SYNTHESIS OF CEPHALOSPORIN R-SULFOXIDES AND THEIR ANTIBACTERIAL PROPERTIES

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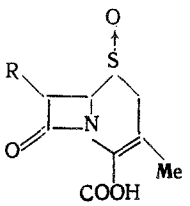
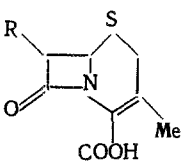
The sulfoxides of cephalosporins are known to exhibit greater antibacterial activity than their unoxidized analogs. It has also been shown that the R-sulfoxides of the cephalosporins are several times more active against gram-positive bacteria than the corresponding S-sulfoxides [9].

Based on the aforementioned statements and as a continuation of our study on the relationship between chemical structure and biological action, we synthesized new cephalosporin R-sulfoxides (Ia-h) which are oxidized analogs of the previously described [3, 4] derivatives of 7-amino-3-desacetoxycephalosporanic acid (7-ADCA), and we studied their antibacterial properties.



Ia-h, III, IV



II, IIa

R = $NHCOCH_2C_6H_3(OMe)_2$-3,4 (Ia), $NHCOCH_2C_6H_3OMe$-3-OPr-4 (Ib), $NHCOCH_2C_6H_3OMe$-3-OBu-4 (Ic), $NHCOC_6H_4OPr$-2-cyclopropyl-1 (Id), $NHCOC_6H_4$-cyclopentyl-1 (Ie) $NHCOC_6H_4OEt$-4-cyclohexyl-1 (If) 2-p-chlorobenzylfuroxyl-5-amino (Ig) mentoxyacetylamino (Ih)

N = CHC_6H_4OH-2 (II, IIa, III), NH_2 (IV), salt II c Me_3CNH_2 (IIa).

The stereospecific synthesis of the R-sulfoxides Ia-h was started from N-salicylidene-7-ADCA (II) (similarly to [10]).

Our attempts to synthesize II, as described in [10], by reacting 7-ADCA with an excess of salicyclic aldehyde in an alcohol medium did not prove successful. In that connection, we applied the method described in [7] to synthesize II with a yield of 70%-80% in which case we obtained the tertbutylamine salt (IIa) instead of the intermediate tert-octylamine salt of II.

Oxidation of II with 85% m-chlorobenzoic acid followed by acid hydrolysis of N-salicylidene-7-ADCA sulfoxide (III) resulted in the formation of 7-ADCA sulfoxide (IV) [5].

The R-sulfoxides of cephalosporins Ia-h were synthesized by the mixed anhydride method of acylating compound IV. The acylating agents were obtained by the method described earlier [1, 2]. The target products Ia-h were isolated in the form of sodium salts whose purity and individual properties were confirmed by TLC. The structure of compounds Ia-h, described in the form of acids, was confirmed by element analysis and IR-spectra which exhibited absorption bands at 1760-1780 cm^{-1} (CO β-lactam), 1710-1735 (CO carboxyl), 1640-1660 (CO amide), and 1020-1040 (sulfoxide group).

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TABLE 1. Properties of 7-ADCA R-Sulfoxides Ia-h

Compound	Yield, %	mp, °C	R_f	Found, %		Empirical formula	Calc., %	
				N	S		N	S
Ia	58,11	75—6	0,68	6,34	7,95	$C_{18}H_{20}N_2O_7S$	6,05	8,07
Ib	43,03	120—1	0,60	6,40	7,90	$C_{20}H_{24}N_2O_7S$	6,66	7,62
Ic	55,57	146—50	0,63	5,86	7,22	$C_{21}H_{26}N_2O_7S$	6,21	7,11
Id	46,38	128—30	0,63	6,84	7,65	$C_{21}H_{24}N_2O_6S$	6,47	7,41
Ie	45,10	175—7	0,81	7,14	8,09	$C_{20}H_{21}N_2O_5S$	6,97	7,98
If	44,75	90—1	0,62	6,41	7,33	$C_{23}H_{28}N_2O_6S$	6,08	6,96
Ig	50,15	102—3	0,74	5,96	7,31	$C_{20}H_{17}ClN_2O_6S$	6,23	7,13
Ih	43,86	100—2	0,68	6,60	6,85	$C_{20}H_{22}N_2O_6S$	6,25	7,16

EXPERIMENTAL (CHEMICAL)

IR-spectra were recorded on a UR-20 (GDR) spectrometer in a paste with petrolatum jelly or KBr pellets. TLC was performed on Silufol UV-254 plates (Czechoslavakia) in a 7:2 n-propanol—water system. Iodine vapor was the developer.

Tertiary Butylamine Salt of N-Salicylidene-7-ADCA (IIa). A 2 g (0.0092 mole) portion of 7-ADCA was suspended in 50-70 ml of water to which tert-butylamine was added to bring the pH up to 8.0-8.5. A 1.1 g (0.0092 mole) portion of salicyclic aldehyde was added dropwise to the resultant solution which was then stirred for 20 to 30 min and left for 2 to 3 h until the precipitate completely separated. The crystals of the tert-butylamine salt IIa were filtered and washed with water. Yield 3 g (86%), mp 215-215°C. Found, %: N 11.03, S 7.98. $C_{19}H_{25}N_3O_4S$. Calculated, %: N 10.79, S 8.19.

N-Salicylidene-7-ADCA (II). A 120 ml portion of abs. CH_2Cl_2 was added to 2 g (0.0056 mole) of tert-butylamine salt of IIa. The mixture was then cooled to 0-5°C on an ice bath after which 1.3 g (0.11 mole) of salicyclic aldehyde and 0.4 ml (0.0056 mole) of CF_3COOH in 6 ml of THF was added. The temperature of the mixture was brought up to room temperature and the mixture was vacuum-evaporated to 1/3 volume at a temperature not exceeding 30°C. After cooling the precipitated crystals were filtered and washed several times with hexane. Yield of II was 1.4 g (80.5%), mp 198-199°C (lit. data [6]: mp 197-198°C; R_f 0.62 (n-propanol—water, 1:1). Found, %: N 8.57, S 10.21. $C_{15}H_{14}N_2O_4S$. Calculated, %: N 8.77, S 10.03. IR spectrum, ν_{max}, cm^{-1}: 1780 (CO β-lactam), 1720 (CO carboxyl), 1675 (CO amide), 1638 (C=N), 1590 (benzylidene); M^+ 318 (mass-spectrometrically).

N-salicylidene-7-ADCA Sulfoxide (III) was obtained in the same manner as described in [5] by reacting 2 g (0.0066 mole) of II in 25 ml of abs. THF with a solution of 1 g (0.007 mole) of m-chloroperbenzoic acid [8] in 10 ml THF with cooling to 8-10°C. Yield of III was 1.5 g (70.6%); R_f 0.45 (1:1 acetone—hexane), mp 175-180°C (with decomposition). Found, %: N 8.13, S 9.39. $C_{15}H_{14}N_2O_5S$. Calculated, %: N 8.37, S 9.58. IR spectrum, ν_{max}, cm^{-1}: 1775 (CO β-lactam), 1715 (CO carboxyl), 1670 (CO amide), 1020, 1040 (sulfoxide group).

7-ADCA Sulfoxide (IV). A 4 N solution of HCl was added with stirring and cooling to 0°C to 1.5 g (0.006 mole) of III until of the latter was dissolved. The reaction mixture was stirred at this temperature for 1-1.5 h, followed by ether extraction and removal of the ether extracts. An 8% solution of $NaHCO_3$ was added to the aq. layer to bring the pH to 2.5-3.0. The precipitated crystals were filtered and washed with water and acetone. Yield of IV was 0.7 g (71.5%), mp 255-260°C (with decomposition). Found, %: N 13.60, S 12.56. $C_8H_{10}N_2O_4S$. Calculated, %: N 13.92, S 12.16. R_f 0.78 (n-propanol—water, 7:2). IR spectrum, ν_{max}, cm^{-1}: 1785 (CO β-lactam), 1725 (CO-carboxyl), 1629 (CO amide), 1030, 1050 (sulfoxide group).

R-Sulfoxides of 7-Substituted 3-Desacetoxycephalosporanic Acids (Ia-h). A 1.2 g (0.024 mole) portion of triethylamine in 12 ml of abs. acetone and 1.5 g (0.014 mole) of ethyl chlorocarbonate in 10 ml of abs. acetone was added to 0.01 mole of an acylating acid [1, 2] in 30 ml of abs. acetone at 0-2°C. The mixture was stirred at room temperature for 0.5 h and the resultant precipitate was filtered off. The filtrate was added upon stirring to 2.3 g (0.01 mole) of IV in 50 ml of acetone and 100 ml of a 3% aq. solution of $NaHCO_3$. The mixture was stirred for 2-3 h at room temperature and left overnight in a refrigerator. The acetone was vacuum-evaporated and the aq. solution was washed with ethyl acetate (EA). The organic layer was separated and 50 ml of EA was added to the aq. layer which was then acidified to pH 2.0

TABLE 2. Antibacterial Activity of Cephalosporin R-Sulfoxides Ia-h

Compound	MGC, μg/ml							
	Staph. 25925	Staph. 209P	Staph. Smith	Staph. 5	E. coli 25922	St. dysenteriae Flexneri 6858	E. typhi 79	Proteus vulgaris
Ia	1,9	1,9	3,9	62,5	>1000	>1000	>1000	>1000
Ib	0,9	1,9	0,9	3,9	>1000	>1000	1000	>1000
Ic	0,12	0,12	0,03	250	>1000	125	62,5	250
Id	31,2	31,2	31,2	1000	62,5	250	125	>1000
Ie	0,48	0,24	0,48	500	500	125	62,5	250
If	0,48	0,48	0,24	250	250	125	31,2	250
Ig	7,8	3,9	3,9	3,9	500	500	250	>250
Ih	0,9	1,9	0,3	500	>1000	500	125	500
Cephalexin	1,56	1,56	1,56	15,6	15,6	3,9	7,8	62,5

with 1 N HCl. The ethyl acetate layer was separated, washed with ice water, dried with Na_2SO_4, and filtered. The filtrate was treated with an 8% $NaHCO_3$ solution to pH 7.0-7.5. The aq. layer was washed with EA and lyophilized. A small portion of the sodium salt was converted to the acid in order to measure the physicochemical constants (Table 1).

EXPERIMENTAL (BIOLOGICAL)

The antibacterial action of the sodium salts of the cephalosporin R-sulfoxides Ia-h were tested by the generally accepted dual series dilution method in beef-extract both (pH 7.2-7.4) against gram-positive staphylococci (reference staphylococci – strain 5) and gram-negative bacilli (E. coli, typhoid, dysentery, and Proteus vulgaris). Microbe load was 20 million microbe bodies per 1 ml of medium. The minimum growth suppression concentration (MGC) was found. Each experiment employed for comparison the medicinal preparation cephalexin which, like the tested compound Ia-h, is a derivative of 7-ADCA.

The compounds' acute toxicities were tested iv on 50 nonpedigree mice weighing 18-20 g.

Our tests showed that the cephalosporin R-sulfoxides are active against susceptible staphylococci (Table 2). The most active of those compounds exceed the activity of cephalexin. Thus, the MGC was 0.03-0.12 μg/ml for the R-sulfoxide of the 3-methoxy-4-butoxyphenylacetamido derivative Ic, 0.24-0.48 for compounds Ie and If, and 1.56 μg/ml for cephalexin. The indicated cephalosporin R-sulfoxides were considerably more active than their nonoxidized analogs for which the MGC was 7.8 μg/ml [1], 62.5-125 μg/ml [4], respectively. The least active of the tested series was the R-sulfoxide of the o-propoxyphenylcyclopropyl derivative Id (MGC 31.2 μg/ml) which turned out to be less active than both cephalexin and nonoxidized analog [4]. The activity of the remaining derivatives of 7-ADCA R-sulfoxide (Ia, Ib, Ig, and Ih) were basically equivalent to the activity of cephalexin and their corresponding nonoxidized analogs.

Compounds Ib and Ig exhibited activity against penicillinase-forming staphylococcus. The cephalosporins Ic, Id, Ie, and If exhibited some activity in comparison to their nonoxidized analogs on the gram-negative test cultures [3, 4].

The tested cephalosporins Ia-h were well tolerated by the white mice at an iv dose of 2000 mg/kg.

Thus, we have found compounds among the tested new derivatives of 7-ADCA R-sulfoxide that are active against susceptible staphylococci and that exceed the activity of cephalexin and its nonoxidized analogs.

LITERATURE CITED

1. Sh. L. Mndzhoyan, M. G. Tsinker, et al., Khim.-farm. Zh., No. 3, 5-10 (1970); Khim.-farm. Zh., No. 5, 15-21 (1970).
2. Sh. L. Mndzhoyan and N. D. Marukhyan, Khim.-farm. Zh., No. 1, 60-63 (1986).
3. Sh. L. Mndzhoyan, M. S. Kramer, et al., Khim.-farm. Zh., No. 4, 446-450 (1986).
4. Sh. L. Mndzhoyan, M. S. Kramer, et al., Khim.-farm. Zh., No. 2, 186-190 (1987).
5. British Patent No. 1,442,993, Chem. Abstr., 80, No. 133,456a (1974).
6. USSR Patent No. 458,131, Otkryt., No. 3 (1975).

7. USA Patent No. 3,867,379, Chem. Abstr., 83, No. 58,843 (1975).
8. K. Donald, Org. Synth., 50, 15-18 (1970).
9. E. H. Flynn, Cephalosporins and Penicillins, New York (1972), Ch. 12, pp. 540, 577.
10. J. J. Koning et al., Recent Advances in the Chemistry of (beta)-lactam Antibiotics, London (1977), Ch. 17, pp 161-166; Chem. Abstr., 88, No. 6760, (1978).

SYNTHESIS AND BIOLOGICAL PROPERTIES OF S-DEOXO ANALOGS OF THE NATURAL ANTIBIOTIC SPARSOMYCIN

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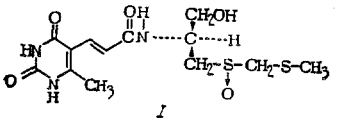
Among pyrimidine derivatives, the antibiotic sparsomycin, which is isolated from Streptomyces sparsogenes by fermentation of beef bouillon [5] and has structure I with an S configuration at the chiral carbon atom [17, 18], is attracting attention.



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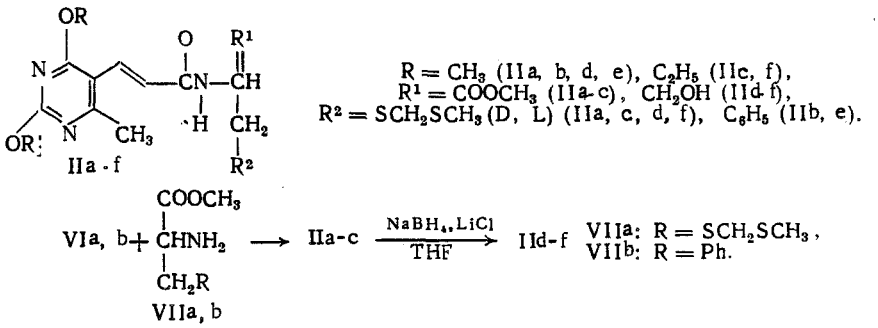
Sparsomycin has a broad spectrum of biological activity – antineoplastic [12], antibacterial [10, 12], fungicidal [12], and antiviral [14]; however, it has a toxic effect on the retina of the eye [7].

The ability of sparsomycin to inhibit the biosynthesis of protein [15] is possibly explained by interaction of the multiple bond with the SH groups of the ribosomal proteins via a reaction of the Michael type [13, 16].

In order to study the biological activity and toxicity of derivatives of S-deoxo sparsomycin we synthesized amides IIa-f.

Compounds IIa-c were obtained by amidation of acids VIa, b with D,L-S-methylthiomethylcysteine methyl ester (VIIa) [8] and D,L-β-phenyl-α-alanine methyl ester (VIIb) [6] by the method of mixed anhydrides with subsequent reduction to amino alcohols IId-f.



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