TABLE 5. Hydrochlorides of Furyl- and Thienylperhydroazepinoalkylsilanes I-VI

Compound	X	m	Yield, %	mp, °C	Found, %			Empirical formula	Calculated, %		
					C	H	N		C	H	N
I	O	1	72	124	56,70	8,52	5,19	$C_{13}H_{25}ClNOSi$	56,80	9,17	5,09
II	O	2	74	159	58,43	9,11	4,88	$C_{14}H_{27}ClNOSi$	58,20	9,42	4,85
III	O	3	62	133	59,35	9,06	4,41	$C_{15}H_{29}ClNOSi$	59,47	9,65	4,62
IV	S	1	76	138	53,13	8,17	4,90	$C_{13}H_{25}ClNSSi$	53,67	8,66	4,81
V	S	2	69	178	55,17	8,51	4,67	$C_{14}H_{27}ClNSSi$	55,13	8,93	4,69
VI	S	3	64	143	56,59	8,73	4,31	$C_{15}H_{29}ClNSSi$	56,48	9,10	4,36

Preparation of Hydrochlorides. To a solution of the amine in ether, an equimolecular amount of a 0.8-N solution of HCl in absolute ether is added with cooling to 0°C. The precipitate which separates is filtered, washed with ether, and recrystallized from ethanol. The yield and the physical properties of the compounds are listed in Tables 3-5.

EXPERIMENTAL PHARMACOLOGICAL SECTION

The studies were carried out on line BALB/c mice of both sexes weighing 20-24 g each. The compounds studied were introduced as aqueous solutions intraperitoneally 30 min before the test was carried out. A corresponding amount of an isotonic solution of sodium chloride was administered to the control animals.

The action of the compounds studied on the central nervous system was evaluated from a set of tests used at present for detecting the neurotropic activity of compounds [2, 3].

The experimental data were processed statistically, and the mean effective (ED_{50}) and mean lethal (LD_{50}) doses were calculated according to Litchfield and Wilkoxon, as well as the mean arithmetic values and standard error of these mean values ($M \pm m$). To estimate the significance of the difference between the mean values, we used Student's criterion. The difference was considered reliable at the probability level of $P \leqslant 0.05$.

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SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF CERTAIN DERIVATIVES OF 1-FORMYL-3H-PYRROLO[2,3-c]CARBAZOLE

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In continuation of work on the synthesis and search for new chemotherapeutic preparations in the pyrrolocarbazole series [1] nucleophilic addition reactions have been studied for 1-formyl-3H-pyrrolo[2,3-c]carbazole, which has been synthesized previously [2].

It was established by us that condensation of aldehyde (I) with acetone in the presence of aluminum oxide proceeds at room temperature and with acetophenone it proceeds in a medium of ethylene glycol in the presence of catalytic amounts of piperidine [3].

Stretching vibrations were observed in the IR spectra of compounds (II) and (III) for a conjugated carbonyl group near 1660 cm^{-1} (for II) and 1640 cm^{-1} (for III), and absorption bands near 1620 cm^{-1} (for II) and 1660 cm^{-1} (for III) were assigned to a C=C double bond.

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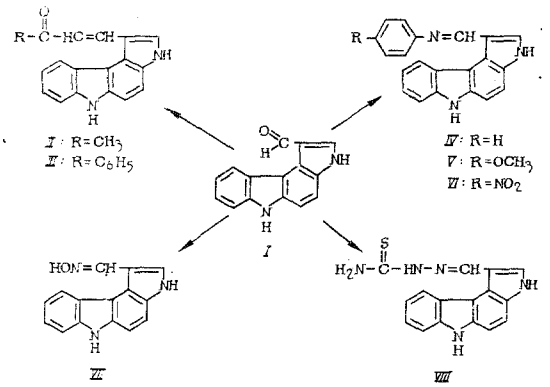

The obtained α,β-unsaturated ketones were characterized by a trans disposition of substituents at the double bond which was confirmed by data of PMR spectra (Table 1). The spin–spin interaction constant for (II) was J_{trans} = 15.9 Hz and for (III) was J_{trans} = 15.2 Hz.

Azomethines have been synthesized by us from aldehyde (I). Aldehyde (I) condensed readily with anilines without a catalyst. On carrying out the same reaction of (I) with p-anisidine and p-nitroaniline in ethanol an alcoholic solution of hydrochloric acid on thionyl chloride was used as catalyst.

IR spectral data on compounds (IV, V, and VI) showed that an absorption band for a conjugated C=N bond was found in the 1625-1615 cm^{-1} region.

The oxime of 1-formyl-3H-pyrrolo[2,3-c]carbazole (VII) was obtained in best yield only on using a strong oxime-forming reagent, viz., a mixture of hydroxylamine hydrochloride and pyridine [4]. Compound (VII) was formed as one geometric isomer.

An absorption band for a hydroxyl group appeared in the region of 3330 cm^{-1} in the IR spectrum of (VII) when taken in a mineral-oil mull, which indicated the participation of the hydroxyl group in the formation of an intermolecular hydrogen bond. In a spectrum taken in methylene chloride at low concentrations of substance there was a sharp absorption band at 3600 cm^{-1} corresponding to a free hydroxyl group. An absorption band near 1635 cm^{-1} was assigned to the stretching vibration of a C=N bond. In the PMR spectrum of compound (VII) the shift towards high field of the 2H signal indicated the syn disposition of the H_{ald} and OH group protons [5].

The thiosemicarbazone of aldehyde (I) was obtained in quantitative yield in the presence of catalytic amounts of an alcoholic solution of hydrochloric acid.

The homogeneity of the obtained compounds was confirmed by data of elemental analysis and TLC and their structures by spectral methods.

EXPERIMENTAL BIOLOGICAL SECTION

The antimicrobial activity of compounds (I, VII, VIII) in relation to certain forms of bacteria and pathogenic fungi was studied by serial dilutions in liquid medium [6]. As is evident from Table 2 compound (I) proved to be practically inactive and at a concentration of 250-500 μg/ml did not suppress the growth of gram-positive or negative bacteria or of pathogenic fungi. The oxime of 1-formyl-3H-pyrrolo[2,3-c]carbazole (VII) possessed marked activity in relation to gram-positive bacteria and fungi. Compound (VIII) took an intermediate position between 1-formyl-3H-pyrrolo[2,3-c]carbazole and its oxime in antimicrobial activity in experiments *in vitro*.

The highest activity for the compounds mentioned was shown in relation to *M. tuberculosis* (strain $H_{37}R_V$). The minimum turberculostatic concentration on Soton medium without protein loading was 7.8-15.6 μg/ml.

EXPERIMENTAL CHEMICAL SECTION

IR spectra were taken on a UR-20 (DDR) instrument in mineral-oil mulls and UV spectra on a Specord UV-vis (CSSR) spectrometer in ethyl alcohol. PMR spectra were taken on a Varian type CFT-20 (USA) instrument with an operating frequency of 80 MHz, internal standard was tetramethylsilane. DMSO-d_6 and acetone-d_6 were used as solvents.

TABLE 1. Chemical Shifts (δ, ppm) and Spin–Spin Interaction Constants (J, Hz) of 1-Formyl-3H-pyrrolo-[2,3-c]carbazole*

Compound	δ, ppm										J, Hz
	2H	3H	4H†	5H†	6H	7H 8H 9H	10H	α-H	β-H	CH_3	
II	8,02	11,1	7,60	7,44	10,5	7,3—7,6	8,54	8,79	6,70	2,42	$J_{\alpha,\beta}=15,9$, $J_{2,3}=3,0$, $J_{4,5}=8,3$, $J_{9,10}=8,4$
III	8,28	11,2	7,67	7,49	10,6	7,2—7,6	8,59	9,12	7,86	—	$J_{\alpha,\beta}=15,2$, $J_{2,3}=3,1$, $J_{4,5}=8,5$, $J_{9,10}=8,7$
IV	8,18	11,1	7,65	7,47	10,5	7,3—7,5	8,67	9,50	—	—	$J_{4,5}=8,7$, $J_{9,10}=8,9$
V	8,08	‡	7,58	7,40	10,3	7,2—7,4	8,64	9,46	—	3,82	$J_{4,5}=8,7$, $J_{c,d}=8,9$** $J_{9,10}=7,0$
VI	8,28	12,5	7,58	7,44	11,5	7,3—7,5	9,20	10,05	—	—	$J_{4,5}=8,9$, $J_{c,d}=9,2$** $J_{2,3}=3,5$, $J_{9,10}=7,3$
VII	7,33	10,9	7,57	7,44	10,4	7,2—7,4	8,47	8,60	10,2	—	$J_{2,3}=2,4$, $J_{4,5}=8,7$, $J_{9,10}=9,5$

*Compounds (II, III, IV, and VII) were taken in acetone-d_6 and (VI) in DMSO-d_6.
†Or vice versa.
‡Rapid NH → ND exchange.
**c and d are protons of the phenyl ring for compounds (V) $\delta_c = 7.56$, $\delta_d = 6.96$ and for compound (VI) $\delta_c = 6.58$, $\delta_d = 9.91$.

TABLE 2. Antimicrobial Activity of 1-Formyl-3H-pyrrolo[2,3-c]carbazole and Its Derivatives

Compound	Staph. aureus	Str. hemoliticus	Cl. diphteriae	Bac. anthracoides	E. coli	Sal. typhi	Shigella dysent	Pr. vulgaris	Ps. aeruginosa	M. tuberculosis	Microsporum lanosum	Frichophyton dypseum	Achorion schonecini	Actynomyces albus	Candida albicans
	minimum inhibitory concentration, μg/ml														
I	>250	>250	>250	>250	>250	>250	>250	>250	>250	15,6	>500	>500	>500	>500	>500
VII	62,5	62,5	31,2	31,2	250	250	250	>250	>250	7,8	125	31,2	62,5	15,6	250
VIII	125	62,5	62,5	62,5	>250	>250	>250	>250	>250	7,8	250	125	>500	>500	>500

1-(3-Keto-1-butenyl)-3H-pyrrolo[2,3-c]carbazole (II). A mixture of aldehyde (0.2 g, 0.0008 mole), aluminum oxide (1.2 g) of Brockmann activity grade II, and acetone (20 ml) was kept at room temperature for a week. It was then chromatographed on a column (silica gel 100/400 μm, eluant was ether–petroleum ether, 1:1). Yield was 0.12 g (52%), mp 210-211°C. IR spectrum, ν, cm^{-1}: 3410 (NH), 1660 (C=O), 1620 (C=C). UV spectrum, λ_{max}, nm (log ε): 229 (4.78), 248 (4.82), 255 (4.84), 278 (4.53), 287 (4.60), 312 (4.47), 339 (4.51), 379 (4.01). Found, %: C 78.8; H 5.1; N 10.2. $C_{18}H_{14}N_2O$. Calculated, %: C 78.9; H 5.2; N 10.3.

1-(3-Keto-3-phenyl-1-propenyl)-3H-pyrrolo[2,3-c]carbazole (III). A few drops of piperidine were added to a solution of aldehyde (I) (0.2 g, 0.008 mole) and acetophenone (0.1 ml, 0.0008 mole) in ethylene glycol (3 ml) and the mixture was heated at a temperature of 160-165°C for 1 h. After cooling, water (5 ml) and acetic acid (0.5 ml) were added to the reaction mixture. The solid which precipitated was filtered off. The product was purified on a column (silica gel 100/250 μm, eluant was petroleum ether–ether, 1:1). Yield was 70 mg (25%), mp 204-205°C. IR spectrum, ν, cm^{-1} 3380, 3360 (NH), 1640 (C=O), 1600 (C=C). UV spectrum, λ_{max} nm (log ε): 235 (4.47), 256 (4.37), 322 (4.44), 424 (4.17). Found, %: C 82.3; H 4.8; N 8.2. $C_{23}H_{16}N_2O$. Calculated, %: C 82.1; H 4.8; N 8.3.

1-(Phenyliminomethyl)-3H-pyrrolo[2,3-c]carbazole (VI). Freshly distilled aniline (3 ml) was added to compound (I) (0.1 g, 0.0004 mole) and the mixture was heated for 3 h. The aniline was distilled off under reduced pressure. After adding ethanol (5 ml) to the reaction mixture the precipitated yellow crystals were filtered off, washed with small portions of alcohol (twice), and then with ether. Yield was 0.1 g (77%) mp 262-263°C (from ethanol). IR spectrum, ν, cm^{-1}: 3420, 3380 (NH), 1625 (C=N). UV spectrum, λ_{max}, nm (log ε): 232 (4.60), 260 (4.24), 274 (4.16), 310 (4.41), 333 (4.36). Found, %: C 81.3; H 4.6; N 13.5. $C_{21}H_{15}N_3$. Calculated, %: C 81.6; H 4.8; N 13.6.

1-(4-Methoxyphenyliminomethyl)-3H-pyrrolo[2,3-c]carbazole (V). p-Anisidine (0.1 g, 0.0008 mole) was added to a solution of aldehyde (I) (0.1 g, 0.0004 mole) in ethanol (20 ml) and a catalytic amount of an alcoholic solution of hydrochloric acid was introduced. The reaction mixture was boiled for 1 h. At the end of the reaction the solvent was partially evaporated. The precipitated yellow solid was filtered off, washed with alcohol, and with ether. Yield was 0.13 g (93%), mp 272-274°C (from ethanol). IR spectrum, ν, cm^{-1}: 3400, 3380 (NH), 1615 (C=N). UV spectrum, λ_{max}, nm (log ε): 205 (4.53), 222 (4.66), 310 (4.42), 339 (4.40). Found, %: N 12.3. $C_{22}H_{17}N_3O$. Calculated, %: N 12.4.

1-(4-Nitrophenyliminomethyl)-3H-pyrrolo[2,3-c]carbazole (VI). p-Nitroaniline (0.11 g, 0.0008 mole) and a catalytic amount of thionyl chloride were added to a solution of aldehyde (I) (0.1 g, 0.0004 mole) in ethanol (20 ml). The reaction mixture was boiled for 1 h. On cooling the precipitated solid was filtered off, washed with alcohol, and dried. Yield was 80 mg (53%). The product darkened at 250°C. Compound (VI) was soluble with difficulty in organic solvents. IR spectrum, ν, cm^{-1}: 3420, 3400 (NH), 1620 (C=N). Found, %: N 15.4. $C_{21}H_{14}N_4O_2$. Calculated, %: N 15.8.

1-Formyl-3H-pyrrolo[2,3-c]carbazole Oxime (VII). A mixture of aldehyde (I) (0.2 g, 0.0008 mole) and hydroxylamine hydrochloride (0.2 g, 0.003 mole) in pyridine (4 ml) was boiled for 6 h. The reaction mass was poured into water. The gray colored solid was separated, washed with water, and dried. It was purified on a column (silica gel 100/250 μm, eluant was ether). Yield was 0.1 g (50%), mp 210-211°C. IR spectrum, ν, cm^{-1}: 3450, 3420 (NH), 3330 (associated OH group), 1635 (C=N). UV spectrum, λ_{max}, nm (log ε): 220 (4.47), 256 (4.34), 274 (4.37), 290 (4.34), 322 (4.23), 333 (4.20). Found, %: C 72.4; H 4.6; N 16.9. $C_{15}H_{11}N_3O$. Calculated, %: C 72.3; H 4.4; N 16.9.

1-Formyl-3H-pyrrolo[2,3-c]carbazole Thiosemicarbazone (VIII). Thiosemicarbazide (0.5 g, 0.003 mole) and a catalytic amount of an ethanolic solution of hydrochloric acid were added to a hot solution of aldehyde (I) (0.2 g, 0.0008 mole) in ethyl alcohol (50 ml) (pH 4.0-5.0). The reaction mixture was boiled for 3 h. The crystals which precipitated on cooling were filtered off, washed thoroughly with water, and dried. Yield was quantitative, mp 254-256°C. IR spectrum, ν, cm^{-1}: 1655 (C=N), 1370 (C=S). Found, %: C 62.1; H 3.9; N 23.0; S 10.3. $C_{16}H_{13}N_5S$. Calculated, %: C 62.5; H 4.2; N 22.8; S 10.4.

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ALLERGENIC PROPERTIES OF MODIFIED FORMS OF TERRILYTIN

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At the present time the effectiveness and potential of using enzyme preparations for various human illnesses has been established [1, 2]. In addition, the positive effect of using enzymes is often reduced due to a series of undesirable consequences, the most important being complications linked with the immune system of the organism, primarily with its allergic retuning which is an important factor in the development of hypersensitivity reactions [3-5].

It is well known that the strength and character of the immune response depends in many respects on the chemical structure of the antigen and the conformation of its molecule [6, 7]. Consequently it is necessary to search for routes and methods of modifying native enzymes giving enzymes with less marked immunogenic and allergenic properties.

The aim of the present work was the study of the allergic potential of preparations of a microbial proteinase of thrombolytic action (terrilytin) obtained by covalent bonding of the native enzyme to polymers of various types.

EXPERIMENTAL

Preparations of terrilytin with a protein content of 72% were used in this work and were obtained in the All-Union Scientific-Research Institute for the Technology of Antibiotics and Enzymes for Medicinal Purposes. Modified forms were also used in which terrilytin was chemically bonded to a copolymer of vinylpyrrolidone and acrolein (molecular weight 30,000) in a 1:1 ratio (VPT), to oxidized rheopolyglucin (molecular weight 30,000) in the same ratio (RPGT), or to human serum albumin at a ratio of enzyme:albumin of 1:5 (AT).

Biological experiments were carried out in guinea pigs of weight 300-400 g and in chinchilla rabbits of weight 2.5-3.0 kg. Allergenic properties of enzymes were assessed by data of active anaphylaxis (AA) and active anaphylaxis on isolated organs (AAIO).

Experiments on AA were performed in guinea pigs and rabbits by the method described by us previously [8]. In AAIO experiments sections of ileum from sensitized and intact guinea pigs were used as the shock organ. Experiments were carried out by the classical Schildt–Dale procedure [9]. The strength of the anaphylactic contraction of gut sections was assessed in relation to the contraction caused by adding a definite amount of acetylcholine (final concentration in vessel was 0.01 μg/ml). In each experiment at least 3 ileum sections were investigated from each animal. The amplitude of the contraction caused by acetylcholine was taken equal to 1. Results of experiments were expressed as an anaphylactic contraction index (ACI) obtained by dividing the size of the contraction caused by adding enzyme by the size of the contraction observed on previously adding the operating dose of acetylcholine.

RESULTS AND DISCUSSION

The investigation was devoted to a comparison of the anaphylactogenic activity of modified terrilytin preparations with that of the active enzyme. Results of experiments are pre-

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