THE SYNTHESIS AND PHARMACOLOGICAL ACTIVITIES OF 1-ISOPROPYL-2-FORMYL-3-AMINOPYRAZOLIDINES

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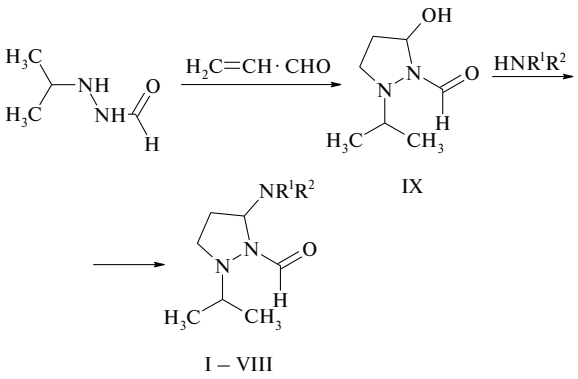
With the aim of identifying safe, high-efficacy substances with anti-inflammatory activity, we have synthesized aminopyrazolidines (compounds I – VIII) from 1-isopropyl-2-formyl-3-hydroxypyrazolidine (compound IX). Compound IX was selected as the initial compound, as it has been shown [1] to have high anti-inflammatory activity combined with low toxicity. Compound IX was synthesized by interaction of acrolein with 1-formyl-2-isopropylhydrazine. The active polyaminal hydroxyl group of compound IX reacts with amines, forming the corresponding 1-isopropyl-2-formyl-3-aminopyrazolidines (compounds I – VIII):



IX

I – VIII

EXPERIMENTAL CHEMICAL PART

PMR spectra were recorded on a Tesla BS-497 (Czechoslovakia) spectrometer with a working frequency of 100 MHz at 25°C in DMSO-d_6 solutions; the internal standard was HMDS. Reaction courses and product purities were monitored by TLC on Silufol UV-254 plates developed with a benzene : acetone (4 : 1) system and detected with iodine vapor.

1-Isopropyl-2-formyl-3-anilinopyrazolidine (compound I). Hydroxypyrazolidine IX (15.8 g, 0.1 mole) in 10 ml of benzene was mixed with an equimolar quantity of aniline; the mixture was heated for 1 h and cooled; the resulting precipitate was collected by filtration and recrystallized from a mixture of ethyl acetate and petroleum ether. The yield was 57% (Table 1). Compounds II – VIII were prepared by the same method, from substituted anilines, benzylamine, and morpholine. Some of the physicochemical properties of the study compounds are presented in Table 1; PMR spectral data are shown in Table 2.

EXPERIMENTAL PHARMACOLOGICAL PART

The acute toxicities of compounds on i.p. dosage was measured as described in [2] in mongrel white mice weighing 18 – 20 g.

Anti-inflammatory activities were studied in a model of acute inflammation edema in the footpads of male rats weighing 180 – 200 g, induced by subplantar administration of 0.1 ml of 0.1% carrageenan 1 h after p.o. doses of study compounds [5]. Oncometric measurements were made of limb volume before and 4 h after experiments started (i.e., at the time of maximum edema). The antiproliferative activities of study compounds were studied in the cotton granuloma model [6]; inflammation was induced by subcutaneous insertion of sterilized cotton balls weighing 15 mg on the back (the procedure was performed under light ether anesthesia). Compounds were given for seven days; on experimental day 8, implanted cotton balls with the surrounding newly formed granulation tissue were removed, dried to constant weight at a temperature of 55 – 60°C, and the weight of newly formed granulation tissue was determined from the difference between the weight of the dried granuloma and the weight of the implanted cotton ball. Study compounds were given p.o. at doses of 1/20 to 1/50 of the LD_{50}. The reference agent was butadione.

Analgesic activity was studied in mongrel white mice using an acetic acid spasm model [6], with measurement of the number of spasms induced by i.p. doses of 3% acetic acid

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TABLE 1. Properties of 1-Isopropyl-2-formyl-3-aminopyrazolidines (Compounds I – VIII)

Compound	R^1	R^2	Yield, %	Melting point, °C (solvent for recrystallization)	Empirical formula
I	C_6H_5	H	57.6	111 – 120*	$C_{13}H_{19}N_3O$
II	*n*-$C_6H_4OCH_3$	H	54.0	98 – 100*	$C_{14}H_{21}N_3O_2$
III	*n*-$C_6H_4NO_2$	H	58.0	200 – 202 (methanol)	$C_{13}H_{18}N_4O_3$
IV	*n*-$C_6H_4CH_3$	H	60.0	169 – 170*	$C_{14}H_{21}N_3O$
V	*n*-$C_6H_4COOCH_2CH_3$	H	60.0	128 – 130 (methanol)	$C_{16}H_{23}N_3O_3$
VI	*n*-$C_6H_4SO_2NH_2$	H	93.0	169 – 170 (methanol)	$C_{13}H_{20}N_4O_3S$
VII	CHC_6H_5	H	78.0	63 – 65*	$C_{14}H_{21}N_3O$
VIII	$–C_2H_4–O–C_2H_4–$		75.0	oil	$C_{11}H_{21}N_3O_2$

* Ethyl acetate – petroleum ether, 1 : 2.

(10 ml/kg) over a 2-min period. Experimental animals received p.o. study compounds at doses of 1/20 to 1/50 LD_{50} 1 h before doses of acetic acid. The analgesic activity of compounds was also studied in terms of the effect on the latent period of tail withdrawal in rats subjected to thermal stimulation [6]. Study compounds and reference agent Analgin (100 mg/kg) were given p.o. 1 h before provocation.

Hypoxia plays an important role in the pathogenesis of inflammation, so we studied the protective effects of study agents in conditions of oxygen starvation. Experiments were conducted on mongrel white mice weighing 18 – 20 g in conditions of acute hypobaric hypoxia modeled using a barometric chamber with a vacuum pump and a working chamber volume of 0.93 m^3 [7]. Animals were placed in the barometric chamber 1 h after p.o. doses of study compounds (1/10 – 1/100 LD_{50}). "Elevation" was at a rate of 50 m/sec to an altitude of 11,000 m above sea level; animals were kept at this altitude for 20 min. The reference agent for these experiments was gutimine, at a dose of 50 mg/kg. The duration of life in these conditions was recorded and expressed as the number of animals surviving at 11,000 m for 20 min.

The ability of study compounds to induce ulcerous lesions was studied in male rats weighing 180 – 200 g using a model based on the destruction of the gastric mucosa [8]. Study agents were given p.o. at a dose of 1/10 LD_{50} for seven days; control animals received distilled water. The reference agent was butadione. Animals were kept in conditions of free access to water and feed from 4 h before to 1 h after administration of compounds or water. On day 8, 4 h after the last dose, animals were sacrificed and the gastric mucosa was examined for the presence of ulcers.

Each compound was studied in 10 animals for each test.

Results were analyzed statistically using the *t* test for significance [9].

LD_{50} values for compounds I, II, III, IV, V, VI, VII, and VIII were 1780, 1940, 3.3, 1120, 3870, 3870, 890, and 980 mg/kg respectively. Thus, all pyrazolidines except compound III had lower toxicity than butadione [3]. Compound III was excluded from further studies because of its toxicity.

TABLE 2. PMR Spectral Data for 1-Isopropyl-2-formyl-3-aminopyrazolidines (Compounds I – VIII)

Compound	Chemical shift, δ, ppm							
	H_3, (1H)	H_4, (2H)	H_5, (2H)	NCH, (1H)	$(CH_3)_2$, (6H)	CHO, (1H)	H_{arom}	H_{aliph}
I	6.02 m	2.43 m	3.53 m	2.47 m	1.20 d, 1.33 d	8.61 s	7.18 – 7.48 (m, 5H)	–
II	5.92 m	2.34 m	3.38 m	2.65 m	1.15 d, 1.28 d	8.58 s	7.03 – 7.12 (m, 4H)	3.97 (s, 3H, OCH_3)
III	6.08 m	2.71 m	3.50 m	2.89 m	1.18 d, 1.39 d	8.64 s	7.35 – 8.37 (m, 4H)	–
IV	5.85 m	2.81 m	3.49 m	3.36 m	1.10 d, 1.33 d	8.48 s	7.04 – 7.18 (m, 4H)	2.39 (s, 3H, CH_3)
V	6.16 m	2.57 m	3.54 m	2.73 m	1.19 d, 1.35 d	8.65 s	7.26 – 8.23 (m, 4H)	4.63 (m, 2H, CH_2), 1.61 (t, 3H, CH_3)
VI	5.84 m	2.42 m	3.24 m	2.69 m	0.93 d, 1.12 d	8.42 s	7.22 – 7.78 (m, 4H)	7.17 (s, 2H, NH_2)
VII	5.52 m	2.31 m	3.93 m	3.07 m	1.72 d, 1.82 d	8.52 s	7.13 – 7.37 (m, 5H)	4.63 (s, 2H, CH_2)
VIII	5.40 m	2.41 m	3.53 m	2.69 m	1.00 d, 1.18 d	8.27 s	–	2.41 [m, 4H, $(CH_2)_2$], 3.18 [m, 4H, $(CH_2)_2$]

Note. PMR spectra of compounds II and VII were recorded in $CDCl_3$, that of VIII was recorded in CD_3OD, and those of the others in DMSO-d_6.

TABLE 3. Anti-inflammatory, Analgesic, and Antihypoxic Activities of Derivatives of 1-Isopropyl-2-formyl-3-aminopyrazolidines.

Compound	Anti-inflammatory activity (% of control)		Analgesic activity (% of control)		Antihypoxic effect	
	Antiexudative effect	Antiproliferative effect	"Acetic acid spasms" model	Tail withdrawal model	Duration of life, % of control	Survival, %
I	94.2	85.8	47.5*	129.2*	198.9*	0.0
II	74.3*	71.7*	99.4*	104.6	234.1*	40.0*
IV	100.2	80.8	30.7*	112.8*	68.2	0.0
V	79.9*	63.3*	60.1*	125.4*	130.7*	0.0
VI	98.1	87.0	55.6*	109.2	154.5*	25.0*
VII	89.6	86.9	54.0*	98.5	102.4	0.0
VIII	69.1*	71.3*	30.7*	131.1*	422.7*	70.0*
Butadione	55.3*	49.1*	…	…	…	…
Analgin	…	…	12.1*	144.6*	…	…
Gutimine	…	…	…	…	409.1*	75.0*

* $p \leq 0.05$ compared with controls.

The data presented in Table 3 show that compounds II, V, and VIII suppress both the exudative and proliferative phases of inflammation, though they were rather less effective than butadione (10 mg/kg). The other compounds had insignificant anti-inflammatory activity.

Most of the study compounds (except for compound II) also had analgesic activity, though less than that of Analgin (Table 3).

Studies of the antihypoxic activity of compounds showed that compounds II, VI, and VIII increased the duration of life animals in conditions of acute hypobaric hypoxia, compound VIII being as effective as gutimine (Table 3).

No study compound had ulcerogenic activity.

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