EXPERIMENTAL PHARMACOLOGICAL SECTION

Tests were carried out on cats narcotized with chlorazole (90 mg/kg intraperitoneally) weighing 2.4-3.8 kg. Blood pressure in the carotid artery was recorded electromanometrically. Transthoracic EKG and respiration were recorded by electrodes inserted under the skin of the thorax on a level with the fourth rib. All recordings were made on a Narko Bio-System physiograph.

Blood flow from the coronary sinus was measured by the method of N. V. Kaverin (1958).

The compounds were dissolved in 50% dimethylacetamide and injected intraperitoneally through a cannula inserted into the femural vein.

The systolic arterial pressure was measured on spontaneously hypertensive rats, SHR strain, using a pneumatic transducer pulse, and recorded on a physiograph. One dose of the compound (10 mg/kg) was tested on 5-7 rats. The test compounds were suspended in an isotonic solution of sodium chloride with Tween 80 and injected into the stomach in doses of 5 ml/kg.

Acute toxicity was studied on female white mice weighing 19-23 g; compounds were injected intraperitoneally into six mice. The animals were observed for 10 days after injection. Acute toxicity (LD_{50}) was determined by the method of Litchfield and Wilcoxon.

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SYNTHESIS AND ANTITUMOR ACTION OF SOME PYRIDO[2,3-b]PYRAZINES

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One of the goals of antitumor research is the synthesis of folic acid antimetabolites [1]. In earlier works on the synthesis of potential folic acid antagonists — compounds with pyrido[2,3-b]pyrazine [2, 3] or pyrido[3,4-b]pyrazine [4] systems — it was reported that some derivatives of pyrido[2,3-b]pyrazine exhibit antitumor activity [3, 5].

The present work describes the synthesis and antitumor action of some new derivatives of pyrido[2,3-b]pyrazine, prepared by the recently developed method for preparing 2,3-diaminopyridine derivatives [6].

The 6-R-pyrido[2,3-b]pyrazines (Ia-d) were synthesized by the cyclization of 6-R-2,3-diaminopyridines with 40% aqueous glyoxal as described in [7]; reaction of the bases Ia-d with methyl iodide in refluxing benzene gave the quaternary salts IIa-d.

The structures of compounds Ia-d were confirmed by NMR spectroscopy (Table 1). The chemical shifts for the H-2 and H-3 protons, together with the low values of the spin—spin

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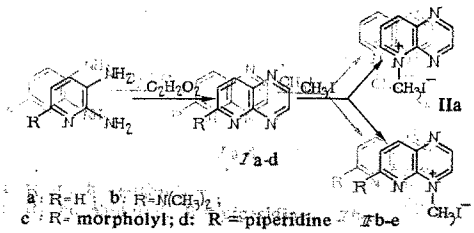
TABLE 1. NMR Spectra of the Pyrido[2,3-b]pyrazines

Compound	Chemical shifts δ (in ppm) and spin–spin coupling constants J (in Hz)								
	H-2	H-3	$J_{2,3}$	H-6	H-7	H-8	$J_{6,7}$	$J_{6,8}$	$J_{7,8}$
Ia	9,12	9,21	1,7	9,25	7,95	8,61	4,1	1,9	8,3
IIa	9,50	9,54	1,9	9,88*	8,55	9,44	5,8	1,6	8,8
Ib	8,53	8,75	2,1		7,40	8,08			9,2
IIb	8,87	9,02*	3,7		7,84	8,30			9,6
Ic	8,59	8,78	2,1		7,57	8,15			9,2
IIc	8,92	9,05*	3,6		7,97	8,36			9,6

*Broad signal.

coupling constants ($J_{2,3}$ = 1.8-3.7 Hz), are characteristic for the pyrazine ring [8].



Three isomers are theoretically possible from the quaternization of pyrido[2,3-b]pyrazines. The structures of the salts IIb and c, i.e., the position of the quaternary nitrogen atom, was determined by NMR spectroscopy. Comparison of the ^1H (Table 1) and ^{13}C NMR spectra for the bases Ia-c and the salts IIa-c shows that quaternization of the nitrogen atom gives rise to a characteristic broadening of the signals of both the α-carbon atom (in relation to the quaternary nitrogen) and also the α-carbon atom protons, and to an increase in the absolute values of the vicinal spin–spin coupling constants for the α-carbon atom protons and displacement of the ^{13}C signals of the α-carbon atoms in the upfield direction.

In the unsubstituted compound IIa, H-6, C-6, and C-10 give rise to broad signals, as do H-3, C-3, and C-10 in the salts with substituents in the 6-position (IIb and c). These broad signals are explained by the spin–spin coupling of the α-carbon atoms and their protons with the $^{14}N^+$ nucleus as the values of $^3J_{^1H,^{14}N}$ and $^1J_{^{13}C,^{14}N}$ are comparable with the frequencies of the quadrupole relaxation of the positively charged nitrogen. A comparison of the vicinal spin–spin coupling constants for compounds Ia-c and IIa-c shows that going from the unsubstituted base Ia to the salt IIa, the constant $J_{6,7}$ increases by 1.7 Hz, and for bases IIb and c, $J_{2,3}$ increases by 1.5-1.6 Hz. The spectrum of Ia differs from that of IIa in that the ^{13}C resonance signals for C-10 ($\Delta\delta$ = 6.94 ppm) and C-6 ($\Delta\delta$ = 2.05 ppm) are shifted downfield. In the spectra of the substituted compounds Ib and c, and IIb and c, there is a downfield shift for C-10 ($\Delta\delta$ = 8.6 ppm) and C-3 ($\Delta\delta$ = 9.2 ppm). The NMR spectra of compounds IIa-c showed no evidence of the presence of isomeric substances (see also [9]).

Thus, substitution in the 6-position of pyrido[2,3-b]pyrazine affects the quaternization center, apparently as a result of steric hindrance.

EXPERIMENTAL BIOLOGICAL SECTION

The antitumor activity of pyrido[2,3-b]pyrazines was studied using nonpedigree mice, $C_{57}Bl_6$ strain, and hybrid BDF mice (males and females were used, depending on the tumor type). Transplanted tumors obtained from the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, were used for the tests: Lewis lung carcinoma, mammary gland adenocarcinoma (AK-755), sarcoma 37 (solid type), and hemocytoblastosis La.

The test compounds were dissolved in water and injected intraperitoneally. For solid tumors, injections were started within 48 h, and for hemoblastosis La within 24 h; injections were given once a day for 5 days. Animals with solid tumors were killed on the 14th day after the transplant, and the tumors were removed and weighed. The percent inhibition of tumor growth (P, %) was calculated from the formula

$$P, \% = \frac{P_k - P_0}{P_k} \cdot 100,$$

TABLE 2. Properties of Pyrido[2,3-b]pyrazines Ia-d and Their Quaternary Salts IIb-d

Compound	R	mp deg C (ethanol)	Found, %	Found, %			Empirical formula	Calculated, %		
				C	H	N		C	H	N
Ib	$N(CH_3)_2$	110—112*	58	62,2	5,7	32,0	$C_9H_{10}N_4$	62,0	5,8	32,1
Ic	Morpholyl	168—170	60	61,0	5,8	26,1	$C_{11}H_{12}N_4O$	61,1	5,6	26,0
Id	Piperidinyl	67—68	50	67,1	6,6	26,2	$C_{12}H_{14}N_4$	67,2	6,6	26,1
IIb	$N(CH_3)_2$	272—274	60	38,0	4,2	18,0	$C_{10}H_{13}IN_4$	38,0	4,1	17,7
IIc	Morpholyl	210—212	63	39,9	4,6	15,5	$C_{12}H_{15}IN_4O$	40,2	4,2	15,6
IId	Piperidinyl	240—241	64	43,6	5,0	16,0	$C_{13}H_{17}IN_4$	43,8	4,8	15,7

*Recrystallized from petroleum ether.

where P_k is the average tumor weight in the control, and P_o in the test animal.

For hemoblastosis La the survival rate of the animals was evaluated, and the percent increase in life span was calculated from the formula

$$T, \% = \left(\frac{a}{b} - 1\right) \cdot 100,$$

where a is the average life span of animals in the test group, and b in the control group.

The results showed that some of the test compounds exhibit moderate antitumor activity. Compounds Ic, IIa, and IIb, in doses of 50-100 mg/kg, inhibit the growth of sarcoma 37 by 50-55%, compound Ib inhibits the growth of Lewis tumor by 60%, and IIb inhibits the growth of tumor AK-755 by 58%.

EXPERIMENTAL CHEMICAL SECTION

NMR spectra were obtained on a Bruker WH-90 (90 MHz) in dimethylsulfoxide-D_6 solution; internal standard tetramethylsilane.

6-R-Pyrido[2,3-b]pyrazines (Ib-d). A solution of 1 ml of 40% aqueous glyoxal in 5 ml of water was added dropwise with mixing to a solution of 0.9 g of 2,3-diamino-6-R-pyridine in 20 ml of water [6], and the reaction mixture heated on the steam bath for 30 min. Compounds Ib-d were separated by continuous extraction with chloroform for 2 h. The extract was dried with sodium sulfate, evaporated to small volume, cooled, and the products (Ib-d) filtered off.

Iodides of 4-Methyl-6-R-Pyrido[2,3-b]pyrazine (IIb-d). A mixture of 0.5 g of 6-R-pyrido[2,3-b]pyrazine (Ib-d) and 50 ml of benzene was heated for 2 h at 60-70°C. After cooling, the crystalline precipitates of IIb-d were filtered off.

The properties of compounds Ib-d and IIb-d are given in Table 2.

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