experiments showed that of all the examined compounds only compound XIV, i.e., 6-amino-2-mercapto-4-oxy-5-(2,2-dimethyl-4-tetrahydropyranyl)pyrimidine, most strongly suppressed spontaneous mutation in the test cultures (see Table 3) in which case it exhibited a protective action whereby it reduced the number of mutations resulting from UV rays by 25% and 20% in comparison to the control (see Table 3).

Thus, the variable degree of observed mutagenic and antimutagenic activity would seem to warrant further study among the tetrahydropyranyl- and tetrahydrothiopyranyl substituted pyrazole and pyrimidine derivatives for the purpose of finding highly active mutagens and antimutagens.

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SYNTHESIS AND ANTI-MONOAMINE OXIDASE ACTIVITY OF N^1-(4-PYRIMIDINOYL)-N^2-BENZYLHYDRAZINES

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Comparison of the therapeutic activity of monoamine oxidase (MAO) inhibitors and other antidepressants shows that with certain forms of depression MAO inhibitors are highly effective [5]. Therefore, despite the possible side effects [5], MAO inhibitors belonging to various classes of organic compounds continue to be of interest to researchers.

It was reported [1, 9] that arylidenehydrazides of 4-pyrimidinecarboxylic acids in experiments in vitro have anti-MAO activity. In order to investigate new MAO inhibitors we carried out the synthesis of the benzylidenehydrazides of 4-pyrimidinecarboxylic acids (II) and studied the possibility of reducing them with sodium borohydride to the corresponding hydrazines (III).

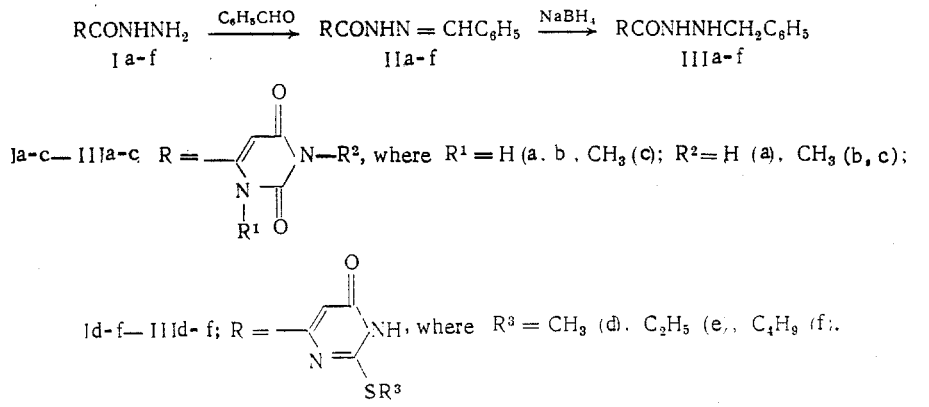

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TABLE 1. Constants and Elemental Analysis Data for Compounds I-III

Compound	Yield, %	mp, °C	Found, %			Empirical formula	Calculated, %		
			C	H	N		C	H	N
Ia	67	>300	39.43	4.17	30.36	$C_6H_6N_4O_3$	39.13	4.37	30.42
Ib	75	209—10	42.19	4.98	28.37	$C_7H_{10}N_4O_3$	42.42	5.08	28.27
IIa	84	>300(with decomp.)	56.01	4.04	21.47	$C_{12}H_{10}N_4O_3$	55.81	3.90	21.69
IIb	60	>300(with decomp.)	57.48	4.11	20.62	$C_{13}H_{12}N_4O_3$	57.35	4.44	20.57
IIc	90	218—9	58.49	5.05	19.67	$C_{14}H_{14}N_4O_3$	58.73	4.92	19.56
IIIa	75	258—60	55.72	4.57	21.61	$C_{12}H_{12}N_4O_3$	55.38	4.67	21.53
IIIb	25*	289—289.5	56.87	5.32	20.57	$C_{13}H_{14}N_4O_3$	56.93	5.15	20.43
IIIc	60	182.5—183	58.30	5.77	19.27	$C_{14}H_{16}N_4O_3$	58.32	5.59	19.43
IIId	75	200—201.5	54.29	5.08	19.43	$C_{13}H_{14}N_4O_2S$	53.78	4.86	19.29
IIIe	64	193—193.5	54.90	5.73	18.33	$C_{14}H_{16}N_4O_2S$	55.25	5.29	18.40
IIIf	25*	143—4	57.70	6.18	17.02	$C_{16}H_{20}N_4O_2S$	57.81	6.06	16.85

Note. Compounds recrystallized as follows: Ib, IIIf) from a water—ethanol mixture; Ic, IIc, IIIa, c-e) from ethanol; IIa, b) from DMF; IIIb) from a DMF—ethanol mixture.
*40% initial hydrazone isolated.

Benzylidenehydrazides II were obtained by condensation of hydrazides I with benzaldehyde.

It is known that the C=N bond in acylhydrazones is readily reduced with sodium borohydride in alcohols [4, 12]. The reduction of hydrazones II using this method did not give the required hydrazines III. In all cases the initial compounds II were isolated from the reaction medium. With the assumption that the reason for this may be that the rate of decomposition of the reducing agent is exceeding the rate of reduction of compounds II, subsequent experiments were carried out in alkaline buffer solution, in which the rate of decomposition of sodium borohydride is lower than in alcohols [6], and in which the products II being reduced are fairly readily soluble. A buffer solution of $Na_2B_4O_7$/NaOH at pH 10.02 was used for this purpose. Experiments showed that the best results were achieved when small portions of an eightfold excess of sodium borohydride were added to the buffer solution of hydrazone II over 3 h with subsequent agitation for 2 h and keeping the reaction mixture for 24 h at 15-20°C. Decreasing the quantity of sodium borohydride, increasing the rate of addition of reducing agent, and having a shorter reaction time for the mixture lead to a decrease in the yield of products III. Increasing the quantity of sodium borohydride, decreasing the rate of addition of reducing agent, and having a longer reaction time for the mixture do not have any significant effects on the yield of the required products III. It was established by means of TLC that at temperatures below 15°C the rate of reducing hydrazones II decreases, and above 20°C hydrolysis appears to start in the reaction mixture and by-products are formed.

The N^1-(4-pyrimidinoyl)-N^2-benzylhydrazines III synthesized are colorless crystalline compounds which have relatively high melting points but lower than those of the initial acylhydrazones II. The PMR spectra of compounds III do not contain a signal from the methine proton in the region 7.95-8.48 ppm, which is characteristic for the initial arylidenehydrazides II, but they have a new signal due to methylene protons in the region 3.98-4.15 ppm, which is evidence for the conversion of arylidenehydrazides II to the corresponding hydrazines III.

EXPERIMENTAL (CHEMICAL)

The course of the reactions and purity of the compounds obtained were monitored by TLC on Silufol plates in the systems ethyl acetate and ethyl acetate—methanol (10:1) respectively for compounds IIIb, d-f, and IIIa, c; UV light and iodine were used for development. PMR spectra were recorded on a BS 487C Tesla spectrometer (80 MHz, Czechoslovakia) at 33°C. The solvent for IIa, b, d, e and IIIa-d was DMSO-d_6, that for IIc, f was CF_3COOH, and that for IIIe, f was $CDCl_3$. HMDS was used as an external standard for compounds IIa, b, d, e and IIIa, b, c, d and as an internal standard for compounds IIc, f and IIIe, f.

The methyl esters of 1-methyl- and 1,3-dimethylorotic acids were synthesized as in [7, 8], orotic acid hydrazide Ia was synthesized according to [11], and benzylidenehydrazides IId-f synthesized according to [1, 9].

1-Methylorotic Acid Hydrazide (Ib). This was obtained from methyl 1-methylorotate in a similar method to that for hydrazide Ia.

TABLE 2. PMR Spectral Data of Compounds II and III

Compound	PMR spectrum, δ, ppm
IIa	6,23 s (1H, CH), 7,25—7,95 m (5H, Ph), 8,48 s (1H, N=CH)
IIb	3,28 s (3H, CH_3), 6,35 s (1H, CH), 7,40—7,88 m (5H, Ph), 8,40 s (1H, N=CH)
IIc	3,00 s (3H, CH_3), 3,13 s (3H, CH_3), 6,00 s (1H, CH), 6,78—7,48 m (5H, Ph), 7,95 s (1H, N=CH)
IId	2,75 s (3H, SCH_3), 6,75 s (1H, CH), 7,38—7,95 m (5H, PH), 8,65 s (1H, N=CH)
IIe	1,45 t (J-6 Hz, 3H, CH_3), 3,50 q (J-6 Hz, 2H, SCH_2), 6,87 s (1H, CH), 7,50—7,95 m (5H, Ph), 8,68 s (1H, N=CH)
IIf	0,60 m (3H, CH_3), 1,28 m (4H, CH_2CH_2), 3,00 M (2H, SCH_2), 7,00 s (1H, CH), 7,01—7,63 m (5H, Ph), 8,15 s (1H, N=CH)
IIIa	4,03 s (2H, NCH_2), 5,95 s (1H, CH), 7,33 s (5H, Ph)
IIIb	3,25 s (3H, CH_3), 4,13 s (2H, NCH_2), 6,23 s (1H, CH), 7,50 s (5H, Ph)
IIIc	3,25 s (3H, CH_3), 3,34 s (3H, CH_3), 4,15 s (2H, NCH_2), 5,78 s (1H, CH), 7,50 s (5H, Ph), 9,98 M (2H, 2NH)
IIId	2,65 s (3H, SCH_3), 4,13 s (2H, NCH_2), 6,68 s (1H, CH), 7,43 s (5H, Ph)
IIIe	1,20 t (J=6 Hz, 3H, CH_3), 2,98 q (J=6 Hz, 2H, SCH_2), 3,98 s (2H, NCH_2), 6,86 s (1H, CH), 7,23 s (5H, Ph), 8,99 M (2H, 2NH)
IIIf	0,83 T (J=6 Hz, 3H, CH_3), 1,45 m (4H, CH_2CH_2), 2,95 t (J=6 Hz, 2H, SCH_2), 4,00 s (2H, NCH_2), 6,88 s (1H, CH), 7,23 s (5H, Ph), 8,94 m (2H, 2NH)

TABLE 3. Anti-monoamine Oxidase Activity of Compounds Studied

Compound	ED_{50}, mg/kg	Inhibition of rat liver MAO activity in vitro		
		inhibitor dose, M	retardation of oxidative deamination, %	P
IIIa	34,0 (18,8—61,5)	$1 \cdot 10^{-3}$	65,1±2,8	<0,001
		$1 \cdot 10^{-4}$	58,1±2,3	<0,001
		$1 \cdot 10^{-5}$	50,1±1,0	<0,001
		$1 \cdot 10^{-6}$	13,7±0,8	<0,01
IIIb	175,0 (97,8—313,3)	$1 \cdot 10^{-3}$	61,5±13,9	<0,001
		$1 \cdot 10^{-4}$	25,5±11,8	<0,01
		$1 \cdot 10^{-5}$	13,7±0,63	<0,01
		$1 \cdot 10^{-6}$	5,0±0,02	<0,02
IIIc	58,0 (31,9—105,6)	$1 \cdot 10^{-3}$	57,7±4,42	<0,001
		$1 \cdot 10^{-4}$	16,6±2,6	<0,01
		$1 \cdot 10^{-5}$	14,7±0,17	<0,05
		$1 \cdot 10^{-6}$	3,0±0,9	<0,1
IIId	44,0 (25,9—71,8)	$1 \cdot 10^{-3}$	68,4±3,5	0,001
		$1 \cdot 10^{-4}$	49,1±0,8	0,001
		$1 \cdot 10^{-5}$	34,0±0,7	0,001
		$1 \cdot 10^{-6}$	1,8±0,04	0,02
IIIe	62,0 (36,6—117,8)	$1 \cdot 10^{-3}$	70,0±1,7	0,001
		$1 \cdot 10^{-4}$	35,8±4,0	0,001
		$1 \cdot 10^{-5}$	15,4±3,0	0,02
		$1 \cdot 10^{-6}$	6,0±0,56	0,02
IIIf	78,0 (43,8—138,4)	$1 \cdot 10^{-3}$	66,3±7,2	0,001
		$1 \cdot 10^{-4}$	39,4±8,7	0,001
		$1 \cdot 10^{-5}$	23,0±7,6	0,02
		$1 \cdot 10^{-6}$	5,0±1,6	0,01
Nialamide	13,0 (7,2—23,4)	$1 \cdot 10^{-3}$	54,4±1,9	0,001
		$1 \cdot 10^{-4}$	46,4±5,3	0,02
		$1 \cdot 10^{-5}$	17,0±0,08	0,05
		$1 \cdot 10^{-6}$	2,0±0,14	0,05

Note. In brackets are the fiducial ED_{50} values when P = 0.05 for interaction with L-dopa in mice.

1,3-Dimethylorotic Acid Hydrazide (Ic). To 9 ml (0.18 mole) of hydrazine hydrate was added a solution of 5.5 g (0.027 mole) of methyl 1,3-dimethylorotate in 10 ml of methanol and the mixture was left at a temperature of 18-20°C for 4 h. The precipitate was filtered off and recrystallized.

Orotic, 1-Methyl-, and 1,3-Dimethylorotic Acid Benzylidene Hydrazides (IIa-c). Hydrazide Ia (0.01 mole) was dissolved in 120 ml of DMF at 80°C, hydrazide Ib (0.01 mole) was dissolved in 70 ml of boiling 80% ethanol, while hydrazide Ic (0.01 mole) was dissolved in 40 ml of boiling ethanol, 1.27 g (0.012 mole) of benzaldehyde was added and the mixture was kept under these conditions for 4 h, cooled, and the product that precipitated out was filtered off and recrystallized.

N^1-(4-Pyrimidinoyl)-N^2-benzylhydrazines (IIIa-f). To a suspension of 0.0038 mole of benzylidenehydrazide II in 45 ml of buffer solution ($Na_2B_4O_7$/NaOH, pH 10.02) with agitation and maintaining the temperature at 15-20°C was added 1.15 g (0.0304 mole) of sodium borohydride in small portions over 3 h; the mixture was agitated for 2 h and left to stand for 24 h. The mixture was acidified to pH 5.0 with conc. HCl. The precipitate was filtered off, washed with cold water (10 ml), and recrystallized.

Data on the compounds is given in Tables 1 and 2.

EXPERIMENTAL (PHARMACOLOGICAL)

The anti-MAO activity of the compounds synthesized was studied in vivo and in vitro. In the first case a method for interacting compounds with L-dopa in mice was used [10]. Four hours after oral administration of the compounds to male white mice of weight 20-25 g, 200 mg/kg of L-dopa was administered i.p. Determination of the anti-MAO activity of the compounds was carried out according to the reaction of the mice to L-dopa. Nialamide was used as a standard.

In the second case homogenates of rat liver tissue were used as the enzyme source. Tyramine hydrochloride served as the substrate. The MAO activity in the tissue homogenates was determined from the quantity of ammonia formed during the enzyme reaction. Incubation of the samples, their fixation, and ammonia determination using Nessler reagent were carried out according to the method of V. Z. Gorkin [2]. Isothermal elimination of ammonia was carried out using a modification of the Conway method [3]. The compounds studied were used at concentrations of $1 \cdot 10^{-3}$-$1 \cdot 10^{-6}$ M in the samples. Inhibition of MAO was denoted in percent relative to the control experiments (without inhibitor).

As can be seen from Table 3, all the compounds IIIa-f investigated have an inhibitory action of MAO in the experiments both in vitro and in vivo. The anti-MAO activity of compounds IIIa-f is due to the structure of the pyrimidine part of the molecule. The orotic acid derivative IIIa is the most active. Introduction of a methyl group into the 3-position of the pyrimidine ring reduces the anti-MAO activity to a greater extent that introduction of two methyl groups into the 1- and 3-positions. The anti-MAO activity is also decreased by introduction of an alkylthio group into the 2-position of the pyrimidine ring, an increase in alkyl chain length having a greater effect. In the experiments in vitro compounds IIIa-f have activity that is superior to nialamide, but in the experiments in vivo they have inferior activity.

Thus, the synthesis of N^1-(4-pyramidinoyl)-N^2-benzylhydrazines has prospective application for research into compounds with high anti-MAO activity.

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