SYNTHESIS OF ACYL DERIVATIVES OF PYRAZOLONE INDUCING THE FORMATION OF ANTIVIRAL INHIBITORS

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Acyl derivatives of aminopyrazolone show an antiphlogistic effect, which is due mainly to an inhibition of the inflammation mediators, a lowering of capillary reactivity, a stabilization of cellular and subcellular structures, and to an inhibitory effect on cellular energy exchange. Additionally, some of these compounds increase the functional activity of human embryo fibroblast culture, reduce all the parameters of the cellular mitotic cycle, and induce the formation of the inhibitors of a series of RNA- and DNA-containing viruses [1-4]. Contrary to interferon, the antiviral inhibitors (AI) that we discovered do not show tissue specificity, are more stable with respect to the proteolytic enzymes, and are inactivated in acidic media [5-7].

In the present work, the synthesis of 33 acyl derivatives of pyrazole is described and the relationship between their AI activity, on the one hand and their chemical structure and antiphlogistic effect, on the other, is studied (Table 1).

Analysis of the effect of antipyrilamides and butadione on AI formation in the experiments on inhibition of the cytopathogenic activity (CPA) of Coxsackie A13 virus, vesicular stomatitis virus (VSV), and adenovirus type 23 in cultures of human embryo fibroblasts (HEF) and chicken embryo fibroblasts (CEF) showed that the series of 4-aminoantipyrine derivatives acylated by aliphatic acids (group A) are active inductors of AI; however, they protect the cells from CPA caused only by RNA-containing viruses (Coxsackie A13 and VSV). The antiviral effect of the group A compounds, as well as their antipyretic and antiphlogistic activities, increases with increase in the number of carbon atoms in the side chain. The most active compounds, acylated by higher fatty acids (antipyrylamides of palmitic and stearic acids), suppress the cytopathogenic effect of viruses Coxsackie A13 and VSV in five and four days, respectively. A decrease in the number of carbon atoms in the acyl residues, as well as the introduction of chlorine atoms, decreases the ability of the compound to protect cells against CPA viruses. The introduction of a stearyl residue into the molecule of 4-methylaminoantipyrine, or in the composition of glycoylaminoantipyrine (group C), also reduces the induction.

Antipyrylamides of the substituted aromatic acids (group B) show, as a rule, higher antipyretic and antiphlogistic activities; the nature and position of the benzyl radical substituents definitely affect the antiviral activity. Compounds of this group show antiviral activity, protecting the cells to various degrees against the CPA of viruses. Contrary to antipyrylamides of group A, the introduction of halogen atoms (chlorine, bromine, iodine) into the aromatic acid radical (group B) increases sharply the ability of the pyrazole derivatives to induce AI on the third day after the virus introduction, which ability is particularly pronounced when two chlorine atoms are present in the phenyl residue (antipyrylamide of 2,4-dichlorobenzoic acid). An increase in the AI induction takes place on the introduction of the electron-seeking substituents, namely bromine and nitro group, into the molecule of salicylic acid antipyrylamide, and two electron-seeking nitro groups in positions 3 and 5 of the phenyl radical. Thus, the antipyrylamides of 3,5-dinitrobenzoic and particularly 2-hydroxy-4-nitrobenzoic acids inhibited the CPA of RNA-containing (Coxsackie A13) and DNA-containing (adenovirus type 23) viruses.

The introduction of alkyl substituents into the amide group of the antipyrylamides does not significantly effect the AI-inducing activity of the compounds, as was the case with the antiphlogistic effect; in this case, the electron donor, or the electron-seeking character

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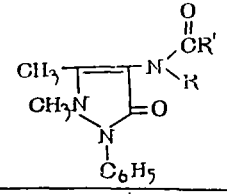
TABLE 1. Acyl Derivatives of 4-Amino- and 4-Methylaminoantipyrine, Their Antiphlogistic Activity, and the Effect on the Formation of Antiviral Inhibitors in Cell Cultures of Chicken Embryo Fibroblasts



R	R'	mp, °C	Infrared spectra, λ_{max}, cm^{-1}				Found, %			Empirical formula	Calculated, %			Antiphlogistic activity*	Viral activity†	
			C=O		NH in amides	other functional groups	C	H	N		C	H	N		Coxsackie A13	adenovirus type 23
			amide group	ring												
Group A																
H	CH_3	198—200	1675	1700	3240	—	—	—	16,87	$C_{13}H_{15}N_3O_2$	—	—	17,14	0,67	3+	4+
H	C_4H_9	174—5	—	—	—	—	67,03	7,35	14,97	$C_{16}H_{21}N_3O_2$	66,55	7,31	14,63	0,70	3+	4+
H	C_5H_{11}	175—6	—	—	—	—	68,16	7,79	13,94	$C_{17}H_{23}N_3O_2$	67,77	7,64	13,95	0,69	2+	4+
H	C_6H_{13}	175—6	—	—	—	—	68,97	7,83	13,81	$C_{18}H_{25}N_3O_2$	68,54	7,94	13,33	0,62	2+	4+
H	$C_{11}H_{23}$	147—8	—	—	—	—	72,01	9,32	11,41	$C_{23}H_{35}N_3O_2$	71,69	9,09	10,91	0,63	3+	4+
H	$C_{15}H_{31}$	142—3	1650	1690	3240	—	73,80	9,64	9,46	$C_{27}H_{43}N_3O_2$	73,46	9,75	9,52	1,10	—	4+
H	$C_{17}H_{35}$	143—4	1645	1690	3240	—	74,60	9,95	8,93	$C_{29}H_{47}N_3O_2$	74,11	10,00	8,94	2,18	—	4+
CH_3	$C_{17}H_{35}$	79,81	1660	1690	—	—	75,15	10,39	8,29	$C_{30}H_{49}N_3O_2$	74,53	10,14	8,69	1,10	2+	4+
H	CH_2Cl	189—90	—	—	—	—	—	—	15,45	$C_{13}H_{14}N_3O_2Cl$	—	—	15,02	0,59	3+	4+
H	$CHCl_2$	238—9	—	—	—	—	49,30	4,26	13,35	$C_{13}H_{13}N_3O_2Cl_2$	49,68	4,14	13,37	0,72	3+	4+
H	CCl_3	230—1	1645	1710	3150, 3230	—	44,30	3,58	12,20	$C_{13}H_{12}N_3O_2Cl_3$	44,70	3,44	12,05	0,72	3+	4+
Group B																
H	C_6H_5	173—4	1650	1680	3310—3320	—	—	—	13,62	$C_{18}H_{17}N_3O_2$	—	—	13,67	0,82	3+	4+
CH_3	C_6H_5	130—1	—	—	—	—	—	—	—	$C_{19}H_{19}N_3O_2$	—	—		0,98	3+	4+
H	C_6H_4OH-o	217—8	1635	1678	3135	3325 (OH)	—	—	12,88	$C_{18}H_{17}N_3O_3$	—	—	13,00	1,77	3+	4+
CH_3	C_6H_4OH-o	198—9	—	—	—	—	—	—	12,41	$C_{19}H_{19}N_3O_3$	—	—	12,46	0,88	2+	4+

TABLE 1 (Continued)

H	$C_6H_3(OH)_2$-2,4	280—1	1620	1635	—	3375 (OH)	64,50	5,16	12,55	$C_{18}H_{17}N_3O_4$	63,71	5,01	12,39	0,97	4+	3+
H	C_6H_3OHBr-2,5	266—7	1634	1674	3135	3340 (OH)	53,48	4,05	10,19	$C_{18}H_{16}N_3O_3Br$	53,73	3,98	10,45	1,06	—	4+
H	$C_6H_3OHNO_2$-2,4	297—8	1636	1683	3190	3150,1572(NO_2) 3350(OH)	59,00	4,53	15,20	$C_{18}H_{16}N_4O_6$	58,70	4,34	15,21	0,88	—	2+
H	$C_6H_4NO_2$-п	207—8	1650	1675	3100, 3250	1350 (NO_2)			16,07	$C_{18}H_{16}N_4O_4$	—	—	15,99	1,10	3+	3+
CH_3	$C_6H_4NO_2$-4	155—6	—	—	—	—			15,19	$C_{19}H_{18}N_4O_4$	—	—	15,30	1,09	3+	4+
H	$C_6H_3(NO_2)_2$-3,5	246—7	1645	1680	3100, 3200,	1350, 1550 (NO_2)	54,00	3,98	1765	$C_{18}H_{25}N_5O_6$	54,40	3,77	17,63	1,96	2+	2+
CH_3	$C_6H_3(NO_2)_2$-3,5	216—7	1650	1676	—	1340, 1520 (NO_2)	55,74	4,12	17,09	$C_{19}H_{17}N_5O_6$	55,47	4,13	17,03	0,95	3+	4+
H	$C_6H_3(NO_2)_2$-2,5	238—9	1650	1685	3150	1350, 1550 (NO_2)	54,62	3,61	17,65	$C_{18}H_{15}N_5O_6$	54,40	3,77	17,63	0,88	4+	4+
CH_3	$C_6H_4NO_2$-2	185—6	—	—	—	—	—	—	15,35	$C_{19}H_{18}N_4O_4$	—	—	15,30	1,31	—	4+
CH_3	$C_6H_4NO_2$-3	123—4	—	—	—	—	—	—	15,42	$C_{19}H_{18}N_4O_4$	—	—	15,30	0,72	3+	4+
H	C_6H_4Cl-2	227—8	1650	1682	3150	—	63,74	5,00	12,64	$C_{18}H_{16}N_3O_2Cl$	62,33	4,78	12,29	1,01	3+	4+
H	C_6H_4Cl-4	226-7	—	—	—	—	—	—	12,44	$C_{18}H_{16}N_3O_2Cl$	—	—	12,29	1,15	3+	4+
H	C_6H_4I-2	250—1	1650	1680	3150	—	50,09	3,89	9,67	$C_{18}H_{16}N_3O_2I$	49,89	3,69	9,70	1,42	3+	4+
H	C_6H_4Br-4	231—2	1655	1665	3190, 3450, 3490	—	53,58	4,62	10,52	$C_{18}H_{16}N_3O_2Br \cdot H_2O$	53,46	4,45	10,40	1,19	3+	4+
H	$C_6H_3Cl_2$-2,4	222—3	1640	1685	3140,	—	54,98	4,25	10,87	$C_{18}H_{15}N_3O_2Cl_2$	57,44	4,00	11,17	1,00	2+	4+
CH_3	$C_6H_4OCH_3$-4	170—1	1665	1670	3170		68,77	6,18	11,78	$C_{20}H_{21}N_3O_3$	68,37	6,00	11,97	0,70	—	3+
						Group C										
H	$CH_2OCOC_{17}H_{35}$	104—5	1660	1710	3220	1750 (C=O of the complex esters)	70,90	9,29	8,44	$C_{31}H_{49}N_3O_4$	70,58	9,30	7,97	1,20	3+	4+
H	$CH_2OCOC_6H_5(NO_2)_2$-3,5	229—30	1650	1700	3100, 3190	1740 (C=O of the complex esters	52,33	4,10	15,05	$C_{20}H_{17}N_5O_8$	52,74	3,73	15,33	1,10	3+	4+
	Butadione (phenylbutazone)													1,0	2+	4+
	Viral control (no compounds added)														4+	4+

*All compounds were administered in a dosage of 100 mg/kg; the butadione activity was taken as the unit.

†Estimated by the inhibition of the cytopathogenic activity in five days: —, no change ±, degeneration of the isolated cells; +, degeneration of 1/4 of the cellular monolayer; 2+, degeneration of 1/2 of the monolayer; 3+, degeneration of 3/4 of all cells; 4+, total degeneration of the tissue culture.

of the phenyl ring substituents, is less pronounced. A clear correlation between the structure of the methylantipyrylamides of the aliphatic and substituted aromatic acids and their antiviral activity could not be detected, although in some cases the replacement of the hydrogen atom in the amide group with a methyl radical led to increased AI-inducing activity. This is the case with the methylantipyrylamide of p-methoxybenzoic acid, which retains the CPA for both the RNA-containing (Coxsackie A13, VSV) and DNA-containing (adenovirus type 23) viruses. An analogous activity, but only against the RNA-containing viruses, is shown by the methylantipyrylamide of o-nitrobenzoic acid.

Further investigation of the antiviral activity of antipyrylamides on the degree of platelet formation in cultures of human embryo fibroblasts and viral hemagglutinin culture (Newcastle disease virus and paragrippe Sendai virus) showed that the highest degree of suppression of the platelets was achieved with the antipyrylamides of palmitic, stearic, 2,4-dichlorobenzoic, and o-chlorobenzoic acids as AI inductors. The suppression of platelet formation (with respect to the control) for a 24-h contact of human embryo fibroblasts with the above compounds was 78.4-89.2%, and for a 48-h contact, it was 83.1-92.0%.

The components of the aminopyrazolone acyl derivatives did not cause the appearance of AI under the conditions of our experiments. Thus, for the appearance of AI induction, the entire molecule of the antipyrylamide is important, while the nature and the position of the substituent in the benzoyl radical at the $C_{(4)}$ carbon atom of the pyrazolone ring are of great significance.

The phenomenon of the formation and isolation of the species-nonspecific AI by cells of human and animal origin under the influence of low-molecular-weight organic compounds, including the pyrazole derivatives, is a prerequisite for obtaining a new class of antiviral agents. Of practical importance in this respect is the sensitivity to the AI activity of the RNA-containing Coxsackie viruses, particularly Coxsackie A13 showing cardiotropism in humans, and Coxsackie A21 inducing epidemic outbursts of respiratory ailments. This becomes even more important when we take into account that there are, presently, no chemotherapeutic agents active against these types of viruses, which are rather insensitive even to interferon [8, 9].

EXPERIMENTAL BIOLOGICAL PART

Evaluation of the antiphlogistic activity of the investigated compounds was carried out using the method showing the reactivity of capillaries to the inflammatory agent [10].

The AI-inducing activity of the compounds (100 or 200 μg/ml depending on the presence of CPA) was determined using a known method [11] with some modifications: The effective dose of the compound in 0.1 ml of the sample was introduced into the cell culture of human or chicken embryo fibroblasts using No. 199 medium (0.8 ml) and the culture was left for 48-72 h at 37°C. The liquid containing the virus (0.1 ml, 100 CPA_{50}) was then added without decanting the medium (No. 199) with the investigated compound; the control tissue cultures were infected with the same dose of the virus. The results were discussed after full degeneration of the control culture cells. The following test samples were used: RNA-containing viruses (VSV, Newcastle disease virus, paragrippe Sendai virus, Coxsackie A13) and DNA-containing viruses (adenovirus type 23), obtained from the collections of the D. I. Ivanovskii Institute of Virology of the Academy of Medical Sciences of the USSR and the Moscow Institute of Viral Preparations.

EXPERIMENTAL CHEMICAL PART

The compounds of groups A and B were prepared by nucleophilic substitution of the hydroxyl in the carboxylic group of the carboxylic acids by a 4-antipyrylamino or a 4-methylantipyrylamino group [12].

A mixture of equimolar amounts of 4-amino- or 4-methylaminoantipyrine and the carboxylic acid in a sufficient amount of anhydrous benzene was heated to 70°C, followed by dropwise addition of the condensing agent, phosphorus trichloride, in an amount one-half of the molar amounts of the amine and the acid. The mixture was refluxed on an oil bath for 2-3 h, the benzene was removed, and the reaction mixture was dried and treated with 10% sodium carbonate solution. After filtration, the residue was washed with water, followed by ether washing, and then recrystallized from ethanol.

The compounds of group C were prepared by nucleophilic substitution of the bromine atom in 4-bromoacetylaminoantipyrine by a carboxylic acid residue. A mixture of equimolar amounts of 4-bromoacetylaminoantipyrine and the sodium salt of the carboxylic acid in absolute ethanol was refluxed for 15 h with continuous stirring. After cooling, the solid mass was filtered, dried, and treated with hot water. The residue was again filtered, washed with water to negative reaction of bromide ions, and recrystallized from alcohol or dioxane.

The compounds prepared were identified by mp, elemental analyses, and selectively by infrared spectra. The data for the synthesized compounds are presented in Table 1.

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SYNTHESIS OF DIACYLHYDRAZINES AND OXADIAZOLES STARTING FROM 3-PHENYL-5-METHYL-4-ISOXAZOYL HYDRAZIDE

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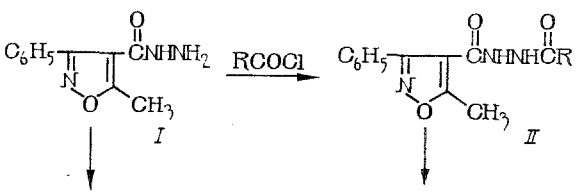
This communication is a continuation of the studies on the synthesis and properties of 3-phenyl-5-methyl-4-isoxazoylhydrazide (I) derivatives [1]. The synthesis and the study of diacylhydrazines (II) and 2- and 2,5-substituted oxadiazoles (III) is reported. These compounds are of interest for biological studies, as some of them were found to show a wide spectrum of physiological activity [2, 3].

The synthesis was carried out according to the following reaction sequence:



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