bunyaviruses), and primary trypsinized chick embryo fibroblasts (with the remaining viruses). The methods used were screening tests, platelet reduction under an agar covering, and in the cases of Sindbis and bunyaviruses, a micromethod.

The basis for the assessment of the results obtained was the chemotherapeutic index (CTI), the ratio of the maximum dose tolerated by the tissue culture (MTC) to the minimum concentration having an antiviral effect within the limits of the commonly-accepted statistical confidence limits (reduction in the viral titer as compared with the untreated control by 1.25 log). A detailed description of the methods of testing and calculation has been given previously [6].

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF 5-(ADAMANT-1-YL)SALICYLIC ACID, 3-(4-HYDROXYPHENYL)ADAMANTANECARBOXYLIC ACID AND SOME OF THEIR DERIVATIVES

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Salicylic acid and its derivatives (acetylsalicylic acid, salicylamide, methyl salicylate, etc.) are used as anti-inflammatory, analgesic, and antiseptic agents [1]. Data on the preparation of adamantyl group-containing derivatives of salicylic acid and their biological activity are not abundant [3, 5]. This prompted us to carry out a search for antibacterial, analgesic and antiinflammatory agents among the derivatives of 5-(adamant-1-yl)-salicylic acid (I) and its structural analog, 3-(4-hydroxyphenyl)adamantanecarboxylic acid (II) (Table 1).

Compounds I and II were obtained for the first time by Stepanov et al. [3]. On the basis of the method described therein for the synthesis of acid I, we found that by increasing the reaction time from 1 h to 13-15 h, the yield of the purified product increases from 30-40 to 85-87%.

According to the data in [3], acid II is obtained by heating 3-bromo-1-adamantanecarboxylic acid (III) with phenol for 1 h. The yield of II, equal to 77%, reported in the article refers to a technical grade product, which after purification is reduced to 10-14%. Our attempts to increase the yield by increasing the duration of the reaction to 10-

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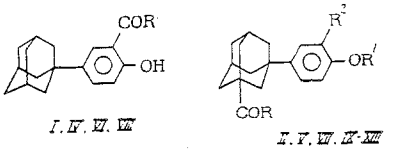
TABLE 1. Characteristics of Compounds IV-XIII

Compound	mp, °C (solvent)	Empirical formula	Mass spectrum M^+
IV	113—115 (methanol)	$C_{18}H_{22}O_3$	286
V	120—122 (heptane-ethyl acetate-benzene, 8:1:1)	$C_{18}H_{22}O_3$	286
VI	336—339 (dioxane)	$C_{17}H_{22}N_2O_2$	286
VII	223—226 (ethanol-water, 1:1)	$C_{17}H_{22}N_2O_2 \cdot {}^1/_2\ H_2O$	286
VIII	220—223 (ethanol-benzene-heptane, 2:2:6)	$C_{23}H_{23}Cl_2NO_2$	416
IX	189—191 (ethyl acetate)	$C_{23}H_{23}Cl_2NO_2$	416
X	149—151 (methanol-water, 1:1)	$C_{19}H_{22}O_4$	314
XI	161—163 (ethanol-water, 7:3)	$C_{25}H_{25}Cl_2NO_3$	458
XII	191—194 (ethanol-water 1:1)	$C_{17}H_{19}BO_3 \cdot {}^1/_2\ H_2O$	
XIII	220,5—222 (dioxane-water, 1:1)	$C_{17}H_{19}O_3$	

22 h were unsuccessful. Taking into account the fact that the presence of the carboxylic group in the adamantyl ring decreases the activity of acid III in the electrophilic substitution reactions, we carried out the adamantylation of phenol in the presence of trifluoracetic acid (TFA) as a catalyst. It was found that the yield of the desired end product II depends on the proportions of the reagents and TFA and on the reaction time. The best results (84%) was obtained on boiling for 48 h compound III, phenol and TFA in a molar ratio of 1:20:10. We used this method for the condensation of the bromo-derivative III with meta-cresol which gave a yield of 3-(2-hydroxy-4-methyl)-phenyladamantanecarboxylic acid of 75%.

The biological properties of I and II have not previously been studied. As the result of our investigation, it was found that acid I has pronounced antifungal and antibacterial (with respect to gram-positive bacteria) properties. The compound had no antiinflammatory and analgesic activity, but such activity was found to exist to a small extent in II (Table 2).

Several derivatives were prepared from compounds I and II to find more active compounds, and their biological activity was studied. Thus, in the treatment of acids I and II with dimethyl sulfate, esters IV and V were synthesized, which were then converted into hydrazides of acids VI and VII.



I, II, III, IV — V, VI, VII, VIII, IX, X, XI, XII, XIII

R = OH (I, II, X, XII, XIII), OMe (IV, V), $NHNH_2$ (VI, VII), $HNC_6H_3Cl_2$ = 2,6 (VIII, IX, XI);
R′ = H (II, V, VII, IX, XII, XIII), Ac (X, XI); R^2 = H (II, V, VII, IX, X, XI), Br (XII), J (XIII)

To obtain the 2,6-dichloroanilide VIII, the chloride of acid I was boiled with 2,6-dichloroaniline in dioxane solution. A similar attempt to synthesize 2,6-dichloroanilide (IX) was unsuccessful, since the chloride of acid II could not be obtained of sufficiently high purity. Therefore, the starting compound II was preliminarily acylated at the phenolic hydroxyl, and the 3-(4-acetoxyphenyl)adamantanecarbonyl chloride was obtained from compound X, and without separation, the latter was treated with 2,6-dichloroaniline to yield the amide XI. The acetyl protection was removed by boiling amide XI in an alcoholic alkali solution. The yield of 2,6-dichloroanilide IX, calculated on the basis of acid II was 39.5%.

The halogen derivatives XII and XIII were synthesized by means of dioxane dibromide and iodine monochloride [4].

EXPERIMENTAL (CHEMICAL)

The mass spectra of the compounds were run on a MAT-112 spectrometer ("Varian", FRG), the PMR spectra on XL-100 and XL-200 spectrometers ("Varian", Switzerland), using TMS in d-acetone as standard. The values found in the elemental analyses correspond to the calculated data.

TABLE 2. Anti-inflammatory, Analgesic Activity and Acute Toxicity of Derivatives of Adamantanecarboxylic Acids I, II, IV, V, VIII-X

Compound	Anti-inflammatory action	Analgesic action	LD_{50}, mg/kg (enterally for mice)
	decrease in edema of paw, %	increase in pain sensitivity threshold, %	
I	0	0	220
II	15±7*	22±15*	1000
IV	14±10*	44±8**	⩾1500
V	0	48±10**	⩾1500
VIII	0	126±20**	⩾1500
IX	0	41±10**	1000
X	15±10*	0	1000
Acetylsalicylic acid	48±6**	80±15**	⩾1500

*Difference in comparison with control unreliable ($p > 0.05$).

**Difference in comparison with control reliable ($p < 0.05$).

Note. Acetylsalicylic acid was used as the reference anti-inflammatory compound.

5-(Adamant-1-yl)salicylic Acid (I). A mixture of 43 g (200 mmoles) of 1-adamantyl bromide, 152.59 g (1105.7 mmoles) of salicylic acid and 2 ml of water was heated at 160-170°C in an inert gas current for 15 h. Water (1.5 liter) was added to the reaction mixture, which was then boiled for 10-15 min. The precipitate was filtered, washed with hot water, and dried. Yield, 47.55 g (87.41%) of I, mp 263.5-264.5°C (dioxane), according to the literature data [3]: 263-264°C.

3-(4-Hydroxyphenyl)adamantanecarboxylic Acid (II). A mixture of 34 g (131 mmoles) of 3-bromo-1-carboxyadamantane, 123 g (11,310 mmoles) of phenol and 314 g (2780 mmoles) of TFA was boiled for 48 h. The reaction mixture was then poured into 1.5 liters of water, the precipitate that separated out was filtered off, washed with hot water, dissolved in 25% NaOH, and the alkaline solution was extracted with ether. The combined ether solution was washed with 25% NaOH. The alkaline solutions were combined and neutralized with HCl. The precipitate that separated out was filtered off, washed with water, and dried. Yield, 30 g (84%) of II, mp 223-225°C (toluene), according to the literature data [3]: 223-225°C.

3-(4-Hydroxy-2-methylphenyl)adamantanecarboxylic acid was obtained in a similar way, mp 194-196°C (benzene—heptane, 3:2). $C_{18}H_{22}O_3$. Mass spectrum, M^+ 286.

Methyl 5-(Adamant-1-yl)salicylate IV. A 2.52 g portion (45 mmoles) of KOH in 15 ml of water and 5.67 g (0.045 mole) of dimethyl sulfate were added to a solution of 8.16 g (30 mmoles) of I in 200 ml of acetone. The reaction mixture was allowed to stand for 20 h at room temperature and the solvent was evaporated under vacuum. A 150 ml portion of water was added to the residue, the mixture was extracted with ether, and the combined ether solution was washed with water, dried over $MgSO_4$, and evaporated under vacuum. Yield, 5.48 g (63.78%) of IV.

Methyl 3-(4-Hydroxyphenyl)adamantanecarboxylate (V) was obtained in a similar way from acid II. The yield of V was 93.6%. PMR spectrum, δ, ppm: 8.83 (s, 1H), 7.20 (d, 2H), 6.81 (d, 2H), 3.61 (s, 3H), 2.03; 1.95; 1.90; 1.89; 1.85 (m, 14H).

TABLE 3. Antimicrobial Activity of Adamantane Carboxylic Acid Derivatives I and IX

Microorganism		MIC, μg/ml	
		I	IX
St. aureus	209-P	31,2	31,2
Str. pyogenes	ATCC 12354	15,6	31,2
B. subtilir	ATCC 6633	15,6	15,6
E. coli	ATCC 25922	250	250
P. vulgaris	ATCC 6896	250	250
P. aeryginosa	ATCC 27853	250	250
M. canis		250	15,6
T. mentagrophytes*		62,5	31,2
C. albicans*		250	31,2

*Clinical strains.

5-(Adamant-1-yl)salicylic acid Hydrazide (VI). A mixture of 2.86 g (10 mmoles) of IV and 0.751 g (15 mmoles) of hydrazine hydrate in 30 ml of ethanol was boiled for 10 h. The solvent was evaporated under vacuum, 50 ml of water was added to the residue, the precipitate was filtered, washed with water, dried, and recrystallized from dioxane. Yield, 1.03 g (36.01%) of VI.

3-(4-Hydroxyphenyl)adamantanecarboxylic Acid Hydrazide (VII). A mixture of 1.8 g (63 mmoles) of V and 3.76 g (75.6 mmoles) of hydrazine hydrate was boiled for 35 h. The treatment was carried out as in the preparation of hydrazine VI.

5-(Adamant-1-yl)salicylic acid 2,6-Dichlorophenylamide (VIII). A mixture of 13 g (47.8 mmoles) of I and 43.03 g (362 mmoles) of $SOCl_2$ was boiled for 1 h, and then the excess of $SOCl_2$ was evaporated under vacuum. A 30 ml portion of petroleum ether was added to the residue, and the mixture was allowed to stand for 36 h at 0-5°C. The precipitate that separated out was filtered off, washed with petroleum ether, and dried. Yield 9.44 g (68%) of 5-(adamant-1-yl)salicyl chloride. The compound was subsequently used without additional purification. Mp 89-97°C.

A mixture 1.31 g (45 mmoles) of 5-(adamant-1-yl)salicyl chloride and 1.46 g (90 mmoles) of 2,6-dichloroaniline in 15 ml of dioxane was boiled for 8 h. The precipitate that separated out after cooling the reaction mixture was filtered off, washed with heptane, and dried. Yield, 1.1 g (58.7% based on the acid chloride) of amide VIII.

3-(4-Acetoxyphenyl)adamantanecarboxylic Acid (X). A 7.85 g portion (100 mmoles) of AcCl was added to 2.72 g (10 mmoles) of II. The reaction mixture was allowed to stand for 16 h at room temperature, and then added in portions to 100 ml of ice water, and the mixture was extracted with ether. The combined ether solution was washed with water, dried over $MgSO_4$, and the solvent was evaporated under vacuum. Yield, 2.93 g (93.2) of X.

3-(4-Acetoxyphenyl)adamantanecarboxylic Acid 2,6-Dichlorophenylamide (XI). A mixture of 5.5 g (17.5 mmoles) of X and 6.25 g (52.5 mmoles) of $SOCl_2$ was boiled for 2 h. Excess of $SOCl_2$ was evaporated under vacuum, and 3.24 g (20 mmoles) of 2,6-dichloroaniline in 15 ml of dioxane was added to the residue. The mixture obtained was boiled for 5 h, the solvent was evaporated under vacuum, and the residue was dissolved in 200 ml of ether. The ether solution was washed with 10% HCl, water, dried over $MgSO_4$, and the solvent was evaporated under vacuum. A 10-15 ml portion of benzene was added to the residue, the precipitate that separated out was filtered off, washed with benzene, and dried. Yield, 3.4 g (42.4%) of XI.

3-(4-Hydroxyphenyl)adamantanecarboxylic Acid 2,6-Dichlorophenylamide (IX). A mixture of 0.6 g (1.31 mmole) of XI and 0.079 g (1.97 mmole) of NaOH in 15 ml of ethanol was boiled for 30 min, and poured into 150 ml of water. The mixture was extracted with ether, the combined ether solution was washed with water, dried over $MgSO_4$ and the solvent was evaporated under vacuum. Yield, 0.45 g (82.7%) of IX. PMR spectrum, δ, ppm: 7.96; 7.92; 7.91; 7.60; 7.56; 7.55; 7.48; 7.45; 7.43 (m, 7H); 2.07; 2.03; 2.01 (m, 14H).

3-(3-Bromo-4-hydroxyphenyl)adamantanecarboxylic Acid (XII). A 0.96 g portion (6 mmoles) of bromine in 10 ml of dioxane was added to a solution of 1.5 g (5.5 mmoles) of II. The reaction mixture was heated for 3 h at 60-65°C, and the solvent was evaporated under vacuum. The residue was dissolved in 35 ml of ether, washed with an aqueous solution of sodium bisulfite, water, and dried over $MgSO_4$. The solvent was evaporated in vacuo. Yield, 1.24 g (63%) of XII. PMR spectrum, δ, ppm: 8.60 (s, 1H), 7.48 (d, 1H), 7.28 (d.d, 1H) 6.95 (d, 1H); 2.1; 1.98; 1.92; 1.87 (m, 14H).

3-(4-Hydroxy-3-iodophenyl)adamantanecarboxylic Acid (XIII). A solution of 4.86 g (30 mmoles) of iodine monochloride in 20 ml of AcOH was added dropwise to a solution of 2.72 g (10 mmoles) of II in 60 ml of glacial AcOH. The reaction mixture was stirred for 2 h, poured into 100 ml of water, and extracted with ether. The ether solution was washed successively with water, a solution of sodium bisulfite, water, a sodium carbonate solution, water again, dried over $CaCl_2$ and the solvent was evaporated. Yield 3.65 g (92%) of XIII. PMR spectrum, δ, ppm: 8.83 (s, 1H), 7.70 (d, 1H), 7.27 (d.d, 1H), 6.90 (d, 1H); 2.06; 1.98; 1.92; 1.86; 1.75 (m, 14H).

EXPERIMENTAL (PHARMACOLOGICAL)

Compounds I, II, IV, VIII-X were tested with respect to parameters characterizing their anti-inflammatory and analgesic activity, and also the acute toxicity.

In experiments on male rats weighing 120-140 g, the influence was studied of the compounds administered enterally in doses comprising 10% of the LD_{50} value on the development of an acute inflammation of a paw, induced by a subplantary administration of 0.1 ml of a 1% solution of carragheen. The magnitude of the paw edema was measured by a "Ugo Basile" pletismometer (Italy), 3 h after the introduction of carragheen.

In experiments on male rats, weighing 130-160 g each, the influence of the compounds was studied on the development of increased pain selectivity during mechanical irritation of the inflamed paw tissues according to [7]. The change in the pain sensitivity threshold was recorded using a "Ugo Basile" analgesimeter 1 h after the subplantary introduction of carragheen. The compounds were administered enterally 1 h before the induction of the inflammation (in the above indicated doses).

The acute toxicity of the compounds was studied on male mice weighing 18-20 g each over 48 h after a single peroral administration in doses of 100-1500 mg/kg. The LD_{50} was calculated graphically according to [6].

The results of the investigation are presented in Table 2. It is seen that the compounds studied in doses comprising 10% of the LD_{50} do not influence the carragheen induced acute inflammatory reaction in rats. At the same time, some of the compounds (IV, V, VIII, XI) cause a statistically reliable increase in the pain sensitivity threshold of the inflamed paw tissues of the rat. Thereby, compounds III, V, IX increase the sensitivity threshold by 41-48% ($p \geq 0.05$) while compound VIII increases it by 126% ($p < 0.05$). The remaining compounds do not cause a reliable change in the pain reactions.

The data obtained show that the adamantanecarboxylic acid derivatives I, II, IV, V, VIII, IX studied do not have an antiinflammatory action, while some of them have analgesic properties. It has thus been found that compounds IV, V, IX are less active than acetylsalicylic acid by a factor of two approximately, while compound VIII has practically the same analgesic activity.

EXPERIMENTAL (MICROBIOLOGICAL)

The antibacterial and antifungal activity of compounds I, II, IV-XIII was studied in vitro by the method of double serial dilutions in a liquid culture medium. The concentration of the compounds, preliminarily dissolved in 0.5 ml of DMSO, was from 250 to 1 μg/ml. The antibacterial activity was determined in a Hottinger bullion with a 120 mg % content of aminic nitrogen, and the antifungal activity — in a Sabouraud bullion with addition of 2% of glucose and 2% of maltose (pH 6.8). The reference strains of gram-positive (*Staphylococcus aureaus* 209-P, *Streptococcus pyogenes* ATCC 12354, *Bacillus subtilis* 6633) and gram-negative bacteria (*Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 6896, *Pseudomonas aeruginosa* ATCC 27853), clinical strains of dermatophytes (*Microsporum canis, Trichophyton mentagrophytes gypseum*) and yeasts (*Candida albicans*) were used as test strains of the microorganisms. The inoculates were prepared from day-old bullion cultures of bacteria and two-week old cultures of fungi. The concentration of the cells in the suspension of the microorganisms was determined according to an optical turbidity standard of 5 units, followed by dilution in the corresponding liquid media to the final inoculate, which was equal to $(1-2)\cdot10^3$ CFU/ml* for bacteria, and about $(2-4)\cdot10^6$ CFU/ml for fungi. The bacteria inoculations were incubated at 35-37°C for 18-20 h, the dermatophyte inoculations — for 6 days and the yeast inoculations — for 20-24 h at 25-28°C [2].

*CFU Colony Forming Units.

Test tubes not containing compounds, to which 0.5% DMSO in a corresponding liquid medium was added, served as control. The incubation conditions of the control were identical to those described for the main experiment.

The activity was appraised visually according to the last test tube in which a visual increase in the growth of the microorganisms was absent, and was characterized by the value of the minimal inhibiting concentration (MIC, μg/ml).

The most active of this group of compounds were found to be the acid I and 3-(4-hydroxyphenyl)adamantanecarboxylic acid 2,6-dichlorophenylamide (IX). They displayed medium activity (MIC 15.6-62.5 μg/ml) with respect to gram-positive bacteria, dermatophytes and yeast (Table 3). Compounds II, IV-VIII, X-XIII do not have antibacterial or antifungal activity (MIC 125-250 μg/ml).

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SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF COORDINATION COMPOUNDS OF SOME 3d-ELEMENTS WITH SCHIFF BASES

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Attention has been given in recent years to directed synthesis, the study of structure and medicobiological properties of coordination compounds of biometals with bioligands having antimicrobial activity [1-3, 5]. These include coordination compounds of 3d-elements with Schiff bases, obtained from aromatic hydroxy-aldehydes, furfural and 5 nitrofurfural [4, 6].

Experimental data are submitted in the present article on the synthesis, establishment of composition, structure, and study of antimicrobial activity of coordination compounds of copper (2+), nickel (2+), cobalt (2+) and zinc with Schiff bases obtained from 2-aminophenol or 4-amino-1,2,4-triazole and 2-hydroxybenzaldehyde, 2-hydroxy-1-naphthaldehyde, furfural and 5-nitrofurfural.

Gram-positive and gram-negative microorganisms served as test microbes in an in vitro experiment. The group of the gram-positive microbes includes *Staph. aureus* strains: Wood 46, Smith, Cowan 1, 209: *Staph. epidermis*, strain 42-a; *B. anthracis*, strains STI and 71/12: B. Cereus, strain 8035. The gram-negative microorganisms are represented by *Proteus vulgaris*, *E. coli*, strain M-17, *S. typhimurium*, *Sh. sonnei* (the S-form). The most active compounds were studied with 20 strains of *Staph. Aureus*, obtained from carriers.

Some of the complexes of the transition metals with Schiff bases obtained from 2-aminophenol were described in [7]; as far as compounds of these metals with ligands based on 4-amino-1,2,4-triazole are concerned, no investiga-

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