SUBSTITUTED AMIDES AND HYDRAZIDES OF MALEIC ACID. I. SYNTHESIS AND BIOLOGICAL ACTIVITY OF o-HYDROXY-, o-AMINOBENZOYLHYDRAZIDES OF MALEIC ACID AND 1H-2,10-DIHYDROPYRIDAZINO[3,2-b]QUINAZOLINE-2,10-DIONE

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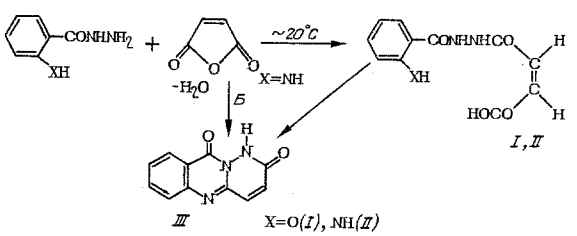
We know that substituted amides of maleic acid (maleamino acids), formed as a result of acylation of arylamines by maleic anhydride [1, 8, 31, 33], display a broad spectrum of biological action [1, 31, 33]. Among them we observe compounds with fungicidal [9, 13], antiartherosclerotic [14, 32, 33], and plant growth regulatory activity [15], and they also can be used as intermediates in synthesis of antimicrobial drugs, insecticides, and fungicides [1, 31].

The substituted (acyl- and sulfonyl-) hydrazides of maleic acid have been much less studied [28, 29]. Preliminary data on the biological activity of these compounds allow us to assess the appearance of new types of physiological action in this series. Thus, data is available on the anti-inflammatory, anticoagulant, hemostatic, hypoglycemic, and antihypoxia effect of arylsulfonylhydrazides of maleic and fumaric acids [3, 22, 23].

It is of interest to investigate the effect on the biological activity of replacement of the arylsulfonyl moiety of substituted hydrazides of malonic acid by an aroyl group. Accordingly, we began a study of the biological properties of compounds in this case, and we observed significant antiarrhythmic, antiaggregational (relative to thrombocytes) [6], and also retardant and growth-stimulating activity in plant specimens [10].

Further search for biologically active substances as potential drugs among substituted amides and hydrazides of maleic acid seem promising. With this goal, we obtained the o-hydroxy- (I) and o-aminobenzoylhydrazide of maleic acid (II) by acylation of the corresponding hydrazides of salicyclic or anthranylic acids with maleic anhydride at room temperature.



The structure of compounds I and II has been confirmed by data from IR and PMR spectroscopy and elemental analysis. In the IR spectra of the arolylhydrazides of maleic acid obtained, there are intense bands from the phenol hydroxyl at 3305 cm^{-1} (compound I) or the antisymmetric or symmetric stretching vibrations of the o-amino group at 3473 and 3382 cm^{-1}, respectively (compound II). The relative location of the latter bands are quite consistent with the calculated values for the primary amino group ($\nu_{sym}^{NH_2}$ = 345.53 + 0.876; $\nu_{asym}^{NH_2}$ = 3388 cm^{-1}) [25], not included in intermolecular and intramolecular hydrogen bonds with the carbonyl amide.

In the PMR spectra of compounds I and II (DMSO-d_6), there are no signals from the protons of the primary amino group of the hydrazide moiety, which in structurally similar hydrazides of anthranylic and salicyclic acids are found respectively at 4.38 and 4.81 ppm [5]. Thus, compounds I and II cannot have the isomeric structure of o-acylhetero-substituted hydrazides

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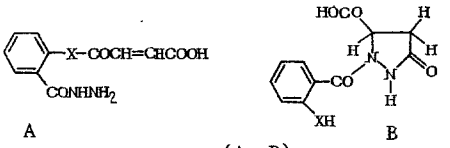
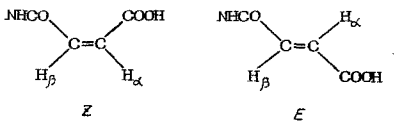
of benzoic acid (A). Hydrazides of salicyclic and anthranilic acids undergo N-acylation by maleic anhydride at the amino group of the hydrazide moiety.



A B

X=O, NH (A, B)

The position of the signals from the two methine protons of the ethylene moiety at 6.34 and 6.40 ppm in the PMR spectra of compounds I and II (DMSO-d_6) confirm the cis configuration of the latter, which is consistent with the calculation according to an additive scheme taking into account the deshielding constants of the substituents in substituted olefins [4, 7].



Z E

$$\delta_{H_\alpha}^{Z(E)} = \delta_{H_2C\text{-}CH_2}^{0} + \sigma_{H_\beta}^{Z(E)} + \sigma_{CONH}^{E(Z)} + \sigma_{COOH}^{gem};$$
$$\delta_{H_\beta}^{Z(E)} = \delta_{H_2C\text{-}CH_2}^{0} + \sigma_{H_\alpha}^{Z(E)} + \sigma_{COOH}^{E(Z)} + \sigma_{CONH}^{gem}$$
$$\delta_{H_\alpha}^{Z} = 6{,}67 \text{ ppm.}, \ \delta_{H_\beta} = 6{,}71 \text{ ppm.}, \ \delta_{H_\alpha}^{E} = 7.25 \text{ ppm.}, \ \delta_{H_\beta}^{E} = 7{,}32 \text{ ppm.}$$

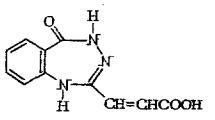
The difference between the experimental and calculated values of the chemical shifts of the protons $|\Delta\delta H_{\alpha(\beta)}|$ for the cis isomer is 0.27-0.27 ppm, while for the trans isomer it is almost three times greater: 0.85-0.92 ppm. Therefore, judging from the calculations, the cis configuration is the most probable for compounds I and II.

The absence of splitting of the interacting protons of the ethylene moiety into two doublets (characteristic of an AB system) in the PMR spectrum is probably due to the insignificant value of $\Delta\nu/J = (\nu H_\beta^Z - \nu H_\alpha^Z)/J$, the calculated value of which is 0.2-0.4 (J = 6-12 Hz [7]). In this case, the lines of the two doublets should practically coincide, and the AB spin system looks like an A_2 system in the spectrum [7].

The absence of splitting in the spectra of the signals from vicinal protons (characteristic for an AX system) also suggests that compounds I and II do not exist as cyclic isomers, 1-aroyl-5-carboxy-3-pyrazolidones (B).

It was established earlier that the o-aminobenzoylhydrazide of phenylacetic acid upon heating in a polyphosphoric acid medium (150-180°C) undergoes heterocyclization with formation of 2-benzyl-4,5-dihydro-1H-1,3,4-benzotriazepin-5-one, displaying anti-inflammatory and anticonvulsant activity with low toxicity [16]. Moreover, data is available indicating that the reaction of the hydrazide of anthranilic acid with succinic anhydride leads (depending on the conditions) to formation of the o-aminobenzoylhydrazide of succinic acid [30] or 1H-2,3,4,10-tetrahydropyridazino[3,2-b]quinazoline-2,10-dione [24, 30]. Pyridazino[3,2-b]quinazolin-10-ones, structurally similar to the latter compound, have been obtained upon condensation of anthranilic acid or its ethyl ester with substituted 3-chloropyridazines [26]. The biological activity of these compounds has not been studied. Considering the possibility of unambiguous chemical behavior of o-hetero substituted aroylhydrazides of maleic acid in heterocyclization reactions and also the presence of biological activity in the conversion products, we carried out cyclization of the o-aminobenzoylhydrazide of maleic acid II by brief heating in acetic acid medium (method A), as a result of which 1H-2,10-dihydropyridazino[3,2-b]quinazoline-2,10-dione (III) was isolated in high yield. The same compound was obtained under analogous conditions by heating the hydrazide of anthranylic acid with maleic anhydride (method B). The o-hydroxybenzoylhydrazide of maleic acid II could not undergo cyclization under these conditions.

In the PMR spectrum of compound II, there are no signals from the protons of the carboxyl group or the two methine groups of the ethylene moiety. In the mass spectrum of this compound, there is a peak of maximum intensity from the molecular ion m/z 213, and there are no peaks from the $M - CO_2^+$ and $M - CO_2 - H$ ions. There data suggest that compound III cannot have the structure of the isomeric 4,5-dihydro-1H,1,3,4-benzotriazepin-5-one (C):



C

Judging from literature data [16], formation of the latter compound could not be initially excluded.

EXPERIMENTAL (CHEMICAL)

The IR spectra of the synthesized compounds were taken on the UR-20 spectrometer (Vaseline mull). The PMR spectra were recorded on the RYa-2310 instrument (60 MHz) in DMSO-d_6 and CF_3COOH; internal standards HMDS and TMS. The mass spectrum was obtained on the Varian MAT-311 instrument in the direct injection mode, emission current 1000 mA, ionizing electron energy 70 eV, vaporizer temperature 180°C. The homogeneity of the componds was confirmed on Silufol UV-254 plates in the system ethylacetate–hexane 5:1; they were visualized with iodine. The elemental analysis data agree satisfactorily with the calculated values.

o-Hydroxybenzoylhydrazide of Maleic Acid (I). A solution of 0.98 g (10 mmoles) maleic anhydride in 30 ml ethylacetate was added with stirring to a solution of 1.52 g (10 mmoles) hydrazide of salicyclic acid in 100 ml ethylacetate. After 2 h, the residue was filtered off and recrystallized from n-butanol. Obtained: 2.15 g (86%) compound I with mp 187-188°C (decomp.). $C_{11}H_{10}N_2O_5$. M 250.23. IR spectrum, ν, cm^{-1} (crystals): 3305 (OH), 3175-3190 (NH amide), 3040-3050 (cis-CH=CH), 1703 (COOH), 1662 (CONH), 1600-1615 (C_6H_4; C=C). PMR spectrum, δ, ppm, DMSO-d_6 (HMDS): 6.40 s (2H, CH=CH), 6.90-8.00 m (4H, C_6H_4), 1075-11.42 br.s (4H, 2NHCO, OHphen., COOH) (δ, NHSO 10.86 ppm in DMSO-d_6 + CF_3COOH, which is consistent with literature data for the hydrazide of salicyclic acid [5]).

o-Aminobenzoylhydrazide of Maleic Acid (II). A solution of 0.98 g (10 mmoles) maleic anhydride in 30 ml ethylacetate was added with stirring to a solution of 1.51 g (10 mmoles) hydrazides of anthranilic acid in 200 ml ethylacetate. After 2 h, the residue was filtered off and recrystallized from isobutanol or a mixture of DMSO–water 5:1. Obtained: 2.30 g (92%) compound II with mp 163-164°C (decomp.). $C_{11}H_{11}N_3O_4$. M 249.24. IR spectrum, ν, cm^{-1} (crystals): 3483 (NH_2, asym), 3382 (NH_2, sym), 3175-3200 (NH amide), 3045-3060 (cis-CH=CH), 1695 (COOH), 1630-1638 (CONH, C=C), 1608-1615 (C_6H_4). PMR spectrum, δ, ppm, DMSO-d_6 (HMDS): 6.34 s (2H, CH=CH) ($\delta_{CH=CH}$ 6.38 ppm in DMSO-d_6 + CF_3COOH), 6.45-7.57 m (6H, C_6H_4, 2NHCO), 8.72-8.95 br.s (2H, NH_2, signal disappears upon addition of CF_3COOH).

1H-2,10-Dihydropyridazino[3,2-b]quinazoline-2,10-dione (III). Method A. A suspension of 2.49 g (10 mmoles) o-aminobenzoylhydrazide of maleic acid II in 50 ml acetic acid was heated with stirring to 60-80°C; compound II in this case goes into solution. It was cooled down to room temperature. The precipitate was filtered off and recrystallized from DMF. Obtained: 1.75 g (82%) compound III. **Method B.** A solution of 0.98 g (10 mmoles) maleic anhydride in 20 ml acetic acid was added with stirring to a solution of 1.51 g (10 mmoles) hydrazide of anthranilic acid in 50 ml acetic acid at a temperature of 60-80°C. After 1 h, the residue was filtered off and recrystallized from DMF. Obtained: 1.70 g (80%) compound III with mp 350°C (decomp.). $C_{11}H_7N_3O_2$. M 213.20. IR spectrum, ν, cm^{-1} (crystals): 3290-3310 (NH amide), 3063 (C=C), 1697 (C^{10} = 0 amide) [21], 1637 (C^2 = 0 amide., C=C). PMR spectrum, δ, ppm, CF_3COOH (TMS): 7.85-8.75 m (7H, C_6H_4, 2CH, NH). Mass spectrum, m/z (I, %), all ion peaks with intensity >3% are given: 213 (100) M^+, 184 (35) M – CO^+, 156 (14) M – 2CO–H^+, 130 (25) M – 2CO–HCN^+ 102 (22) $C_6H_4CN^+$, 76 (18) $C_6H_4^+$.

EXPERIMENTAL (BIOLOGICAL)

We studied the antimicrobial, anti-inflammatory, antidepressant, and anticonvulsant activity of the synthesized compounds.

The acute toxicity LD_{50} of compounds I-III were determined by the method of Pershin [18] with intraperitoneal injection into white mice of mass 16-24 g in the form of a suspension in 2% starch slurry.

The antimicrobial activity of the compounds with respect to the reference strains of *E. coli* M_{17} and golden staphylococcus *St. aureus* P-209 were determined by the standard method of twofold series dilutions in meat peptone broth [18] for bacterial load of 250 thousand microbial units per ml solution. As the effective dose, we took the minimum inhibiting concentration (MIC) of the compound: the maximum dilution leading to complete suppression of development of the test microbes. We compared the antimicrobial activity of the compounds obtained (Table 1) with the activity of the antimicrobial drug ethacridine lactate, widely used in medicine [11].

The anti-inflammatory action of the compounds was studied in the model of acute inflammatory edema induced by subplantar injection of a 0.1 ml 1% aqueous solution of carrageenan into the hind paw of white rats of mass 180-200 g. We assessed the anti-inflammatory action from the degree of retardation of exudation (in % relative to the control) upon intraperitoneal injection of the compounds in the form of a suspension in a 2% starch slurry. We compared the effect with amidopyrine, active in a dose of 100 mg/kg [2], and mefenamic acid in a dose of 50 mg/kg (Table 1).

TABLE 1. Antimicrobial and Anti-Inflammatory Activity of the Synthesized Compounds

Compound	Antimicrobial activity, MIC, μg/ml		Compound	LD_{50}, mg/kg	Dose, mg/kg	Equitoxic dose, arb. units	Anti-inflammatory activity	
	E. coli M_{17}	S. aureus P-209					av. increment in rat paw vol, % of original	retardation of exudation, % of control
I	1000	1000	II	1500	50	0,033	85,1	24,5
			III	1000	50	0,05	78,5	30,4
II	500	500	Control – 2%					
III	500	500	starch slime	—	—	—	112,7	—
Ethacridine			Amidopyrine	249	100	0,40	42,0	62,7
lactate	2000	500	Mefenamic acid	150	50	0,33	46,0	59,2

TABLE 2. Antidepressant Activity of o-Aminobenzoylhydrazide of Maleic Acid II

Compound	LD_{50}, mg/kg	Dose, mg/kg	Equitoxic dose, arb. un. $\left(\frac{\text{dose}}{LD_{50}}\right)$	Antidepressant activity			Change in reserpin-induced hypotherm., °C
				immob. time, sec.		effect, % of con.	
				after 6 min	after 30 min		
II	1500	10	0,007	139,1±35,1	1399,0±63,6	31,6	—2,55
Control – 2% starch slime	—	—	—	203,4±17,6	1490,1±90,8	—	—3,50
Amitryptiline	76	10	0,13	146,2±20,8	1353,0±81,8	77,3	—2,01

The antidepressant activity of compound II was investigated using the swimming test [20] on male white mice of mass 16-22 g, which were injected intraperitoneally with the compound (first group of experimental mice), amitryptiline [11] (second group of mice) in doses of 10 mg/kg in the form of a suspension in a 2% starch slurry, and also equivolume amounts of the latter (control group of mice) 30 min before the test. We assessed the antidepressant action from the immobilization time for the animals in 6 and 30 min of swimming. We compared the changes with the control and with amitryptiline. We also determined the effect of the compound on hypothermia in white mice induced by injecting them intraperitoneally with reserpine in a dose of 2 mg/kg 4 h before the experiment [12] (Table 2).

The anticonvulsive activity of the compounds was determined using the maximum electric shock test [19] on white mice of mass 18-22 g with intraperitoneal injection in a 2% starch slurry.

The acute toxicity of the tested compounds is greater than 1000 mg/kg (Tables 1 and 2); they are much less toxic than the comparison drugs: ethacridine lactate (LD_{50} 70 mg/kg), amidopyrine (LD_{50} 249 mg/kg [2], mefenamic acid (LD_{50} 150 mg/kg) and amitryptyline (LD_{50} 76 mg/kg). Thus, the compounds are practically nontoxic; the white mice tolerated well a single intraperitoneal injection of the drugs in doses of 1000-1500 mg/kg.

All the investigated compounds display weak bacteriostatic action with respect to both strains of test microbes for a minimum inhibiting concentration of 500-1000 μg/ml (Table 1), which is not inferior in activity to ethacridine lactate. Compounds II and III display a weak anti-inflammatory effect in a dose of 50 mg/kg, less than 0.033 and 0.05 LD_{50} of these compounds, while amidopyrine and mefenamic acid are active in significantly larger equitoxic doses of respectively 0.40 and 0.33 LD_{50} (Table 1). Antidepressant action is displayed in the o-aminobenzoylhydrazide of maleic acid II; this compound is inferior to amitryptiline in activity, but is effective in an almost 19 times smaller equitoxic dose (Table 2). In all the investigated compounds, no anticonvulsive effect is displayed in doses up to 600 mg/kg.

REFERENCES

1. USSR Inventor's Certificate 182141 (1966); Ref. Zh. Khim., No. 10 N 514 P (1967).
2. I. S. Berdinskii, O. G. Min'kina, and A. F. Goleneva, Biological Effect of Products of Organic Synthesis and Natural Compounds [in Russian], Perm' (1982), pp. 56-62.
3. V. N. Vydashenko, V. I. Makurina, and I. S. Grishchenko, "Scientific and technical progress and optimization of drug production processes," Abstracts, All-Union Scientific Conference, L'vov (1987), pp. 139-140.

4. B. V. Ioffe, R. R. Kostikov, and V. V. Razin, Physical Methods for Determination of the Structure of Organic Compounds [in Russian], Moscow (1984), p. 72.
5. L. M. Kapkan, A. Yu. Chervinskii, and E. V. Titov, Ukr. Khim. Zh., **47** , No. 10, 1088-1092 (1981).
6. V. O. Koz'minykh, B. Ya. Syropyatov, S. Yu. Solodnikov, and Yu. S. Andreichikov, "Synthesis, pharmacology, and clinical aspects of new psychotropic and cardiovascular drugs," Abstracts, Volgograd (1989), pp. 31-32.
7. A. I. Kol'tsov and B. A. Ershov, Nuclear Magnetic Resonance in Organic Chemistry [in Russian], Leningrad (1968), pp. 45, 58.
8. A. L. Lifits, A. A. Veitsman, and L. Ya. Distanova, Methods for Obtaining Chemical Reagents and Drugs [in Russian], Moscow (1970), No. 22, pp. 114-117 (1970).
9. N. Kh. Masksurov, D. Z. Mukhtarova, A. M. Abramova, and Kh. G. Maksumova, Manuscript Dep. VINITI 18.02.1976, No. 516-76 Dep., Tashkent (1976).
10. V. B. Martsenyuk, V. O. Koz'minykh, and Yu. S. Andreichikov, Geographical Aspects of the USSR Food Program (Chernozem-Poor Western Urals) [in Russian], Perm' (1990), pp. 122-126.
11. M. D. Mashkovskii, Drugs [in Russian], Vol. 1, p. 96; Vol. 2, pp. 398, 409 (1985).
12. M. D. Mashkovskii, N. I. Andreeva, and A. I. Polezhaeva, Pharmacology of Antidepressants [in Russian], Moscow (1983), pp. 196-197.
13. U.S. Pat. 2779704 (1957); Ref. Zh. Khim., No. 65476 P (1958).
14. Neth. Pat. 108413 (1964); Ref. Zh. Khim., No. 22 N 239 P (1966).
15. U.S. Pat. 3326659 (1967); Ref. Zh. Khim., No. 15 N 659 P (1968).
16. U.S. Pat. 3542767 (1970); Ref. Zh. Khim., No. 15 N 489 P (1971).
17. Belg. Pat. 713387 (1971); Ref. Zh. Khim., No. 24 N 364 P (1972).
18. G. N. Pershin, Methods of Experimental Chemotherapy [in Russian], Moscow (1971), pp. 100, 109-117, 318.
19. K. S. Raevskii, Farmakol. Toksikol., No. 4, 495-497 (1961).
20. D. Yu. Rusakov and A. V. Val'dman, Farmakol. Toksikol., No. 5, 107-111 (1983).
21. R. A. Slavinskaya, N. A. Dolgova, T. A. Kovaleva, V. Z. Gabdrakipov, and G. A. Yuldasheva, Zh. Obshch. Khim., **60**, No. 1, 2364-2369 (1990).
22. E. M. Sopel'nik, S. V. Stavnichuk, E. M. Knyaz', E. K. Ryadnykh, and S. V. Popov, "Increase in the quality of drug relief for ambulatory and nonambulatory patients based on acceleration of scientific and technical progress in light of the resolutions of the 27th Congress of the Communist Party of the Soviet Union," Abstracts of the 4th All-Union Congress of Pharmacists, Kazan' (1986), pp. 388-389.
23. V. P. Chernykh, S. V. Stavnichuk, E. M. Knyaz', L. F. Checherskaya, S. B. Popov, and A. E. Shevchenko, "Optimization of drug supply and a way to enhance the effectiveness of pharmaceutical science," Abstracts, Republic Scientific Conference, Khar'kov (1986), p. 123.
24. V. Balasubramaniyan and N. P. Argade, Indian J. Chem., **27B**, No. 10, 906-908 (1988).
25. L. J. Bellamy and T. Williams, Spectrochim. Acta, No. 9, 341-344 (1957).
26. H. Beyer and C.-E. Völcker, Chem. Ber., **97**, No. 2, 390-395 (1964).
27. M. R. Chaurasia and S. K. Sharma, Arch. Pharm., **315**, No. 4, 377-381 (1982).
28. H. Feuer and H. Rubinstein, J. Am. Chem. Soc., **80**, No. 21, 5873-5877 (1958).
29. H. Feuer and J. P. Asunskis, J. Org. Chem., **27**, No. 12, 4684-4686 (1962).
30. M. Ghelardoni and V. Pestellini, Ann. Chim. (Ital.), **64**, No. 7-8, 445-453 (1974).
31. G. M. Gherasim and I. Zugravescu, Rev. Chim. (RSR), **32**, No. 11, 1047-1054 (1981).
32. Kang-Chien Liu, Tong-Bao Chen, Hon-Yi Jan, and Ching-Yun Shin, J. Chin. Chem. Soc., **20**, No. 3, 163-169 (1973).
33. E. M. Schultz, W. A. Bolhofer, A. Augenblick, J. B. Bicking, Ch. N. Habicker, J. K. Horner, J. Kwong, and A. Pietruszkiewicz, J. Med. Chem., **10**, No. 4, 717-724 (1967).