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SYNTHESIS AND PHARMACOLOGICAL ACTIVITY OF N-SUBSTITUTED 2-AZASPINACEAMINE

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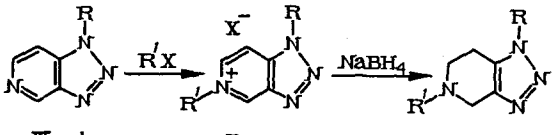
Derivatives of 2-azaspinaceamine or 4,5,6,7-tetrahydro-1,2,3-triazolo[4,5-c]pyridine were first described in 1964 [1], but the data of investigators on their biological activity have not hitherto been included in the literature. At the same time, in the study of the biogenic spinaceamine – the near analog 4,5,6,7-tetrahydrotriazolo[4,5-c]pyridine, its protective bacteriostatic influence in relation to the surface of the amphibian skin was established [2-4]. Some derivatives of spinaceamine showed antiviral activity [5], anticonvulsant activity [6], and antisecretory and antiulcer activity [7]. Special attention is merited by the detection of marked hypotensive effect in the series of 5-hydroxyethylspinaceamines, which may be combined with anticonvulsant, sedative, soporific, and antihypoxic action [8] for different compounds. The manifestation of such a type of activity by spinaceamines as well as the high molecular–structural similarity with 2-azaspinaceamines induced us to investigate the biological activity of the latter. However, the route of synthesis mentioned was found to be practically unsuitable for the isolation of simple derivatives of 2-azaspinaceamine due to the significant explosion hazard of the azides, as well as the low yields of the object compounds [1].

By analogy with the isolation of derivatives of spinaceamine [5], we developed a simple method for the synthesis of substituted 2-azaspinaceamine (Ia-g) by the reduction, using sodium borohydride, of the quaternary salts (IIa-g), readily formed in turn by the heating of 1-methyl- or 1-phenyl-1H-triazolo[4,5-c]pyridines (IIIa, b) [9, 10] with alkyl(benzyl) halides (Table 1).



IIIa, b IIa-g Ia-g

R = Me (Ia–e, IIa–e, IIIa) Ph (If, g, IIe, g, IIIb);
R^1 = Me (Ia,g, IIa,g), Br (Ib, IIb), Bu (Ic, IIc), CH_2PH (Id, IId), $HOCH_2CH_2$ (Ie,f, IIe,f), X = Cl (IId–f), I (IIa–c,g)

The PMR spectra of 2-azaspinacemaines (Table 2) are similar to the PMR spectra of spinaceamines [5] and, apart from signals of the substituents R and R′, they only have signals of protons at the saturated carbon atoms; this unambiguously confirms the structure both of the salts (IIa-g) and of the bases (Ia-g) presented here. The derivatives of 2-azaspinaceamine (Ia-g), obtained by us, are low-melting substances, in consequence of which they were characterized in the form of dihydrochlorides. The 2-azaspinaceamines (Ia-g) were also investigated in just this stable salt form for biological activity.

EXPERIMENTAL (CHEMICAL)

The PMR spectra of the compounds obtained were registered on the "Tesla" spectrometer (60 MHz, Czechoslovakia) in CF_3COOH with TMS as the internal standard.

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TABLE 1. Quaternary Salts of Derivatives of Triazolo-[4,5-c]pyridine (IIa-g) and Dihydrochlorides of 1,5-Disubstituted 2-Azaspinaceamine (Ia-g)

Compound	Yield, %	mp, °C	Empirical formula
Ia	94	239-1	$C_7H_{12}N_4 \cdot 2HCl$
Ib	97	220-2	$C_9H_{16}N_4 \cdot 2HCl$
Ic	95	174-5	$C_{10}H_{18}N_4 \cdot 2HCl$
Id	96	246-8	$C_{13}H_{16}N_4 \cdot 2HCl$
Ie	94	159-60	$C_8H_{14}N_4O \cdot 2HCl$
If	96	202-4	$C_{13}H_{16}N_4O \cdot 2HCl$
Ig	98	249-51	$C_{12}H_{14}N_4 \cdot 2HCl$
IIa	98	221-3	$C_7H_9IN_4$
IIb	96	154-6	$C_9H_{13}IN_4$
IIc	95	134-6	$C_{10}H_{15}IN_4$
IId	98	139-41	$C_{13}H_{13}ClN_4$
IIe	97	167-9	$C_8H_{11}ClN_4O$
IIf	91	212-4	$C_{13}H_{13}ClN_4O$
IIg	83	188-90	$C_{12}H_{11}IN_4$

TABLE 2. PMR Spectra of Derivatives of 4,5,6,7-Tetrahydrotriazolo[4,5-c]pyridine (Ia-g)

Compound	Chemical shift, δ, ppm				
	N^1-R	4-CH_2	N^5-R'	6-CH_2	7-CH_2
Ia	4,25 (s, 3H, CH_3)	5,10, q	3,26(s, 3H, CH_3)	4,01, t	3,53, t
Ib	4,17(s, 3H, CH_3)	5,01, t	1,00(t, 3H, CH_3), 1,90(q, 2H, β-CH_2), 3,91(t, 2H, α-CH_2)	3,46,t	3,37, t
Ic	4,20 (s, 3H, CH_3)	5,10, t	1,00(t, 3H, CH_3), 1, 45q(2H, α-CH_2), 2,11(t, 2H, β-CH_2), 4,03(t, 2H, γ -CH_2)	3,70, t	3,57, t
Id	4,20 (s, 3H, CH_3)	4,88, t	4,68(s, 2H, CH_2), 7,41(s, 5H, C_6H_5)	3,83, t	3,55, t
Ie	4,21 (s, 3H, CH_3)	4,98, t	3,95(t, 2H, β-CH_2), 5,28(t, 2H, α-CH_2)	3,85, t	3,58, t
If	7,76 (s, 5H, C_6H_5)	5,10, t	4,41(t, 2H, β-CH_2), 5,30(t, 2H, α-CH_2)	4,21, t	3,63, t
Ig	7,68 (s, 5H, C_6H_5)	5,25, q	3,35(s, 3P, CH_3)	3,98, t	3,60, t

The values of the elemental analyses found correspond with the calculated values.

1,5-Disubstituted Triazolo[4,5-c]pyridinium Halides (IIa-g). Method A. To the solution of 10 mmole of the base (III) in 15 ml of abs. alcohol are added 15 mmole of the corresponding alkyl(benzyl) halide. The mixture is heated at the boiling temperature for 3 h. The excess of the alkyl halide and the solvent is distilled off in the vacuum of a water-jet pump, and the salt obtained is crystallized from alcohol.

Method B. The mixture of 10 mmole of the base (III) and 15 mmole of ethylene chlorohydrin is heated with weak boiling for 3 h. The excess of the ethylene chlorohydrin is distilled off in the vacuum of a water-jet pump; the residue is washed with ether and triturated with acetone (2 portions of 5 ml each). The salt obtained is crystallized from isopropanol.

1,5-Disubstituted 2-Azaspinaceamine Dihydrochlorides (Ia-g). To the solution of 10 mmole of the salt (IIa-g) in 50 ml of alcohol is added, in parts with stirring in the course of 1.5 h at room temperature, 0.57 g (15 mmole) of $NaBH_4$. The stirring is stopped, and the residue is filtered off after 16-20 h and washed with 5 ml of alcohol. The total filtrate is concentrated at 50-60°C in the vacuum of a water-jet pump. The residue is extracted with benzene; the extract is dried with solid alkali, and the solvent is distilled off. The resulting oil is dissolved in 8 ml of alcohol prior to the addition of 2 ml of concentrated HCl to the solution and the evaporation to dryness. The salt obtained is recrystallized from alcohol.

EXPERIMENTAL (BIOLOGICAL)

The compounds synthesized were investigated for seven types of pharmacological activity: the hypotensive, spasmolytic, sedative, soporific, anticonvulsant, myorelaxant, and antihypoxic. Moreover, the acute toxicity of derivatives of 2-aza-spinaceamine was studied. Experiments were carried out on white hybrid mice, rats, and cats.

The influence of the compounds (Ia-g) on the systemic arterial pressure (SAP) and the frequency of cardiac contractions (FCC) was studied in acute experiments on white hybrid rats, of both sexes and the mass 170-220 g, and cats of the mass 3.2-4.3 kg, anesthetized with 50 mg/kg of pentobarbital sodium ip. The investigated substances were introduced iv in doses equal

TABLE 3. Influence of Derivatives of 2-Azaspinaceamine on the SAP of Anesthetized Rats

Compound	LD_{50}, mg/kg	ED_{20}, mg/kg	Duration of action at the dose of ED_{20}, min	LD_{50}/ED_{20}
a	780	66	15	11.8
b	745	Not active	-	-
c	726	85	15	8,54
d	472	14.3	5-10	33.0
e	504	72	10	7.0
f	480	35.9	15-30	13,4
g	122	6,8	30	17,9
Dibazol	310	22,1	-	14.0

TABLE 4. Neurotropic, Myorelaxant, and Antihypoxic Activity of the Compounds (d, f, g) with Their ip Introduction at the Dose of 0.1 LD_{50} (M ± m)

Compound (I)	Spontaneous motor activity		Duration			
	horizontal	vertical	of hexanol sleep, min	of life after the introduction of Corazol, min	of holding of load, sec	of life in the pressured volume, min
d	27,0 ± 1,0*	17,8 ± 1,8	63,7 ± 3,0*	2.95 ± 0,09	11,3 ± 0,38	58,7 ± 3,0
f	26,2 ± 2,1*	3,8 ± 1,6*	63,4 ± 10,0	5,29 ± 0,9	9,8 ± 1,2*	86,4 ± 3,1*
g	31,5 ± 1,0	30,3 ± 3,1	236,7 ± 10,8*	4,52 ± 0,23*	18,6 ± 3,0	67,6 ± 4,7
Control	34,4 ± 2,7	24,2 ± 5,2	41,9 ± 3,7	3,17 ± 0,05	20,0 ± 4,2	56,0 ± 1,9

*Differences were statistically significant ($p \leq 0.05$) in relation to the control.

to 1/100 and 1/30 of the LD_{50}. The SAP was measured using a mercury manometer on the common carotid artery. The hypotensive activity of the investigated compounds was evaluated according to the effective dose lowering the SAP by 20%, i.e. from the ED_{20}. Moreover, the duration of the hypotensive effect of the compounds synthesized was registered as well as the breadth of the therapeutic action, equal to the ratio of the acute toxicity (LD_{50}) to the ED_{20} (i.e. the therapeutic index was calculated).

The spasmolytic action of the derivatives of 2-azaspinaceamine (Id, f, g) was studied on isolated segments of the small intestine of white hybrid rats according to the method of Ya. I. Khadzhai and coauthors [11, 19]. The spasmolytic activity of the substance was evaluated from the effective concentration giving a halving in the intensity of the barium-induced spasm (the EC_{50}, M). The EC_{50} was calculated on the microcomputer "Élektronika MK 51" using the standard method of least squares [12] with the coordinates "percentage of suppression of spasm–logarithm of concentration" [13].

The neurotropic action of the compounds (Id, f, g) was evaluated in white hybrid mice of both sexes having the mass of 20-22 g according to the following forms of activity: sedative, soporific, and anticonvulsant. The neurotropic effects of the investigated substances were registered at 30-40 min after their ip introduction at the dose equal to 0.1 LD_{50}.

The sedative action was studied from the change of the spontaneous motor activity in a box of the size 30 × 30 × 20 cm, the floor of which was broken into four squares. Upright positions and the number of squares crossed were registered in the course of 2 min [14].

The influence of the compounds on the duration of hexenal-induced narcosis and the anticonvulsant action by the antagonism to Corazol using the model of Corazol-induced spasms were also investigated [14, 15].

The myorelaxant activity was evaluated by the method of I. S. Raevskii [15]. The time in the course of which the mouse held a strip of metallic netting, of the mass equal to its body mass, in the front paws was thereby noted with the immobilization of the animal in the vertical position by the tail.

The antihypoxic properties were studied using the model of hypercapnic hypoxia in white hybrid mice of both sexes having the mass of 20-22 g by placing them in a pressured volume of 250 ml [16].

The results of the experiments were treated by the method of variation statistics utilizing the Student's t-criterion on the microcomputer "Élektronika MK 51" [17].

The acute toxicity was determined on white hybrid mice of both sexes having the mass of 16-20 g. The investigated substances were introduced ip. The death of the mice was registered in the course of 24 h. The LD_{50} values were calculated graphically by the method of Miller and Teitner [17].

It was established that the compounds (Id, f, g) decrease the SAP in rats after the iv introduction (Table 3), whereby the hypotensive effect of the compound (Id) is transient (5-10 min), and the effect of the two other derivatives of 2-azaspinaceamine – (If, g) – is more prolonged (the SAP decreases in the course of 15-30 min). The compound (Id) has a large therapeutic breadth of action (see Table 3). The compounds (Id) and (Ig) surpassed dibazol in the magnitude of their depressor effect by factors of 1.5 and 3.3 correspondingly, and the substance (If) was inferior to dibazol in its activity.

The derivatives of 2-azaspinaceamine (Ia-g) can be referred to the moderately toxic according to the criteria of I. V. Sanotskii and coauthors [18].

It was established that the compound (Id) lowers the spontaneous motor activity (Table 4) and also increases the duration of hexenal-induced sleep. The compound (If) lowers the spontaneous motor and exploratory activity and exerts marked myorelaxant action, halving the time of holding of the load in relation to the control, as well as marked antihypoxic action using the model of hypercapnic hypoxia (cf. Table 4). In contrast to other derivatives of 2-azaspinaceamine, the compound (Ig) has high neurotropic activity: it increases the duration of hexenal-induced sleep by a factor of 5.6 and the duration of life after the introduction of Corazol by a factor of 1.4, and it decreases the intensity of Corazol-induced spasms in the animals (see Table 4). Therefore, compound (Ig) potentiates the action of soporific agents and exerts anticonvulsant activity.

Compounds of the given series show insignificant spasmolytic activity, suppressing the spasm of the intestine induced by barium chloride only at concentrations of 10^{-3}-10^{-4} M.

Therefore, the synthesized derivatives of 2-azaspinaceamine exert hypotensive, psychosedative, myorelaxant, and antihypoxic action. The further synthesis and study of the cardiovascular and neurotropic action of the new derivatives of 2-azaspinaceamine can be considered expedient.

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