SYNTHESIS AND PHARMACOLOGICAL ACTIVITIES OF AMIDOXIMES OF (2-OXOPYRROLIDINO)ALKANOIC ACIDS AND THEIR O-ACYL DERIVATIVES*

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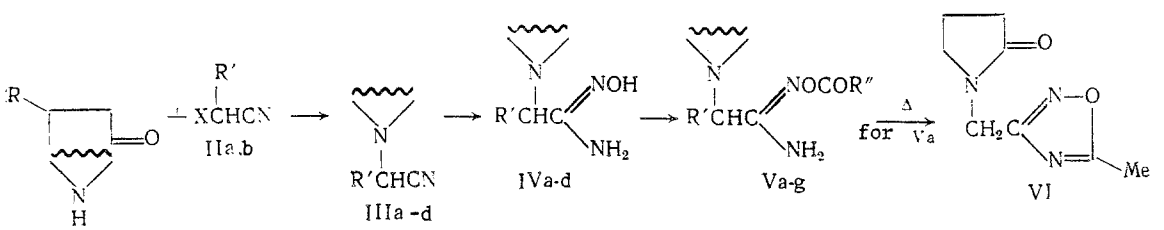
UDC 615.214.31.012.1

It is known that amides and hydrazides of (2-oxopyrrolidino)alkanoic acids have nootropic activity (piracetam, etiracetam, pramiracetam, durpracetam [7]). It was shown earlier that small doses of the amide of (2-oxo-4-phenylpyrrolidino)acetic acid show psychostimulating action [2].

Among the amidoximes of carboxylic acids, which are structural analogs of amides in which the oxygen group of the amide is substituted by a hydroxyimino moiety, a number of psychotropic agents have been found (naprodoxime, trizoxime) [4, 7]. With a view to searching for new effective nootropic preparations and investigating the influence of structural factors (introduction of the amidoxime group, the presence of a phenyl substituent at the 4-position of the pyrrolidine ring, and the nature of the acid radical at the oximino moiety) on the activity, we have synthesized the amidoximes of (2-oxopyrrolidino)alkanoic acids (IVa-d) and their O-acyl derivatives (Va-g), and studied their antihypoxic and antiamnesic properties.

The synthesis of compounds IV was realized by starting from pyrrolidone-2 (Ia) and 4-phenylpyrrolidone-2 (Ib) according to the following scheme:



R = H (Ia, IIIa,b, Va-g), Ph (Ib, IIIc, d, IVc, d);
R′ = H (IIa, IIIa, b, IVa, c, Va, c, e-g), Me (IIb, IIIb, c, IVb, c, Vd, c);
R″ = Me (Va, b), Ph (Vc, d), C_6H_4Cl-2 (Ve), C_6H_5Cl-4 (Vf), $CH(C_3H_7)_2$ (Vg), X = Cl (IIa), Br (IIg)

Alkylation of the sodium salts of pyrrolidones Ia, b with the nitriles of α-halocarboxylic acids IIa, b yielded the nitriles of (2-oxopyrrolidino)alkanoic acids (IIIa-d), which were converted to amidoximes IVa-d by reaction with $H_2NOH{\cdot}HCl$ in the presence of EtONa. Acylation of amidoximes IVa,b with acetic anhydride in dioxane leads to O-acetylamidoximes Va, b. The O-benzoyl derivatives Vc-f were prepared by the reaction of the amidoximes with the corresponding benzoyl chlorides in pyridine. The O-valproyl derivative Vg was obtained with the carbodiimide method. Thermolysis of O-acetylamidoxime Va yields 1,2,4-oxadiazole derivative VI. The structures of the prepared compounds were confirmed by IR and PMR spectral data.

*From the section "Search for New Drugs"

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0091-150X/89/2309-0779$12.50

EXPERIMENTAL (CHEMICAL)

IR spectra were recorded on a Perkin-Elmer 457 (USA) spectrometer and PMR spectra on a Varian T-60 (USA) spectrometer. Found and calculated values of elemental analyses matched.

The nitrile of 2-Oxopyrrolidino acetic acid (IIIa) was prepared according to [5].

Nitrile of 2-(2-Oxopyrrolidino)propionic Acid (IIIb). To a solution of 4.3 g (0.05 mole) of pyrrolidone-2 (Ia) in 15 ml of dry toluene was added 1 g (0.045 mole) of sodium. The mixture was refluxed for 4 h, cooled, and 6 g (0.045 mole) of nitrile IIb in 5 ml of toluene was added dropwise at 0°C. The mixture was heated at 70-80°C for 5 h, the precipitate was filtered off, the filtrate was evaporated under vacuum, and the residue was distilled. Yield 1.2 g (19.4%) of IIIb, bp 126°C/6 mm Hg. IR spectrum (film), ν_{max}, cm^{-1}: 2240 (C≡N), 1690 (C=O).

Nitrile of (2-oxo-4-phenylpyrrolidino)acetic Acid (IIIc). To a suspension of 0.5 g (0.022 mole) of NaH in 5 ml of dry DMF was added a solution of 3.2 g (0.02 mole) of 4-phenylpyrrolidone-2 (Ib) in 20 ml of DMF, the mixture was stirred at 20°C for 2 h, then a solution of 1.65 g (0.022 mole) of IIa in 2.5 ml of DMF was added, the mixture was stirred at 20°C for 2 h, and allowed to stand overnight. The mixture was poured out in 10 ml of water and extracted with benzene. The extract was evaporated under vacuum and the residue was chromatographed over Al_2O_3 (activity stage II according to Brockmann; eluent $CHCl_3$). Yield 2.3 g (57%) of IIIc, bp 204-206°C/5 mm Hg, $n_D^{23.5}$ 1.5565, $C_{12}H_{12}N_2O$. IR spectrum (film), ν_{max}, cm^{-1}: 2250 (C≡N), 1695 (C=O).

Nitrile of 2-(2-oxo-4-phenylpyrrolidino)propionic Acid (IIId). To a suspension of 0.5 g (0.022 mole) of NaH in 10 ml of absolute dioxane was added dropwise a solution of 3.2 g (0.02 mole) of Ib in 100 ml of dioxane, the mixture was heated at 90°C for 2 h, cooled, and then 2.8 g (0.02 mole) of nitrile IIb was added. The reaction mixture was heated at 80-90°C for 2.5 h, filtered, the filtrate was evaporated under vacuum, and the residue was chromatographed over Al_2O_3 (activity stage II according to Brockmann; eluent $CHCl_3$). Yield 1.6 g (37.2%) of nitrile IIId, which without further purification was used for the preparation of amidoxime IVd.

Amidoximes of (2-Oxopyrrolidino)alkanoic Acids (IVa-d). General Method. To a mixture of 0.013 mole of the corresponding nitrile and 0.013 mole of $H_2NOH{\cdot}HCl$ in 7-10 ml of absolute alcohol is added a solution of NaOEt, prepared from 0.013 mole of Na in 25 ml of absolute alcohol, while keeping the pH of the reaction mixture at 7. The mixture is stirred at 20°C for 1 h, allowed to stand for 16 h, the precipitate (in the cases of IVa, b) is filtered off, washed with alcohol, and the filtrate is evaporated to yield amidoximes IVa, b. In the cases of amidoximes IVc, d, the reaction mixture is filtered, the precipitate is washed with water, and crystallized from the appropriate solvent. IR spectrum of IVa (in paraffin oil), ν_{max}, cm^{-1}: 3485 (NH_2, asymm.), 3395 (NH_2, symm.), 3160-3300 OH, bonded), 1665 (C=O), 1625 (C=N). IR spectrum of IVc ($CHCl_3$), ν_{max}, cm^{-1}: 3580 (OH), 3500 (NH_2), 1680 (C=O). PMR spectrum of IVa (D_2O), δ, ppm: 2.45 m (2H, 4-CH_2), 2.88 m (2H, 3-CH_2), 3.92 m (2H, 5-CH_2), 4.32 s (2H, CH_2—N). PMR spectrum of IVb (D_2O), δ, ppm: 1.25 d (3H, CH_3—CH), 1.95 dd (2H, 4-CH_2), 2.34 m (2H, 3-CH_2), 3.27 m (2H, 5-CH_2), 4.78 q (1H, CH), 4.85 br. s (2H, NH_2), 8.62 br. s (OH).

O-Acetylamidoximes of (2-Oxopyrrolidino)acetic Acid (Va) and 2-(2-Oxopyrrolidino)propionic Acid (Vb). General Method. To a suspension of 0.01 mole of the amidoxime in 3 ml of dry dioxane is added at 0-5°C a solution of 0.015 mole of Ac_2O in 2 ml of dioxane, the mixture is stirred at the same temperature for 30 min, a solution of 40 ml of heptane is added, and the mixture is set aside for about 16 h. The precipitate is filtered off, washed with heptane, and crystallized from the appropriate solvent to give O-acetyl derivatives Va, b. IR spectrum of Va (in paraffin oil), ν_{max}, cm^{-1}: 3180-3380 (NH_2), 1742 (COO), 1669 (C=O), 1655 (C=N). PMR spectrum of Va ($CDCl_3$), δ, ppm: 2.02 m (2H, 4-CH_2), 2.08 s (3H, CH_3), 2.31 m (2H, 3-CH_2), 3.41 m (2H, 5-CH_2), 3.94 s (2H, CH_2-N), 5.43 br. s (2H, NH_2).

O-Benzoylamidoximes (Vc-f). General Method. To a solution of 0.01 mole of amidoxime in 15 ml of dry pyridine is added dropwise at 0-3°C 0.01 mole of the acid chloride of the appropriate benzoic acid, the mixture is stirred for 1 h, and allowed to stand for about 16 h. The reaction mixture is poured out in water and extracted with $CHCl_3$. The extract is evaporated under vacuum, the residue is washed with ether, and crystallized from the appropriate solvent to yield O-acetylamidoximes Vc-f. IR spectrum of Vc (in paraffin oil), ν_{max}, cm^{-1}: 3460 and 3360 (NH_2), 1730 (C=O), 1680 (C=O), 1625 (C=N). IR spectrum of Vf (in paraffin oil), ν_{max}, cm^{-1}: 3365 and 3310 (NH_2), 3180 (NH_2, bonded), 1755 (COO), 1680 (C=O), 1640 (C=N).

TABLE 1. Characteristics of the Amidoximes of (2-Oxopyrrolidino)alkanoic Acids and Their O-Acyl Derivatives

Compound	Yield, %	mp, °C (cryst. solvent)	Emperical formula
IVa	68	135—6 (ethanol)	$C_6H_{11}N_3O_2$
IVb	48,6	144—5 (abs. ethanol)	$C_7H_{13}N_3O_2$
IVc	80,0	176—7 (ethanol)	$C_{12}H_{15}N_3O_2$
IVd	48,4	143,5—45 (ethanol)	$C_{13}H_{17}N_3O_2$
Va	85,4	130—1 (ethanol)	$C_8H_{13}N_3O_3$
Vb	89,2	136,5—38 (isopropanol)	$C_9H_{15}N_3O_3$
Vc	74,7	149,5—50,5 (abs. ethanol)	$C_{13}H_{15}N_3O_3$
Vd	76,4	112,4—3,5 (benzene—petr. ether	$C_{14}H_{17}N_3O_3$
Ve	60,7	121—2 (abs. ethanol)	$C_{13}H_{14}ClN_3O_3$
Vf	75,0	160—1 (ethanol)	$C_{13}H_{14}ClN_3O_3$
Vg	72,0	131 (ethyl acetate)	$C_{14}H_{25}N_3O_3$

TABLE 2. Antiamnesic Activity of the Amidoximes of (2-Oxopyrrolidino)alkanoic Acids and Their O-Acyl Derivatives (at a dose of 300 mg/kg) according to the CRPA Retention Test

Compound	Retention of CRPA, with	
	latency time	time spent in the illuminated compartment of the chamber
Control (training)	72,8 (59,7—85,9)	103,3 (97,4—109,2)
Control (training + MES)	62,0 (50,1—73,9)	68,0 (66,1—79,9)
Piracetam	71,8 (56,6—87,0)	82,5 (67,3—97,7)*
IVa	60,5 (48,1—72,9)	71,0 (58,8—83,4)
IVc	112,7 (105,0—120,4)***	112,7 (105,0—120,4)***
IVd	104,3 (91,7—116,8)**	113,1 (107,2—119,0)***
Va	105,0 (89,2—120,4)***	110,0 (99,4—120,6)***
Vb	50,1 (36,5—63,7)*	85,1 (75,8—94,4)***
Vc	82,5 (70,1—95,0)	83,5 (71,1—95,9)
Ve	77,5 (61,6—93,4)	101,6 (92,8—110,4)
Vf	58,1 (42,9—73,3)	71,8 (58,6—85,0)
Vg	71,0 (47,4—94,6)	109,6 (104,2—115,0)***

* $P < 0,05$.
** $P = 0,01$.
*** $P < 0,01$.

Note. Here and in Tables 3 and 5 in parentheses we give the range of the variations.

O-Dipropylacetylamidoxime of (2-Oxopyrrolidino)acetic Acid (Vg). To a solution of 1.57 g (0.01 mole) of amidoxime IVa and 1.44 g (0.01 mole) of dipropylacetic acid in 25 ml of waterfree pyridine is added 2.06 g (0.01 mole) of dicyclohexylcarbodiimide, the mixture is stirred at 20°C for 10 h, filtered, the filtrate is diluted with 200 ml of heptane, and the precipitate is filtered off to yield valproyl derivative Vg. PMR spectrum ($CDCl_3$), δ, ppm: 0.81 t (6H, $2CH_2$), 1.0-1.70 m (10H, $2CH_2CH_2$ and 4-CH_2), 2.12 dd (1H, CH), 2.37 m (2H, 3-CH_2), 3.44 m (2H, 5-CH_2), 3.96 s (2H, CH_2N), 5.35 br. s (2H, NH_2).

Characteristics of compounds IVa-d and Va-g are summarized in Table 1.

3-(2-Oxopyrrolidinomethyl)-5-methyl-1,2,4-oxadiazole (VI). One and eight tenths grams (0.009 mole) of the O-acetylamidoxime of (2-Oxopyrrolidino)acetic acid was heated at 150°C for 25 min and the melt was distilled under vacuum. Yield 1.1 g (63%) of VI, bp 140-144°C/3 mm Hg. **Oxalate:** a solution of 0.64 g (0.0071 mole) of the base in 15 ml of dry ether was treated with oxalic acid to yield the oxalate of VI, yield 1.35 g (70%), mp 99-100°C (from ether), $C_8H_{11}N_3O_2 \cdot C_2H_2O_4$. PMR spectrum ($CCl_4$), δ, ppm: 1.95-2.35 m (4H, 4-CH_2 and 3-CH_2), 2.55 s (3H, CH_3), 3.52 m (2H, 5-CH_2), 4.48 s (2H, CH_2—N).

TABLE 3. Antiamnesic Activity of Compound Va in the Test with Scopolamine-Induced Amnesia

Compound	Dose, mg/kg	Retention of CRPA, with	
		latency time	time spent in the illuminated compartment of the chamber
Scopolamine (control)	2.5	17,2 (15,6—18,8)	27,2 (22,9—31,5)
Piracetam + scopolamine	400 2,5	26,0 (16,8—35,2)	35,0 (25,3—44,7)
Piracetam + scopolamine	800 2,5	60,5 (48,6—72,4)	69,5 (58,1—80,9)*
VA + scopolamine	400 2,5	15,5 (12,3—18,7)	32,5 (19,3—25,7)

* $P < 0,05$.

TABLE 4. Antiamnesic Activity of Compound Va at a Dose of 100 mg/kg in the Active Avoidance Test in Case of Amnesia Evoked by Electroshock in Rats (M ± m)

Compound	Number of conditioned reactions, %			
	1st day	2nd day	3rd day	4th day
Control + MES	29,0±2,3	49,0±4,3	58,0±2,5	68,0±3,3
VA + MES	28,0±2,5	54,0±4,0	65,0±3,1*	77,0±2,6*
Control + MES	23,0±2,1	31,0±6,2	40,0±4,2	43,0±3,9
Piracetam + MES	23,0±2,1	50,0±5,1*	63,0±6,9**	73,0±3,6**

* $P < 0,05$.
** $P < 0,01$.

EXPERIMENTAL (PHARMACOLOGICAL)

Experiments were carried out with white mongrel male mice weighing 20-22 g and with male Wistar rats weighing 140-200 g. The compounds were administered intraperitoneally, 40 min before starting the experiment. The antiamnesic activity was determined under the conditions of the method of the conditioned response of passive avoidance (CRPA) in mice using as amnesic agent the effect of the maximal electroconvulsant seizure or the anticholinergic scopolamine. The experiment chamber consisted of two compartments that were connected by an opening: an illuminated large one and a small dark one with an electrode area. The animals were placed in the illuminated compartment with their tail to the opening. We recorded the times of staying in the illuminated and dark compartments of the experiment chamber over a two-minute interval. Then in the dark compartment the animal got an electric irritation of the skin by means of an electrode current (0.4 mA) to let it run into the illuminated compartment. The animal was considered to be trained when it did not enter the dark compartment for 30 sec. The retention test was carried out after 24 h. The animal was placed in the illuminated compartment with its tail to the opening and we recorded the latency of entering the dark compartment and also the times of staying in the illuminated and dark compartments of the experiment chamber for a two-minute interval. The maximal electroshock (MES) was applied immediately after the training session by means of corneal electrodes (50 Hz, 50 mA, 0.2 sec). Administration of scopolamine was carried out at a dose of 2.5 mg/kg, 15 min before the CRPA training session.

The antiamnesic activity according to the active avoidance test using an electroshock as amnesic influence was determined in rats according to [6].

The antihypoxic action of the compounds was determined in mice under the conditions of the model of hypobarametric hypoxia in a pressure room at a "lift" with a rate of 50 m/sec to a "site" at 11,000 meters (we recorded the number of surviving animals after an exposure of 30 min and the average life of the animals in the pressure room),

TABLE 5. Antihypoxic Properties of the Amidoximes of (2-Oxopyrrolidino)alkanoic Acids and Their Derivatives

Compound	Dose, mg/kg	Hypobarometric hypoxia in the pressure chamber		Hypoxia with hypercapnia in a closed space
		life span in the pressure chamber, % relative to control	% of surviving animals after 30 min exposure	life span, % relative to control
Control	—	100 (55,6—114,4)	33	100 (88,8—111,2)
Piracetam	200	120 (22—217)	16	—
	300	—	—	135,7 (123,6—147,8)
IVc	300	81,4 (40,8—122,0)	16	—
	600	—	—	201,7 (185,7—217,7)**
IVd	300	144,2 (102,2—186,1)	50	—
Va	300	177,7* (135,8—218,7)	66	98,8
Vb	300	161,13	33	—
Vℓ	300	26,0	0	—

* $P = 0,05$.
** $P < 0,01$.

according to the model of hypoxia with hypercapnia in a hermetically sealed space (we recorded the lifespans of the animals in an hermetically sealed beaker with a volume of 200 ml), and according to the model of hypoxia at normal barometric pressure (nitrogen atmosphere).

As compound for comparison we used piracetam. Statistical processing of the results was done by calculating the arithmetic means with a confidence interval at p = 0.05, and also by using the criterium of Wilcoxon—Mann—Whitney [1, 3].

The results of the investigations of the antiamnesic activities are summarized in Tables 2-4. During the investigation of the antiamnesic activity with the CRPA test with amnesia evoked by the application of MES after a training session (see Table 2) it was found that piracetam at a dose of 200 mg/kg did not have the capacity to prevent the development of retrograde amnesia. On increasing the dose to 300 mg/kg the protecting properties of the preparation clearly appeared; the time spent in the illuminated compartment in the retention was reliably increased. Amidoximes IV and V were also studied at a dose of 300 mg/kg.

The largest antiamnesic effect was found in compounds IVc, d, Va, b, and Vf. These compounds showed a more pronounced effect than piracetam. However, compound Vb possessed the capacity to increase the action of the maximal electroshock seizure by increasing the percentage of deaths of the animals. Compound IVe showed the least pronounced protecting effect. Compounds IVa, Vc, and Vf did not have this property.

During the investigation of one of the active compounds, Va, in the test with amnesia evoked by previous administration of the anticholinergic scopolamine (see Table 3), it was found that at a dose of 300 mg/kg it did not prevent the development of amnesia. The preparation for comparison, piracetam, also appeared to be ineffective at a dose of 300 mg/kg in the test mentioned and showed antiamnesic properties only at a dose of 800 mg/kg.

Investigation of the antihypoxic properties of the compounds at dose of 300 mg/kg (Table 5) with the test of hypobarometric hypoxia in the pressure chamber showed that compound Va has distinct antihypoxic properties and increases the average life of the animals in comparison with the control and piracetam, and the number of surviving animals. Compound Vb also increased the average life span, but to a lesser degree than Va. The number of surviving animals was not increased as compared with the control group. Compounds IVc and Ve did not show antihypoxic properties in this test. Compound IVd showed antihypoxic activity and increased both the average life spans of the animals in the pressure chamber and the number of surviving animals; however, with respect to its antihypoxic properties it was inferior to compound Va.

Investigation of the antihypoxic activity of the compounds according to the test of hypoxia with hypercapnia in the pressure chamber showed that compound Va does not have a protective effect at a dose of 300 mg/kg in this test. When the dose is increased to 600 mg/kg, compound Va shows distinct antihypoxic activity and increases the average lifespan of the animals in the pressure room twofold in comparison with the control group.

Compound Va was tested for its antihypoxic activity according to the model of hypoxia at normal pressure (nitrogen atmosphere) at doses of 30, 100, and 300 mg/kg. In this case compound Va displayed protective activity against

hypoxia only at a dose of 100 mg/kg. Increased doses led to disappearance of the effect. The reference preparation pyracetam displayed antihypoxic activity only at a dose of 300 mg/kg. Thus, according to this model compound Va is three times as active as pyracetam, while both compounds showed a dose-independent effect.

It was established that intraperitoneal administration of compound IVc at a dose of 1,000 mg/kg caused the death of 100% of the animals after 1 h. When compound IVd was administered at a dose of 600 mg/kg, after 30 min lying on the side was observed in 50% of the animals, and after 35 min the death of 16% of the animals, and in the rest of the animals convulsive twitching, Straub's pose, and death after 60 min. When compound Va was administered at a dose of 1,000 mg/kg any toxic phenomenon was absent in the animals. With compound VI at a dose of 300 mg/kg, after 10 min death was observed in 50% of the animals and the rest of the animals were lying on their sides.

Thus, introduction of the amidoxime moiety into the amide group of the piracetam molecule leads to loss of the antiamnesic activity, but in the case of 4-phenylpiracetam introduction of the amidoxime group leads to development of distinct antiamnesic properties surpassing the analogous properties of piracetam. In the case of introduction of an O-ortho-chlorobenzoyl group into the amidoxime radical, antiamnesic activity without antihypoxic activity is found. However, in case of introduction of an O-benzoyl or an O-para-chlorobenzoyl group into the amidoxime radical, an antiamnesic effect is absent. The largest antiamnesic activity concurrent with distinct antihypoxic activity and low toxicity shows compound Va, of which the nootropic properties, with respect to strength, are almost equal to the specific activity of piracetam.

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