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STUDIES OF SEMISYNTHETIC CEPHALOSPORINS.

SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF CERTAIN 7-SUBSTITUTED 3-METHYL-3-CEPHEM-4-CARBOXYLIC ACIDS

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Over the last decade, intensive research has been carried out in the area of semisynthetic cephalosporins, which, similarly to penicillins, belong to the class of β-lactam antibiotics. Compared with penicillins, the cephalosporin derivatives have several advantages, so that the synthesis of new cephalosporins is a very hopeful and urgent subject.

For a directed search for new antibacterial preparations and for the clarification of the relationship between chemical structures and biological activity it was of interest to introduce into the amino group of 7-aminodesacetoxycephalosporanic acid (7-ADCA) such acyl groups, which, as has already been shown [2-4], in combination with a penicillin ring would have not only a certain antibacterial activity, but also low toxicity and pecillinase- and acid-stability.

For the synthesis of the new cephalosporin derivatives (I-XII), we chose the acid chloride method [5], consisting in direct reaction of 7-ADCA with acid chlorides in the presence of $NaHCO_3$ in acetone at low temperatures (method A). As acylating agents, acid chlorides of various carboxylic acids were used [2-4], obtained by known methods, i.e., by boiling of the acids with $SOCl_2$ in absolute benzene. In general, all the acid chlorides were distilled *in vacuo*, and in the case of resinification during vacuum distillation, they were introduced into the acylation reaction directly after multiple washing absolute benzene.

During recent years, the silyl methods [1] have been extensively used in the chemistry of β-lactam antibiotics. To increase the yields of cephalosporins I-XII, we used silyl protection of the carbonyl group in 7-ADCA, followed by the acylation of its trimethylsilyl ester by acid chlorides (method B). The convenience of this method consists in the ease of removal of the trimethylsilyl protective group during the treatment of the reaction mixture.

The desired end products I-XII were isolated in the form of sodium salts, whose purity and individual state was confirmed by the TLC method (Table 1).

The structure of the cephalosporin derivatives synthesized, characterized in the form of acids, was confirmed by elemental analysis and by spectrometry. In all the IR spectra, absorption bands were observed at 1760-1780 cm^{-1} (a β-lactam CO group), 1710-1725 cm^{-1} (a carboxylic CO group), 1640-1660 cm^{-1} (an amide CO group), 3300-3340 cm^{-1} (NH); in the mass spectra there were peaks of molecular ions and several characteristic fragmentary ions.

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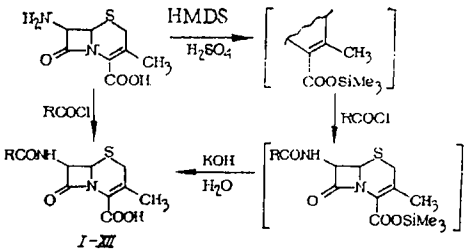

I: R = $CH_2C_6H_3(OMe)_2$-3, 4; II: R = $CH_2C_6H_3OMe$-3-OPr-4;
III: R = $CH_2C_6H_3OMe$-3-OPr-*i*-4; IV: R = $CH_2C_6H_3OMe$-3-Bu-4;
V: R = $CH_2C_6H_3OMe$-3-Bu-*i*-4; VI: R = 2-bromofurye-5; VII: R = 2-benzylfuryl-5;
VIII: R = 2-p-chlorobenzylfuryl5; IX: R = 2-p-methylbenzyltetrahydrofuryl-5;
X: R = (4-p-methylbenzyltetrahydrofuryl-5); XI: R-menthoxymethyl
XII: R = (menthoxy)(di-tert-butyl)methyl

EXPERIMENTAL (CHEMICAL)

The IR spectra were run on a UR-20 spectrophotometer (GDR) in paste with mineral oil or in KBr tablets, the mass spectra were measured on a MX-1303 mass spectrometer (USSR) with a direct introduction of the sample into the ionic source at a temperature 20-30°C below the melting point of the compound studied. The TLC was carried out on Silufol UV-254 plates (CSSR) in a 3:1 n-propanol–water system, with iodine vapors as developer.

7-Substituted 3-Methyl-2-cephem-4-carboxylic Acids (I-XII). Method A. A mixture of 2.1 g (0.01 mole) of 7-ADCA, 2.8 g (0.033 mole) of $NaHCO_3$ in 80 ml of water and 60 ml of acetone is cooled to 0-2°C, and 0.01 mole of a carboxylic acid chloride in 20 ml of absolute acetone is added dropwise with stirring. Stirring is continued at this temperature for 3-4 h, and the mixture is left to stand overnight in a refrigerator. Acetone is evaporated to dryness, the aqueous solution is washed with ethyl acetate (EA), the organic layer is discarded, and to the aqueous layer 50 ml of EA are added, and the mixture is acidified in the presence of the latter by 1 N HCl to pH 2.0. The EA layer is separated, washed with ice water, dried over anhydrous Na_2SO_4, filtered, and treated with 8% $NaHCO_3$ to pH 6.0-7.0. The aqueous layer is washed with EA, and lyophylized. To determine the physicochemical constants, a small portion of the sodium salt is converted into an acid (Table 1). Mass spectrum, m/z, I: 392 (M^+), 374, 348, 262, 158, 140; VII: 398 (M^+), 380, 354, 268, 158, 140.

Method B. Two drops of concentrated H_2SO_4 and 1.6 g (0.01 mole) of hexamethyldisilazane (HMDS) are added to a suspension of 2.1 g (0.01 mole) of 7-ADCA in 30 ml of absolute dichloroethane. The mixture is boiled for 4-5 h to complete dissolution of the precipitate. A 0.79 g portion (0.01 mole) of pyridine and 0.01 mole of a carboxylic acid chloride in 5 ml of dichloroethane are added, with stirring, to the solution obtained, cooled to −10°C. The solution is stirred for two more hours at the same temperature and for 1 h at room temperature. The reaction mixture is then diluted with 30 ml of water and neutralized with 1 N NaOH to pH 7. The aqueous layer is separated, washed with 30 ml of EA, acidified with 1 N HCl to pH 2.0, and the oil that separates, is extracted with EA. Further treatment is carried out according to method A.

TABLE 1. Properties of 7-Substituted 3-Methyl-3-cephem-4-carboxylic Acids I-XII

Compound	Yield, %		mp(dec), °C	R_f	Found, %		Emperical formula	Calculated, %	
	method A	method B			N	S		N	S
I	54,5	80,4	135—6	0,72	7,26	8,30	$C_{18}H_{20}N_2O_6S$	7,13	8,17
II	56,5	76,5	100—1	0,62	6,28	7,50	$C_{20}H_{24}N_2O_6S$	6,66	7,62
III	60,3	85,5	140—1	0,65	6,37	7,64	$C_{20}H_{24}N_2O_6S$	6,66	7,62
IV	52,5	83,8	134—6	0,60	6,31	7,48	$C_{21}H_{26}N_2O_6S$	6,45	7,38
V	47,4	70,6	170—1	0,65	6,40	7,81	$C_{20}H_{24}N_2O_6S$	6,66	7,62
VI	50,0	70,2	116—7	0,58	7,31	8,01	$C_{13}H_{11}BrN_2O_5S$	7,23	8,28
VII	65,5	86,0	90—2	0,57	7,19	7,79	$C_{20}H_{18}N_2O_5S$	7,03	8,04
VIII	55,6	80,5	107—9	0,60	6,88	7,60	$C_{20}H_{17}ClN_2O_5S$	6,47	7,40
IX	50,4	80,3	75—5	0,70	6,50	7,90	$C_{21}H_{24}N_2O_5S$	6,72	7,69
X	56,0	73,5	56—7	0,75	6,02	7,50	$C_{23}H_{28}N_2O_5S$	6,30	7,21
XI	58,5	78,0	59—60	0,64	6,47	7,61	$C_{20}H_{30}N_2O_5S$	6,82	7,81
XII	60,4	83,2	120—2	0,62	5,54	6,30	$C_{28}H_{46}N_2O_5S$	5,66	6,48

TABLE 2. Antibacterial Activity and Toxicity of 7-Substituted Desacetoxycephalosporins I-XII

Compound	MIC, μg/ml		MED, mg/kg
	Staph. aureus 209p	Staph. aureus 5	
I	0,96	7,8	1500
II	0,012	7,8	1500
III	3,9	31,2	1000
IV	7,8	125	250
V	0,96	125	1000
VI	250	500	2500
VII	0,78	125	125
VIII	7,8	125	250
IX	0,78	31,2	125
X	0,12	5000	500
XI	0,12	7,8	250
XII	7,8	7,8	500
Cephalexin	1,56	7,8	...

EXPERIMENTAL (BIOLOGICAL)

The antibacterial activity of desacetoxycephalosporins I-XII was studied by the method of double dilutions of a meat-peptone bullion (pH 7.2-7.4) at a microbial load of $2 \cdot 10^6$ microbial bodies per ml of medium. In the experiments gram-positive staphylococci (penicillinase-sensitive, 3 strains; stable, 7 strains) and gram-positive bacilli (*Escherichia coli*, *Salmonella typhosa*, *Bacillus dysenteriae*, *Bacillus pyocyneus*, and *Proteus*) were used.

In all the experiments, the minimum inhibiting concentration (MIC) of cephalexin was determined for comparison with the compounds I-XII studied. The experiments were repeated not less than 3 times. The maximally endurable doses (MED) were determined in acute experiments on white nonpedigree mice (8-10 times for each preparation), weighing 18-20 g each, using intravenous administration. In all, 105 animals were used.

The compounds studied did not display pronounced antibacterial activity with respect to gram-negative microorganisms. Depending on the microorganism and compound, their MIC are within 125-1000 μg/ml, while cephalexin inhibits their growth in a concentration of 7.8-31.2 μg/ml. On the whole the compounds are inactive with respect to clinical strains. With respect to strains of Staphylococcus sensitive to benzylpenicillin, they have, in general, a certain activity, exceeding that of cephalexin. Table 2 shows the MIC indexes of cephalosporin derivatives with respect to one of the strains of *Staphylococcus*, *Staph. aureus* 209p (the same relationships are, in general, revealed for other sensitive strains;, and also with respect to one of penicillinase-producing strains — *Staph. aureus* 5.

Table 2 shows that compounds I-IV inhibit the growth of Staphylococcus in a concentration of 0.012-7.8 μg/ml. Of these, the most active is compound II, containing methoxy groups in the 3-position of the benzyl radical and propoxy groups in position 4. This compound is more active than its penicillanic analog, whose MIC is equal to 0.48 μg/ml. Replacement of the propoxy group in the benzyl radical by the isopropoxy group (III) leads to increase in MIC and decrease in MED. A similar dependence exists in the penicillanic analog of III, but to a lesser extent. In the series of benzyl-substituted 7-ADCA, the least active is compound IV, but this compound is also twice as active as its penicillanic analog, although it is much inferior to it with respect to endurance [2]. The same alkoxy groups introduced into a phenyl radical (V) somewhat decrease the MIC of the compound and increase the MED from 250 to 1000 mg/kg.

Another group of cephalosphorins studied were compounds containing substituted furan and tetrahydrofuran rings (VI-X). The bromofuran derivative has no antibacterial activity, but is the least toxic compound. Substitution of the bromine atom by benzyl radical (VII, VIII) leads to a decrease in MIC as well as in MED. When the furan ring is replaced by the tetrahydrofuran ring (IX, X), the endurance of the compounds is not improved, but their MIC is somewhat decreased, especially in the case of X — up to 0.12 μg/ml. With respect to antibacterial properties, compounds V-X are inferior to the penicillin analogs [3], and, except for V, are more toxic than the latter.

7-Menthoxymethyl- and 7-menthoxy (α,α-diisobutyl)methylcephalosporins XI, XII, displaying antibacterial activity, are more toxic than their penicillin analogs [4]. The antibacterial effect with respect to the penicillinase-forming strain *Staphylococcus*, the *Staph. aureus* 5, characteristic of compounds XI, XII, is comparable with the action of cephalexin.

It should be noted that benzyl derivatives I-III display a similar action with respect to *Staph. aureus* 5.

Thus, among the compounds studied, the benzyl-substituted desacetoxycephalosporins, having a low toxicity and a higher antibacterial activity than their penicillin analogs, deserve most attention.

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POLYMER COMPLEXES OF GOSSYPOL AND THEIR ANTIVIRAL ACTIVITY

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Gossypol — 2,2-di(1,6,7-trihydroxy-3-methyl-5-isopropyl-8-aldehydronaphthol) — is well known as an effective antiviral agent. The antiviral activity of gossypol is manifested with respect to a whole series of myxo- and herpes viruses [2]. However, gossypol is practically insoluble in water. Therefore, it is currently used clinically for the treatment of certain diseases of viral etiology only in the form of a 3% liniment [5].

In this work we describe a method of producing complexes of natural gossypol with a water-soluble, relatively nontoxic polymer, polyvinylpyrrolidone (PVP); their toxicity and antiviral activity were studied in cell cultures of primary trypsinized chick embryo fibroblasts (CEF).

The complexes were produced by mixing solutions of PVP and pharmacopoeia-pure gossypol. The compounds obtained, at low gossypol contents (up to 3%), in contrast to the initial gossypol, are readily soluble in water. For an evaluation of the nature of the bond between gossypol and PVP we used the methods of UV and IR spectroscopy.

In the UV spectra of the complexes, a decrease of almost a whole order of magnitude was observed in the intensity of the absorption bands at 290 and 375 nm, corresponding to the phenol and aldehyde groups of gossypol. In the IR spectra, together with the absorption band at 1670 cm^{-1}, characteristic of the absorption of the carbonyl group of the amide bond of the initial polymer, absorption bands (shoulder) appear in the region of 1740-1750 cm^{-1}, which was previously assigned to the amide carbonyl group, bonded by a hydrogen bond [1, 3].

TABLE 1. Polymer Complexes of Gossypol

Substance	Molecular weight of initial polymer	Yield, %	Gossypol content	
			wt. %	mole %
I	2000	93	3,0	0,60
II	2000	86	2,3	0,50
III	2000	81	1,2	0,26
IV	12600	87	3,0	0,60

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