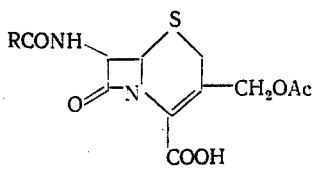
It is generally recognized that the purpose of goal-oriented research in the synthesis of semi-synthetic cephalosporins is to select substituents and their combinations to provide optimal biomedical properties. The literature indicates [4] that the substituent in the $C_{(3)}$ cephem ring plays a significant role, and also notes that with a substituent at the same $C_{(7)}$ radical, derivatives of 7-aminocephalosporanic acid (7-ACA) are more active than analogs of 7-amino-3-desacetoxycephalosporanic acid (7-ADCA).

As a continuation of our study of the relationship between chemical structure and biological activity for the purpose of increasing antibiotic activity, we undertook the synthesis of new semi-synthetic cephalosporins (I-XV) which differ from the previously synthesized compounds [1] of this class by the presence of an acetoxymethyl group in position 3 instead of a methyl group.



I—XV

I: R=$CH_2C_6H_3(OMe)_2$-3,4; II: R=$CH_2C_6H_3$OMe-3-OEt-4; III: R=$CH_2C_6H_3$-OMe-3-OPr-4; IV: R=$CH_2C_6H_3$OMe-3-OPr-iso-4; V: R=$CH_2C_6H_3$OMe-3-Bu-4; VI: R=2-bromofuryl-5; VII: R=menthoxymethyl; VIII: R=(menthoxy)(di-tert-butyl)methyl; IX: R=C_6H_4OPr-4-cyclopropyl-1; X: R=C_6H_4OPr-2-cyclopropyl-1; XI: R=C_6H_4OEt-2-cyclopropyl-1; XII: R=C_6H_5-cyclopentyl-1; XIII: R=C_6H_4OMe-4-cyclopentyl-1; XIV: R=C_6H_4OPr-iso-4-cyclopentyl-1; XV: R=C_6H_4OBu-3-cyclopentyl-1.

The starting substance for the synthesis of this group of compounds was 7-ACA which was acylated by the chloroanhydride method (A) to obtain compounds I-VIII, and by the mixed anhydride method (B) to obtain compounds IX-XV. The synthesis of the acylating agents has been described earlier [2, 3].

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TABLE 1. Properties of 7-Substituted Cephalosporanic Acids I-XV

Compound	Yield, %	mp, °C (decomp.)	R_f	Found, %		Empirical formula	Calculated, %	
				N	S		N	S
I	49,8	62—3	0,51	5,85	7,45	$C_{20}H_{22}N_2O_8S$	6,21	7,11
II	53,5	59—60	0,50	6,25	6,75	$C_{21}H_{25}N_2O_8S$	6,06	6,90
III	62,3	70—2	0,52	6,00	6,65	$C_{22}H_{26}N_2O_8S$	5,85	6,70
IV	53,0	68—70	0,54	5,46	7,05	$C_{22}H_{26}N_2O_8S$	5,85	6,70
V	68,9	79—80	0,53	5,42	6,20	$C_{23}H_{28}N_2O_8S$	5,71	6,50
VI	54,5	110	0,53	6,19	7,28	$C_{15}H_{13}N_2O_7SBr$	6,29	7,20
VII	38,4	59—60	0,51	5,85	6,38	$C_{22}H_{32}N_2O_7S$	5,97	6,83
VIII	60,0	113—5	0,50	5,00	5,80	$C_{30}H_{48}N_2O_7S$	4,82	5,52
IX	52,0	85—6	0,49	6,36	6,83	$C_{23}H_{26}N_2O_7S$	5,90	6,76
X	43,4	65—70	0,78	5,44	6,50	$C_{23}H_{25}N_2O_7S$	5,88	6,73
XI	61,4	62—4	0,57	6,57	7,11	$C_{22}H_{24}N_2O_7S$	6,08	6,96
XII	62,5	85—7	0,49	6,10	7,40	$C_{22}H_{24}N_2O_8S$	6,24	7,15
XIII	45,3	75—80	0,53	6,39	7,24	$C_{23}H_{26}N_2O_7S$	5,90	6,76
XIV	53,4	60—2	0,53	5,80	6,60	$C_{25}H_{30}N_2O_7S$	5,57	6,38
XV	52,3	55—60	0,53	5,81	6,40	$C_{26}H_{32}N_2O_7S$	5,41	6,20

The structure of the synthesized cephalosporins was confirmed by element analysis and IR-spectra data which indicated the presence of group C=O absorption bands at 1760-1780 cm^{-1} (β-lactam), 1710-1740 cm^{-1} (acetoxymethyl), 1670-1680 cm^{-1} (amide), and 1610-1625 cm^{-1} (carboxyl).

The purity and identity of compounds I-XV were confirmed by TLC.

EXPERIMENTAL (CHEMISTRY)

IR-spectra were recorded on a UR-20 spectrometer (GDR) in a petroleum jelly paste or KBr pellets. TLC was performed on Silufol UV-254 plates (Czechoslovakia) in a 4:3 acetone—hexane system. Iodine vapor was used as the developer.

7-Substituted Acetamidocephalosporanic Acids (I-VIII). Method A. A mixture of 2.7 g (0.01 mole) of 7-ACA, 2.5 g (0.03 mole) of $NaHCO_3$ in 80 ml of water and 60 ml of acetone was cooled to 0-2°C. A 0.01 mole portion of carboxylic acid anhydride in 20 ml of abs. acetone was added dropwise to the mixture while stirring. Stirring was continued at this temperature for 3 to 4 h after which the mixture was left overnight in a refrigerator. The acetone was vacuum-evaporated, the aqueous solution was washed with ethyl acetate (EA), and the organic layer was separated. A 50 ml portion of EA was added to the aq. solution which was then acidified with 1 N HCl to pH 2.0. The EA layer was then separated, washed with ice water, dried over anhydrous Na_2SO_4, and filtered. The cephalosporin acid was separated by evaporating the EA and triturating with hexane or petroleum ether (Table 1).

Method B (IX-XV). A 1.2 g (0.012 mole) portion of Et_3N in 25 ml of abs. acetone and 1.5 g (0.014 mole) of ClCOOEt in 20 ml of abs. acetone was added to 0.01 mole of 1-alkoxyphenylcycloalkane-1-carboxylic acid in 50 ml of abs. acetone at 0-2°C. The mixture was stirred for 2 h at room temperature and the resultant precipitate was filtered off. The filtrate was added upon stirring to 2.7 g (0.01 mole) of 7-ACA in 70 ml of acetone and 100 ml of a 3% aq. solution of $NaHCO_3$ which was then stirred for 3 to 4 h. Subsequent treatment followed the same procedure as in method A (see Table 1).

Sodium Salts of Compounds (I-XV). An 8% aq. $NaHCO_3$ solution was carefully added to the cephalosporin acid filtrate obtained above to bring the pH to 7.0-7.5. The aq. layer was then separated, washed with EA, and lyophilized.

EXPERIMENTAL (BIOLOGY)

The antibacterial activity of cephalosporins I-XV in the form of sodium salts was tested by the series dilution method on a beef-extract broth (pH 7.2-7.4) at a microbial load of $2 \cdot 10^6$ microbe bodies per 1 ml of medium. The compounds were tested against gram-positive Staphylococcus (penicillin-sensitive strains 25923, 209-P, Smith; penicillinase-forming strain 5) and gram-negative bacilli (Salmonella typhi, Shigella, Pseudomonas pyocanea, and Proteus).

In all of the experiments we compared the minimum suppressive concentration (MSC) of cephalothin to the compounds under examination. The experiments were run no less than three times. The maximum tolerated doses (MTD) were determined by iv injection of the test com-

TABLE 2. Antibacterial Activity of 7-Substituted Cephalosporins I-XV

Compound	Minimal suppressive concentration, μg/ml					
	Staphylococcus aureus				Sh. dysent. Flexneri 6858	S. typhi 79
	Smith	209-P	25923	5		
I	0,24	0,24	0,24	3,9	62,5	125
II	0,24	0,12	0,12	3,9	125	125
III	0,9	0,48	0,48	15,6	250	250
IV	0,48	0,12	0,12	0,9	125	125
V	7,8	3,9	7,8	15,6	125	125
VI	7,8	3,9	1,9	3,9	31,2	125
VII	0,24	0,12	0,12	0,48	31,2	62,5
VIII	15,6	7,8	7,8	31,2	250	250
IX	7,8	3,9	3,9	15,6	125	125
X	3,9	0,9	1,9	7,8	125	62,5
XI	1,9	1,9	1,9	3,9	125	250
XII	15,6	15,6	15,6	31,2	125	125
XIII	7,8	3,9	3,9	15,6	62,5	125
XIV	15,6	7,8	7,8	15,6	62,5	62,5
XV	7,8	7,8	7,8	15,6	62,5	62,5
Cephalothin	0,12	0,06	0,06	0,48	31,2	7,8

pounds in acute experiments on white non-pedigreed mice weighing 18-20 g. A total of 80 animals were used.

As can be seen from Table 2, the dialkoxybenzyl- and methoxy-substituted cephalosporins I-IV and VII, just as cephalothin, exhibit pronounced antibacterial activity against Staphylococcus strains that are sensitive to benzylpenicillin. The MSC of compounds V and VIII is somewhat higher. They are followed in degree of antibacterial activity by the alkoxyphenylcyclopropyl derivatives IX-XI and the bromofuran derivative VI. The least active of the compounds were the alkoxyphenylcyclopentyl substituted XII-XV compounds.

The activity of the tested cephalosporins against the penicillinase-producing St. aureus 5 strain of Staphylococcus was the same as against the Staphylococci that do not form penicillinase. Compounds IV and VII, whose MSC were 0.9 and 0.48 μg/ml respectively, exhibited pronounced antibacterial activity which was equivalent to that of cephalothin.

Compounds I-XV at a concentration of 62.5-125 μg/ml were less effective than cephalothin in suppressing the growth of Shigella and Salmonella typhi, but like cephalothin, were not effective against Pseudomonas and Proteus, i.e., MSC > 250 μg/ml.

The most active cephalosporins I-IV and VII also turned out to be the least toxic: Their MTD were within the range of 1500-2000 mg/kg whereas the MTD of the less active compounds fell within the range of 200-1500 mg/kg.

The new 7-ACA derivatives I-XV were basically more active than their analogs, the derivatives of 7-ADCA [1] which were obtained from the same carboxylic acids. Compounds I-XV exhibited the greatest activity against penicillinase-forming Staphylococcus whose growth was suppressed at a concentration of 3.9-15.6 μg/ml whereas its growth was suppressed by the corresponding 7-ADCA derivatives at a concentration of 125-500 μg/ml. The extent to which the benzyl-substituted 7-ACA and 7-ADCA compounds were effective against sensitive Staphylococcus depended upon the substituents on the benzene ring. Thus, the MSC of compound VI against Staph. 209-P was 0.12 μg/ml whereas the MSC of the derivative 7-ADCA with the same substituent was 3.9 μg/ml. The bromofuran derivative of 7-ACA VI whose MSC was 1.9 μg/ml turned out to be much more active than its analog, and the MSC of the 7-ADCA was 500 μg/ml. A similar characteristic was observed in the case of the phenylcycloalkane-substituted cephalosporins.

Thus, our study of new semisynthetic cephalosporins obtained from 7-ACA demonstrated that these compounds exhibit pronounced antibacterial activity and a low level of toxicity. We have also shown that they exhibit a higher degree of anti-stephylococcal activity than the corresponding desacetoxycephalosporins.

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BIOLOGICAL ACTIVITY OF TRANSFORMED STEROIDS. XXIII.* 20-KETOPREGNANES CONDENSED WITH A THREE-MEMBERED HETEROCYCLIC RING AT THE 16α,17α-POSITION

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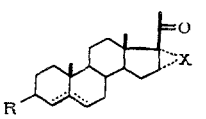
In continuation of our studies on the biological activity of systematic series of transformed steroids, we turned our attention to the series of 20-ketopregnanes (I-V), condensed at the 16α,17α-position with a three-membered ring (epoxy, epimine, episulfide) with Δ^5-3β-hydroxy, Δ^5-3β-acetoxy-, Δ^4-3-keto groupings in rings A and B.



R=OH (Ia, IIa, IIIa, IVa, Va), OAc (Ib, IIIb, Vb), O (Ic, IIb, IIIc, IVb); X=O (Ia-c), NH (IIa,b), NAc (IIIa-c), NCOOEt (IVa, b), S (Va,b); Δ^5 (Ia,b, IIa, IIIa,b, IVa, Va,b); Δ^4 (Ic, IIb, IIIc, IVb).

These compounds are starting materials in the synthesis of various biologically active 16α,17α-disubstituted 20-ketosteroids, including five-membered heterocycles [20], but they themselves have practically not been yet studied from the biological point of view. Meanwhile, it is known that while 16α,17α-epoxyprogesterone (Ic) has no appreciable gestagenic activity [18, 22], its carbocyclic analog, 16α,17α-cyclopropanoprogesterone (VI) has a high gestagenic effect, evaluated as 4 points on the Mac-Feil scale, as well as a contraceptive activity, which is only somewhat lower than that of megestrol acetate [23].

It appeared to be desirable to carry out a screening of compounds I-V for different types of activity. The compounds studied were synthesized according to the literature data from $\Delta^{5,16}$-dehydropregnenolone 3-acetate, by carrying out its epoxidation Ia [21], epimination IIa and IVa [7, 16], and subsequent transformations described in [6] for the preparation of Va. Their Δ^4-3-keto derivatives Ic [15], IIb [16], IIIc [16] and IVb were obtained by the Oppenauer oxidation of the corresponding Δ^5-3-hydroxy derivatives. The N-acetyl- and N-carbethoxyepimino steroids IIIa-c and IVa were synthesized by conventional methods of esterification with acetic anhydride or ethyl chloroformate.

*For Communication XXII, see [9].

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