SYNTHESIS AND ANTIVIRAL ACTIVITY OF CERTAIN ALIPHATIC AMIDES OF N-SUBSTITUTED α-PYRROLIDINE CARBOXYLIC ACIDS

A. S. Lebedeva, N. S. Tolmacheva,
N. A. Yushchenko, A. M. Likhosherstov,
and A. P. Skoldinov

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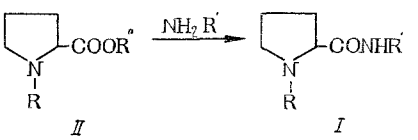
According to the available literature data, mainly Japanese authors, a series of substances belonging to various classes of organic compounds and containing long aliphatic radicals possess antiviral activity (q.v. for example [1,2]). Similar activity was also noted in a series of amides of proline [3].

In connection with a search for substances active against influenza viruses we prepared certain aliphatic amides of N-substituted α-pyrrolidine carboxylic acids (I), among them some containing long aliphatic chains, and studied their antiviral activity in respect to influenza virus A. The synthesis of the amides was carried out by two methods. Method A was the reaction of the corresponding alkyl esters of the N-substituted α-pyrrolidine carboxylic acids (II), prepared by the method we reported earlier [4], with primary amine and simultaneous distillation of the alcohol formed as a result of the reaction.



II I

This method was used for the preparation of the amides (I) with different radicals R and R'.

A simpler method of synthesis (method B) was used for the preparation of the amides (I, R=R') with the same radical on the pyrrolidine and amide nitrogen atoms, the reaction of alkyl esters of α-bromo-δ-chlorovaleric acid (III) with primary amines in an inert solvent:

$$CH_2ClCH_2CH_2CHBrCOOR'' + NH_2R' = I\ (R = R').$$

The yields, constants, and analyses for the substances prepared by these methods are given in Table 1.

The antiviral activity was studied both in vitro and in vivo experiments on chicken embryos innoculated with influenza virus A (PR8). The test results are given in Table 2. Of the amides of N-substituted α-pyrrolidine carboxylic acids (I) studied, compounds No. 5,6, and 10 showed virulicidal action in respect to influenza virus A (PR8). The antiviral activity of the substances studied is primarily dependent on the length of the alkyl chain on the amide nitrogen atom. Thus, antiviral activity is absent for compounds having alkyl chains of C_{10} and below and appears for compounds with a C_{14} alkyl chain.

The antiviral activity is also dependent on the nature of the radical on the pyrrolidine nitrogen atom. For example, compound No. 10 in the in vitro experiments on chicken embryos showed the most powerful inhibiting action in respect to the virus. However it will be necessary to carry out more comprehensive studies in order to explain the effect of the nature of the radical on the pyrrolidine nitrogen atom.

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TABLE 1. Amides of N-Substituted α-Pyrrolidine Carboxylic Acids (I)

Compound No.	R	R'	Method of preparation	Yield (in %)	Boiling point (in degrees)	n_D^{20}	Found (in %)		Empirical formula	Calculated (in %)	
							C	H		C	H
1	CH_3	n-C_4H_9	А	35	107—8 (3 mm)	1,4691	65,34	10,70	$C_9H_{18}N_2O$	65,16	10,94
2	CH_3	n-C_6H_{13}	А	93	123—4 (1,5 mm)	1,4688	67,45	11,34	$C_{12}H_{24}N_2O$	67,89	11,40
3	CH_3	n-C_7H_{15}	А	96	134—5 (2 mm)	1,4678	68,78	11,60	$C_{13}H_{26}N_2O$	68,96	11,58
4	CH_3	n-$C_{10}H_{21}$	А	90	163—4 (3 mm)	1,4477	71,15	12,25	$C_{16}H_{32}N_2O$	71,60	12,01
5	CH_3	n-$C_{14}H_{29}$	А	92	213—4 (4 mm)	—	73,96	12,63	$C_{20}H_{40}N_2O$	74,03	12,43
6	CH_3	n-$C_{16}H_{33}$	А	95	Mp. 38—40 210—1 (2 mm)	—	75,25	12,47	$C_{22}H_{44}N_2O$	74,94	12,58
7	n-C_4H_9	n-C_4H_9	Б	48,9	Mp. 42—43 96—8 (1 mm)	1,4673	68,71	11,31	$C_{13}H_{26}N_2O$	69,00	11,58
8	iso-C_4H_9	iso-C_4H_9	Б	31	131 (4 mm)	1,4633	68,87	11,51	$C_{13}H_{26}N_2O$	69,00	11,58
9	iso-C_5H_{11}	iso-C_5H_{11}	Б	40,2	148—51 (3 mm)	1,4652	70,55	11,65	$C_{15}H_{30}N_2O$	70,84	11,89
10	$C_6H_5CH_2$	n-$C_{14}H_{29}$	А	94	215—7 (1 mm)	—	77,84	11,26	$C_{26}H_{44}N_2O$	77,96	11,08
11	$CH_2CH{=}CH_2$	$CH_2CH{=}CH_2$	Б	42	Mp. 34—36 107—8 (2 mm)	1,4915	67,92	9,38	$C_{11}H_8N_2O$	68,03	9,34

EXPERIMENTAL

Amides of N-Substituted α-Pyrrolidine Carboxylic Acids (I). A. A mixture of 1 mole of the ethyl or methyl ester of the N-substituted α-pyrrolidine carboxylic acid and 1 mole of the primary amine was heated for 5 h at 195-200°C simultaneously distilling off the alcohol formed. The obtained amide was distilled in vacuum. The yield was 85.95% (q.v. No. 1-6, 10, Table 1).

B. A mixture of 1 mole of the methyl or ethyl ester of α-bromo-δ-chlorovaleric acid and 3.5 mole of primary amine was boiled for 6 h in an inert solvent and then cooled. The precipitate was filtered off and the filtrate made alkaline with a saturated aqueous solution of potassium carbonate. The organic layer was evaporated. The residue was distilled in vacuum. The yield of the corresponding amide was 50-70% (q.v. No. 7-9, 11, Table 1).

Method of Study of the Antiviral Activity in the Experiments in Vitro

Such a quantity of the test substances was added to 2 ml of a physiological solution of virus A (PR8) in a dilution of 10^{-3} so that their concentration was 50 or 100 μg/ml. The mixture was maintained for 3 h at 37°. Then each mixture was diluted to 10^{-8}. Four embryos were inoculated with each dilution of the appropriate sample. The presence of virus in the sample was determined after 48 h of incubation by establishment of a hemagglutination reaction (HGR) with a 1% suspension of chicken erythrocytes. For the value of the infection titer the highest dilution was used in which virus was detected in 2 out of 4 embryos on inoculation. A physiological solution of virus without compounds was used as control.

The test results are given in Table 2.

Method of Study of Antiviral Activity in Experiments in Vivo

The antiviral activity in vivo was studied in chicken embryos and mice. The substances under test were introduced into chicken embryos at the maximum transferable concentrations and then after 15-20 min, 1, 10, or 100 infection doses of virus A (PR8) were introduced. Conclusions concerning the intensity of multiplication of the virus were drawn from the presence of virus in each embryo and from the infection titer which were appropriately determined by establishment of the HGR with a 1% suspension of chicken erythrocytes and titration of tenfold dilutions of the material in fresh chicken embryos. The results of the tests are given in Table 2.

In the latter series of experiments the inhibition effect of compounds No. 5, 6, and 10 were determined for the influenza infection of white mice. Animals of weight 20-22 g were innoculated under light ether narcosis with 10 LD_{50} of virus in a volume of 0.05 ml of physiological solution. The compounds under test were introduced intraperitoneally for the first time 30 min from inoculation; then after two days, once a day in doses of 50 mg/kg (compounds No. 5 and 6) and 12.5 mg/kg (for compound No. 10); these are the maximum transferable doses for this method of introduction.

TABLE 2. Antiviral Activity of Amides of N-Substituted α-Pyrrolidine Carboxylic Acids (I)

Compound No.	R	R′	Virus neutralizing activity in experiments in vitro		In vivo activity in chicken embryos			
			Concentration of substance in μg/ml	Infection titer	Dose of comp. (mg/embryo)	Amount of virus (in LD_{50})	% Embryo-of with virus	Infection titer
2	CH_3	C_6H_{13}	100	10^{-8}	0,5	1	50	10^{-7}
3	CH_3	C_7H_{15}	100	10^{-8}	0,5	1	60	10^{-7}
4	CH_3	$C_{10}H_{21}$	100	10^{-8}	0,3	1	65	10^{-7}
5	CH_3	$C_{14}H_{29}$	50	0	1	1	13	10^{-4}
						10	90	10^{-8}
6	CH_3	$C_{16}H_{33}$	50	0	1	1	19	10^{-4}
						10	83	10^{-7}
7	n-C_4H_9	n-C_4H_9	100	10^{-7}	2	1	65	10^{-7}
8	iso-C_4H_9	iso-C_4H_9	100	10^{-8}	2	1	65	10^{-7}
10	$C_6H_5CH_2$	$C_{14}H_{29}$	50	0	2	1	6	10^{-3}
						10	30	10^{-5}
						100	100	10^{-8}
	Control			10^{-8}	—	1	60	10^{-7}
						10	90	10^{-8}
						100	100	10^{-8}

CONCLUSIONS

1. A series of amides of N-substituted α-pyrrolidine carboxylic acids are prepared for comparachemotherapeutic tests.

2. The antiviral activity of amides of N-substituted α-pyrrolidine carboxylic acids are studied with respect to influenza virus type A. A connection between the chemical structure of the amides and their antiviral activity is followed in experiments in vitro and in vivo.

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