SUBSTITUTED AMIDES AND HYDRAZIDES OF MALEIC ACID. II.* SYNTHESIS AND BIOLOGICAL ACTIVITY OF ARYLIDENE- AND DIARYLMETHYLENEPHYDRAZIDES OF MALEIC ACID

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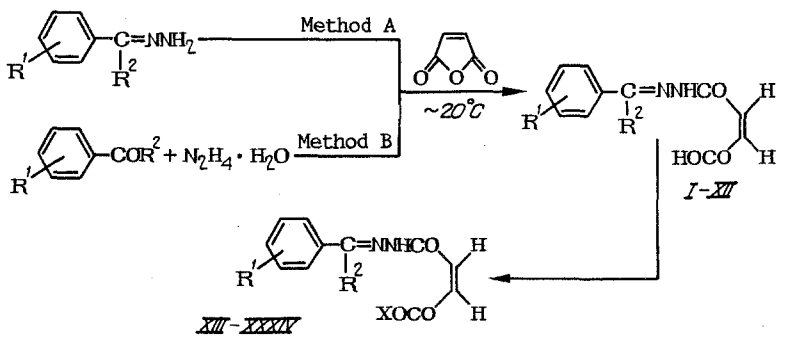
We know that ethylenedicarboxylic acids (fumaric, cis-aconitic) are natural metabolites for exchange of substances in the organism and take part in bioenzymatic processes [8]. It has been suggested that modification of their structure can be used to introduce a number of pharmocophore maleyl and furmaroyl groups. Data are available suggesting that arylsulfonylhydrazides of maleic and fumaric acids have anti-inflammtory, anticoagulant, hemostatic, hypoglycemic, and antihypoxic action [2, 15, 18, 19]. We estalbished that replacement of the arylsulfonyl fragment of substituted hydrazides of maleic acid by o-hydroxy- or o-aminobenzoyl leads to the appearance of bacteriostatic and antidepressant activity with a weak anti-inflammatory effect [4]. These compounds have no anticonvulsant effect [4]. Recently in a study of the biological properties of some methylene- and acylhydrazides of maleic acid, we observed significant antiarrhythmic and antiaggregational (with respect to thrombocytes) activity [3]. Preliminary data are also available concerning the retardant and growth-stimulating effect of the these compounds with respect to monocotyledonous plants [9, 10]. Thus, judging from the literature data and the results of our investigations, sulfonyl- and acylhydrazides of maleic acid have a broad spectrum of biological action.

Further search for biologically active compounds (potential drugs) among substituted hydrazides of maleic acid seems promising. With this goal, we have obtained arylidene- and diarylmethylenehydrazides of maleic acid I-XII by acylation of the corresponding hydrazones of aromatic aldehydes or arylketones (method A) or method B (without separation of hydrazones from the mxiture of diarylketones and hydrazine hydrate) by maleic anhydride at room temperature. Ammonium, lithium, sodium, potassium, calcium, magnesium, and nickel salts of benzylidene- and diarylmethylenehydrazides of maleic acid (XIII–XXV, XXVIII–XXXIV) were synthesized by treatment of the acids I, VI–XI with the corresponding metal hydroxides or ammonium hydroxide in ethanol medium. Subsequent reaction of the potassium salt of diphenylmethulenehydrazide of maleic acid XXIII with methyl iodide or ethyl iodide allowed us to obtain esters of the acid VI: compounds XXVI and XXVII.



R^1=H (I, IV, VI, XII–XXVII), o-CH_3 (VII, XXVIII), p-CH_3 (VIII, XXIX, XXX), p-CH_3O (IX, XXXI, XXXII), p-Br (X, XXXIII), p-$(CH_3)_2N$ (XI, XXXIV), m-NO_2 (II), p-NO_2 (III, V); R^2=H (I–III, XIII–XIX), CH_3 (IV, V), C_6H_5 (VI–X, XX–XXXIII), p-$(CH_3)_2NC_6H_4$ (XI, XXXIV), C_6H_5CO (XII); X=CH_3 (XXVI), C_2H_5 (XXVII), NH_4 (XIII, XX, XXVIII, XXIX, XXXI, XXXIII, XXXIV), Li (XIV, XXI, XXX, XXXII), Na (XV, XXII), K (XVI, XXIII), 1/2 Ca (XVII, XXIV), 1/2 Mg (XVIII, XXV), 1/2 Ni (XIX).

*For Communication 1, see [4].

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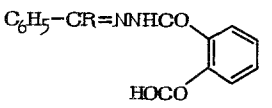
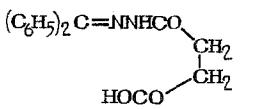
The physicochemical characteristics of arylidene- and diarylmethylenehydrazides of maleic acid and their salts are presented in tables 1 and 2. The structure of the substances obtained has been confirmed by IR and PMR spectroscopy data. The spectral characteristics of acids I-XII are presented in Table 1.

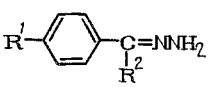
The position of the signals from the two interacting methine protons of the ethylene moiety at 6.25-6.48 and 6.50-7.04 ppm in the PMR spectra of compounds I, III-XII (DMSO-d_6, see Table 1) confirms the cis-configuration of the latter, which is consistent with the calculation according to an additive scheme (taking into account the shielding constants of the substituents in substituted olefins) which we performed earlier in [4]. The splitting of the methine protons of the AB spin system for these compounds is characterized by the spin–spin coupling constant J = 12.0-12.8 Hz, the magnitude of which is consistent with tabulated values for cis-olefins [6]. Compound II is difficultly soluble in DMSO; we had to heat the mxiture almost to boiling. We can probably explain the approximately 1.2 ppm downfield shift of the methine proton signals in the spectrum compared with the signals of its structural analogs (see Table 1) by formation of the trans isomer under these conditions. The difference between the epxerimental and calculated values of $\Delta\delta H_{\alpha,\beta}$ [4] for the cis isomer is 0.99-1.19 ppm, while for the trans isomer it is 0.41-0.58 ppm; i.e., judging from the calculations, this is probably the trans olefin.



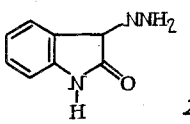
XXXV, XXXVI



XXXVII



XXXVIII-XLI



XLIII

$(C_6H_5)_2C{=}\overset{+}{N}NH_3Br^-$

XLII

$C_6H_5{-}\underset{|}{C}{=}N{-}N{=}CR{-}C_6H_5$

XLIV, XLV

R=H (XXXV, XLIV), C_6H_5 (XXXVI, XLV); R^1=H (XXXVIII–XL), p-$(CH_3)_2N$ (XLI); R^2=H (XXXVIII), C_6H_5 (XXXIX), p-BrC_6H_4 (XL), p-$(CH_3)_2NC_6H_4$ (XLI).

In order to study the effect on the biological activity of replacement of the maleyl group by the similar moieties of phthalic and succinic acids, and also in order to isolate the pharmacophores of the hydrazone part of the molecule, we obtained arylidenehydrazides of phthalic acid XXXV, XXXVI, the diphenylmethylenehydrazide of succinic acid XXXVII, and also hydrazones and azines of benzaldehyde, diarylketones XXXVIII–XLII, XLIV, XLV and the 3-hydrazone of isatin XLIII.

EXPERIMENTAL (CHEMICAL)

The IR spectra of the syntheiszed compounds were taken on the UR-20 spectrometer (vaseline mull). The PMR spectra were recorded on the RYa-2310 instrument (60 MHz) in $CDCl_3$ $(CD_3)_2CO$, DMSO-d_6 and CF_3COOH, internal standards HMS and TMS. The course of the reactions was monitored and the purity of the compounds was determined on Silufol UV-254 plates in a 5:1 ethylacetate–hexaen system, visualized with iodine. The characteristics of the compounds obtained are presented in Tables 1 and 2. The elemental analysis data correspond to the calculated values.

Arylidene- and Diarylmethylenehydrazides of Maleic Acid (I-XII). Method A. A solution of 20 millimoles of the corresponding hydrazone in 100-200 ml ethylacetate at room temperature was added to a solution of 1.96 g (20 millimoles) of maleic anhydride in 50 ml ethylacetate. We used hydrazones of: benzaldehyde XXXVIII, m-nitrobenzaldehyde, p-nitrobenzaldehyde [20], p-nitroacetophenone [24], benzophenone XXXIX [20], p-bromobenzophenone XL, bis-(p-dimethylaminobenzophenone) XLI or benzyl (dibenzoyl) [14]. The reaction mixture was allowed to stand for 2-3 h. The residue was filtered (or in the case of reaction with benzyl

TABLE 1. Physicochemical and Spectral Characteristics of Substituted Methylenehydrazides of Maleic, Phthalic, and Succinic Acids I-XII, XXXV-XXXVII

Compound	Method	Yield, %	mp, °C (decomp.)	Empirical formula	IR spectrum, ν, cm^{-1}, crystals	PMR spectrum, δ, ppm, DMSO-d_6
I	*A*	94	183—184*	$C_{11}H_{10}N_2O_3$	3225 (HNCO), 1700 (COOH), 1637, 1615 (CONH, C=C)	6,34; 7,01 two d (2H, CH=CH, J 12,2 Hz), 7,46—8,10 m (5H, C_6H_5), 8,32s (1H, CH), 11,75 br.s (1H, NH)
II	*A*	87	196—198	$C_{11}H_9N_3O_5$	3210 (NHCO), 1702 (COOH), 1615—1640 (CONH, C=C)	7,66; 7,90 two d (2H, CH=CH), 8,13—8,58 m (4H, C_6H_4), 8,75s (1H, CH)**
III	*A*	92	273—275	$C_{11}H_9N_3O_5$	3217 (NHCO), 1698 (COOH), 1620—1645 (CONH, C=C)	6,35; 6,88 two d (2H, CH=CH), J 12,8 Hz), 7,65—8,22 m (5H, CH, C_6H_4), 11,72 br.s (1H, NH)
IV	B	74	165—167	$C_{12}H_{12}N_2O_3$	3250 (NHCO), 1712 (COOH), 1620—1650 (CONH, C=C)	2,28s (3H, CH_3), 6,48; 7,04 two d (2H, CH=CH, J 12,0 Hz), 7,40—7,78 m (5H, C_6H_5), 10,94 br.s (1H, NH)
V	*A*	93	175—177	$C_{12}H_{11}N_3O_5$	3185 (NHCO), 1718 (COOH). 1670 (CONH), 1635 (C=C)	2,30s (3H, CH_3), 6,25; 6,98 two d (2H, CH=CH), J 12,6 Hz), 7,83—8,25 m (4H, C_6H_4), 11,03 br.s (1H, NH)
VI	*A*	85	170—171	$C_{17}H_{14}N_2O_3$	3208 (NHCO), 1716 (COOH), 1590—1632 (CONH, C=C, C_6H_5)	6,27; 6,53 two d (2H, CH=CH, J 12,2 Hz), 7,18—7,70 m (10 H, $2C_6H_5$), 9,78 br.s (1H, NH)***
VII	B	52	169—170	$C_{18}H_{16}N_2O_3$	3207 (NHCO), 1720 (COOH), 1590—1625 (CONH, C=C, Ar)	2,02s (3H, CH_3), 6,38 br.s (2H, CH=CH), 7,10—7,62 m (9H, C_6H_5, C_6H_4), 10,55 br.s (1H, NH)
VIII	B	68	182—183	$C_{18}H_{16}N_2O_3$	3242 (NHCO), 1713 (COOH), 1590—1635 (CONH, C=C, Ar)	2,28s (3H, CH_3), 6,28; 6,55 two d (2H, CH=CH), J 12,6 Hz), 7,00, 7,80 m (9H, C_6H_5, C_6H_4), 10,77 br.s (1H, NH)
IX	B	60	173—174	$C_{18}H_{16}N_2O_4$	3248 (NHCO); 1714 (COOH), 1580—1620 (CONH, C=C, Ar)	3,77; 3,83 два с (3H, CH_3O), 6,27; 6,50 two d (2H, CH=CH, J 12,0 Hz), 7,00—7,60 m (9H, C_6H_5, C_6H_4), 10,77 br.s (1H, NH)
X	*A*	91	197—198	$C_{17}H_{13}BrN_2O_3$	3218 (NHCO), 1713 (COOH), 1590—1630 (CONH, C=C, Ar)	6,28; 6,53 two d (2H, CH=CH, J 12,2 Hz), 7,30—7,70 m (9H, C_6H_5, C_6H_4), 9,95 br.s (1H, NH)
XI	*A*	94	206—207	$C_{21}H_{24}N_4O_3$	3208 (NHCO), 1710 (COOH), 1600—1617 (CONH, C=C)	2,97c (12H, $4CH_3$), 6,35—7,43 group of signals (10H, CH=CH, $2C_6H_4$), 9,33 br.s (1H, NH)**
XII	*A*	77	129—130	$C_{18}H_{14}N_2O_4$	3213 (NHCO), 1692 (COOH) 1657 (CONH), 1580—1620 (C_6H_5CO, C_6H_5, C=C)	6,25; 7,00 two d (2H, CH=CH, J 12,2 Hz), 7,45—7,85 m (10H, $2C_6H_5$), 11,64 br.s (1H, NH)
XXXV	—	70	153—154	$C_{15}H_{12}N_2O_3$	3244 (NHCO), 1724 (COOH), 1636 (CONH)	7,38—8,30 m (10H, C_6H_5, C_6H_4, CH), 10,43 br.s (1H, NH)**
XXXVI	—	84	177—178	$C_{21}H_{16}N_2O_3$	3120 (NHCO), 1716 (COOH), 1628 (CONH)	7,23—7,94 m (14H, $2C_6H_5$, C_6H_4), 10,35 br.s (1H, NH), 12,85 br.s (1H, OH)
XXXVII	—	88	185—186	$C_{17}H_{16}N_2O_3$	3165 (NHCO), 1729 (COOH), 1652 (CONH)	2,96 br.s (4H, $2CH_2$), 7,32—7,73 m (10H, $2C_6H_5$), 10,05 br.s (1H, NH), 12,20 br.s (1H, OH)

*mp 176°C [11], 183°C [21], 192-193°C [22]. **The compound is difficultly soluble in DMSO. ***Solution in CF_3COOH (TMS): 6.31 sec (2H, CH=CH).

TABLE 2. Physicochemical Characterisitcs of Salts and Esters of Benzylidene- and Diarylmethylenehydrazides of Maleic Acid XIII-XXXVI

Compound	Yield, %	mp, °C (decomp.)	Empirical formula
XIII	80	186—188	$C_{11}H_{13}N_3O_3$
XIV	68	136—138	$C_{11}H_9N_2O_3Li$
XV	94	250—252	$C_{11}H_9N_2O_3Na$
XVI	73	276—278	$C_{11}H_9N_2O_3K$
XVII	72	>300	$C_{22}H_{18}N_4O_6Ca$
XVIII	64	112—115	$C_{22}H_{18}N_4O_6Mg$
XIX	77	>350	$C_{22}H_{18}N_4O_6Ni$
XX	79	177—179	$S_{17}H_{17}N_3O_3$
XXI	85	111—113	$C_{17}H_{13}N_2O_3Li$
XXII	71	148—150	$C_{17}H_{13}N_2O_3Na$
XXIII	70	233—235	$C_{17}H_{13}N_2O_3K$
XXIV	90	210—213	$C_{34}H_{26}N_4O_6Ca$
XXV	88	177—180	$C_{34}H_{26}N_4O_6Mg$
XXVI	93	152—153*	$C_{18}H_{16}N_2O_3$
XXVII	85	114—115*	$C_{19}H_{18}N_2O_3$
XXVIII	84	171—173	$C_{18}H_{19}N_3O_3$
XXIX	92	144—146	$C_{18}H_{19}N_3O_3$
XXX	74	120—122	$C_{18}H_{15}N_2O_3Li$
XXXI	95	152—154	$C_{18}H_{19}N_3O_4$
XXXII	86	161—163	$C_{18}H_{15}N_2O_4Li$
XXXIII	90	146—148	$C_{17}H_{16}BrN_3O_3$
XXXIV	87	205—208	$C_{21}H_{27}N_5O_3$

*Melts without decomposition.

hydrazone, the residue was evaporated and treated) and recrystallized from ethanol (compounds VI-X, XII), acetone (compound I), n-butanol (compounds II, XI) or a mixture of 5:1 DMSO—water (III, V). The crystals of compounds III, V were dried in a desiccator over P_2O_5.

Method B. A mixture of 20 millimoles of the corresponding arylketones (acetophenone, o-methylbenzophenone, p-methylbenzophenone, or p-methoxybenzophenone) and 20 ml 70% hydrazine hydrate was boiled for 8-10 h (in the case of acetophenone, with addition of 0.5 g barium oxide), cooled, and extracted with 3 × 30 ml ethylacetate. The organic layer was washed with water and dried with anhydrous magnesium sulfate. The solution obtained was added with mixing to a solution of 1.96 g (20 millimoles) maleic anhydride in 50 ml ethylacetate, then treated analogously to method A (in the case of reaction with o-methylbenzophenone, the solvent was evaporated). The result was recrystallized from ethanol (compound VII-IX) or acetone (IV).

Salts of Benzylidene- and Diarylmethylenehydrazides of Maleic Acid (XIII—XXV, XXVIII—XXXIV). 10 ml of a 25% solution of ammonia (to obtain salts XIII, XX, XXVII, XXIX, XXXI, XXXIII, XXXIV) of a solution of 10 millimoles of lithium, sodium, or potassium hydroxide in 50 ml water (for salts XIV-XVI, XXI-XXIII, XXX, XXXII), or a solution of 10 millimoles of potassium, magnesium, or nickel chlorides (for salts XVII-XIX, XXIV, XXV) was added with stirring ot a suspension of 10 millimoles of acids I, VIXI in 150-200 ml ethanol at a temperature of 50-70°C. The solvent was evaporated and the residue of compounds XVII, XIX, XXIV, XXV was filtered. The result was recrystallized from ethanol (compounds XIII-XV, XX, XXI, XXVIII-XXIV), water (XVI-XVIII, XXII-XXV), or a mixture of DMSO-water, 5:1 (XIX). The crystals of compounds XVI-XIX, XXII-XXV were held for 2-3 days over P_2O_5 in a desiccator.

An attempt to obtain the previously described compound I according to a one-step method proposed in patent [11] was unsuccessful. We only isolated the heterocyclization product: 6-hydroxy-2,3-dihydro-3-pyridazinone. The latter probably is formed in the presence of an excess of the hydrazine hydrate (unreacted with the benzaldehyde), which for this reason must be removed (as suggested in our method B). We also encountered similar difficulties in the synthesis of this compound according to the Feuer method [22]; it was not possible to obtain the acyclic hydrazide of maleic acid described in this paper even with better cooling of the reaction mixture.

Esters of Diphenylmethylenehydrazide of Maleic Acid (XXVI, XXVII). 10 ml methyl iodide was added to a suspension of 3.32 g (10 millimoles) of the potassium salt of diphenylmethylenehydrazide of maleic acid XXIII in 50 ml methylethylketone and boiled for 3 h. The solution was filtered, the filtrate was evaporated, and the residue was recrystallized from ethanol (compound XXVI) or a 1:1 benzene—hexane mixture (XXVII).

TABLE 3. Antimicrobial Activity of Synthesized Compounds

Compound	Minimal inhibitory concentration MIC, μg	
	E. coli M_{17}	S. aureus P-209
I	500	250
II	1000	500
III	500	250
IV	500	500
VI	Inactive	1000
VII	1000	500
VIII	1000	1000
IX	1000	62,5
X	500	250
XI	1000	1000
XIV	Inactive	Inactive
XV	Inactive	Inactive
XVI	1000	1000
XVII	1000	500
XVIII	Inactive	1000
XIX	Inactive	Inactive
XXII	1000	1000
XXIII	1000	1000
XXIV	1000	500
XXV	Inactive	1000
XXVI	1000	125
XXVII	Inactive	15,6
XXVIII	Inactive	Inactive
XXIX	Inactive	1000
XXX	Inactive	1000
XXXIII	1000	1000
XLII	500	500
XLY	500	500
Ethacridine lactate*	2000	500

*LD_{50} 70(63-78) mg/kg.

Methyl Ester of Diphenylmethylenehydrazide of Maleic Acid (XXVI). IR spectrum, ν, cm^{-1} (crystals): 3250 (NHCO); 3055 (C=C); 1738 ($COOCH_3$); 1681 (C=C); 1647 (CONH). PMR spectrum, δ, ppm $(CD_3)_2CO$ (HMDS): 3.62 sec (3H, $COOCH_3$), 6.34 7.06 two d (2H, CH=CH, I 12.3 Hz, 7.30-7.67 m (10 H, $2C_6H_5$), 10.10 broad s (1H, NH).

Ethyl Ester of Diphenylmethylenehydrazide of Maleic Acid (XXVII). IR spectrum: ν, cm^{-1} (crystals): 3257 (NHCO); 3063 (C=C); 1720 ($COOC_2H_5$), 1675 (C=C); 1635 (CONH): PMR spectrum, δ, ppm, $CDCl_3$ (HMDS): 1.21 t (3H, CH_3), 4.20 q (2H, CH_2), 6.30, 6.67 two d (2H, CH=CH, J 12.2 Hz), 7.28-7.65 m (10H2C_6H_5), 10.87 broad s (1H, NH).

Arylidenehydrazides of Phthalic Acid (XXXXV, XXXVI). A solution of 10 millimoles hydrazone of benzaldehyde XXXVIII or benzophenone XXXIX in 50-100 ml ethylacetate at room temperature was added with stirring to a solution of 1.48 g (10 millimoles) phthalic anhydride in 80 ml ethylacetate. After 2 h, the residue was filtered and recrystallized from benzene.

Diphenylmethylenehydrazide of Succinic Acid (XXXVII). A solution of 1.96 g (10 millimoles) hydrazone of benzophenone in 70 ml chloroform at room temperature was added to a solution of 1.00 g (10 millimoles) succinic anhydride in 80 ml chloroform. After 2 h, the residue was filtered and recrystallized from ethanol.

Hydrazone of p-Bromobenzophenone (XL) and Hydrazone of Bis(p-dimethylaminobenzophenone) (XLI). A mixture of 0.1 moles of the corresponding diarylketones (p-bromobenzophenone or p-dimethylaminobenzophenone) and 80 ml 70% hydrazine-hydrate was boiled for 4-5 h and then cooled. The residue was filtered and recrystallized from ethanol. Compound XL: m.p. 102-103°C, yield 71%; compound XLI: m.p. 146-148°C, yield 83%.

Using the familiar technique, we obtained hydrazones of benzaldehyde XXXVIII, m-nitrobenzaldehyde, p-nitrobenzaldehyde [20], p-nitroacetophenone [24], benzophenone XXXIX [20], benzyl [14], 3-hydrazone of isatin XLIII [23], and also azines of benzaldehyde and benzophenone XLIV, XLV [20].

Hydrobromide of Benzophenone Hydrazone (XLIII). Hydrogen bromide was passed through a solution of 9.8 g (50 millimoles) benzophenone hydrazone in 200 ml benzene at room temperature for 10 min. The residue was filtered and recrystallized from methanol. Obtained: 11.0 g

TABLE 4. Acute Toxicity and Anticonvulsant Activity of Synthesized Compounds

Compound	Acute toxicity LD_{50}, mg/kg	Anticonvulsant effect, maximum electric shock test	
		ED_{50}, mg/kg*	nominal range of action LD_{50}/ED_{50}, arb.units
I	870(674—1122)	Inactive	—
IV	500(409—610)	Inactive	—
VI	440(317—612)	210(174—254)**	2,1
VII	340(261—442)	Inactive	—
VIII	290(243—345)	»	—
IX	140(170—182)	»	
X	411(284—594)	»	—
XI	2900(2265—3712)	»	—
XIV	2010(1887—2141)***	4	8
XX	420(406—434)***	»	—
XXI	770(746—794)***	»	—
XXII	645(600—693)***	4	8
XXIV	458(330—595)	»	—
XXV	792(625—1070)	»	—
XXVI	900(790—1026)	490(376—637)**	1,8
XXVII	388(193—781)	Inactive	—
XXXI	620(559—688)***	»	—
XXXV	About 300	»	—
XXXVI	355(290—420)	283(145—552)	1,3
XXXVII	About 250	Inactive	—
XXXVIII	136(92—202)	172(99—298)	0,8
XXXIX	134(103—172)	175(130—234)	0,8
XL	283(212—377)	Inactive	—
XLI	1086(791—1490)	Inactive	—
XLII	123(77—197)	»	—
XLIII	>3000	420(365—483)[4]*	>7,1
XLIV	>600	Inactive	—
XLV	1241(548—2336)	»	—
Hexamidine	340(288—401)	90(79—103)[5]*	3,8

*All the compounds (except XXXV, XLIII-XLV, doses 30 mg/kg) were tested at doses up to 600 mg/kg. **Peak effect, 120 min. ***For intravenous injection. [4]**Peak effect, 60 min. [5]**Peak effect, 240 min.

(80%) compound XLII with m.p. 203-205°C (decomp.). IR spectrum: ν, cm^{-1} (crystals): 3210 ($^{+}NH_3$), 1610, 1565-1599 (C=N, C_6H_5).

EXPERIMENTAL (BIOLOGICAL)

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

The acute toxcity LD_{50} of the compounds obtained was determined by the method of G. N. Pershin [12] with intraperitoneal or intravenous injection into white mice of mass 16-25 g in the form of a suspension in 2% starch slime or in the form of an aqueous solution. Statistical treatment of the experimental data was done according to the Litchfield and Wilcoxon method [1] for p = 0.05.

The antimicrobial activity of the compounds with respect to the reference strains of Escherichia coli E. coli M_{17} and golden staphylococcus S. aureus P-209 were determined by the standard method of two-fold series dilutions in meat peptone broth [12] for bacterial load of 250 thousand microbial units per ml solution. As the effective dose, we took the minimal inhibitory concentration (MIC) of the compound: the maximum dilution leading to complete suppression of devleopment of the test microbes. We compared the antimicrobial activity of the compounds obtained (Table 3) with the activity of an antimicrobial drug used in medicine, ethacridine lactate [7].

The anticonvulsant activity of the compounds was determined using the maximum electric shock test [13] on white mice of mass 18-24 g with intraperitoneal injection of the compared in 2% starch slime. We compared the effect with hexamidine [7] (Table 4).

TABLE 5. Anti-Inflammatory Activity of Synthesized Compounds

Compound	Dose, mg/kg	Equitoxic dose (dose/LD_{50}), arb. units	Anti-inflammatary effect	
			average increase in rat paw volume, % of original	retardation of exudation, % of control
II	50	—	120,4	+13,8
III	50	—	207,4	No retardation
V	50	—	171,8	Same
VII	50	0,15	90,3	+14,0
XXXVII	50	0,20	85,4	+24,2
XLII	50	0,41	67,5	+40,1
Control — 2% starch slime	—	—	112,7 (139,7)	—
Mefenamic acid	50	0,33	46,0	+59,2

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 160-200 g. We assessed the anti-inflammatory action from the degree of retardation of exudation (in % relatie to the control) upon intraperitoneal injection of the compounds in the form of a suspension in 2% starch slime in a dose of 50 mg/kg; we compared the effect with mefenamic acid [7, 16] (Table 5).

The acute toxicity of the tested compounds lies in the range from 123 mg/kg (compound XLII) to more than 3000 mg/kg (hydrazone XLIII). According to the classification presented in the monograph by S. Franke [17], sixteen compounds (IV, VI-X, XX, XXIV, XXVII, XXXV-XL, XLII) can be classified as moderately toxic, and the rest can be classified as low-toxicity. Moreover, many of the compounds obtained are much less toxic than the reference drugs for the studied biological activity: ethacridine lactate (LD_{50}, 70.0 mg/kg), hexamidine (LD_{50}, 340 mg/kg), or mefenamic acid (LD_{50}, 150 mg/kg [16]). In a number of substituted methylenehydrazides of maleic acid, the toxicity gradually increases from benzylidenehydrazide I through α-methylbenzylidenehydrazide IV to diarylmethylenehydrazides VI-X, which is promoted by introduction of a second substituent (alkyl or aryl) in the α-position of the benzylidene moiety (see Table 4). The appearance of substituents on one of the benzene rings of diarylmethylenehydrazides of maleic acid also leads to an increase in the toxic properties in the order $H < o\text{-}CH_3 < n\text{-}CH_3 < n\text{-}CH_3O$, but the presence of a halogen atom (bromine) slightly reduces the toxicity. The electron-donor dimethylamino group, introduced in the para position of both benzene rings, promotes a sharp decrease in toxicity. Among the esters of diphenylmethylenehydrazide of maleic acid XXVI, XXVII, the toxicity increase from the methyl to ethyl ester. Pronounced and numerically close toxic properties are inherent to arylidenehydrazides of phthalic and succinic acids XXXV-XXXVII; upon going to them from methylenehydrazides of maleic acid, we do not observe a decrease in toxicity.

In a number of model hydrazones of benzaldehyde and diarylketones XXXVIII-XLI, which represent, as we suggest, the pharmacophore unit of the molecules of aryl-substituted methylenehydrazides of maleic, phthalic, and succinic acids, an analogous regularity is not noted, possibly to some extent due to the small number of compounds studied. However, the low toxicity of the hydrazone of bis(p-dimethylaminobenzophenone) XLI compared with other studied hydrazones allows us to suggest the exisence of a close correlation.

The toxicity of hydrazones of benzaldehyde XXXVIII, benzophenone XXXIX, and the hydrobromide of the hydrazone of benzophenone XLII has practically identically high values. This is probably due to the presence of the toxic hydrazine (LD_{50} 62 mg/kg [5]) in the equilibrium system

$$2C_6H_5C(R)=NNH_2 \underset{\text{incl. } [H^+]}{\rightleftharpoons} C_6H_5C(R)=N-N= \\ =C(R)C_6H_5+N_2H_4 \ (R=H, C_6H_5).$$

The azines of benzaldehyde and benzophenone XLIV, XLV, in constrast to their hydrazones, are much less toxic (see Table 4). Thus substitution of a single hydrogen atom of the primary amino group of the hydrazones by an acyl moiety with formation of maleyl-, phthaloyl- and succinoylhydrazones leads to a decrease in toxicity. Substitution of both hydrogen atoms in formation of the azines promotes an even greater reduction in the toxic properties.

As a result of study of the antimicrobial activity of the synthesized compounds (see Table 3), we established that most of them have a bacteriostatic effect for a MIC from 15.6 to 1000 μg/ml. The most active with rspect to the golden staphylococcus strain proved to be the acid IX and the ethyl ester XXVII; the salts of methylenehydrazides of maleic acid display weak antimicrobial action or are inactive compared with the corresponding acids or esters.

Among the 28 investigated compounds, anticonvulsant action is observed in six substances with different structures (see Table 4). In a number of diarylmethylenehydrazides of maleic acid, a weak effect was displayed by the acid VI and its methyl ester XXVI; both substances display less activity and a narrower range of action than hexamidine.

Introduction of substituents into the benzene ring, substitution of one of them by a hydrogen atom or a methyl group, and also lengthening of the alkyl chain of the ester moiety (the ethyl group) leads to disappearance of the effect. Upon substitution of the maleyl moiety of the diphenylmethylenehydrazide of maleic acid VI by phthaloyl, the activity is slightly reduced; the range of action in this case decreased by a factor of 1.6. Transition to a succinoyl fragment leads to loss of activity.

The anticonvulsant effect of hydrazones of benzaldehyde XXXVIII and benzophenone XXXIX is observed on the background of a general toxic effect, probably due to the presence of equilibrium toxic hydrazine. Moreover, the diphenylmethylenehydrazine moiety $(C_6H_5)_2C{=}NNH$ can be considered as a pharmacophore group within the corresponding maleyl- and phthaloyl-hydrazides, since the latter display anticonvulsant action. The benzylidenehydrazine unit $C_6H_5CH{=}N{-}NH$ does not impart anticonvulsant activity to the compounds containing the residues of these dicarboxylic acids. As in the case of diarylmethylenehydrazides of maleic acid, introduction of substituents onto one of the benzene rings of the hydrazone of benzophenone leads to loss of activity. Formation of a salt with hydrogen bromide analogously affects the activity. It is interesting that the low-toxicity 3-hydrazone of isatin XLIII has a significant range of anticonvulsant action. Probably the 3-ylidene-2-indolinone moiety also is a pharmacophore group for anticonvuslants, which is confirmed by Popp's investigatiosn [25, 26].

Some of the investigated compounds displayed weak anti-inflammatory activity, less than observed in mefenamic acid (see Table 5). From preliminary data, a number of salts of diaryl-methylenehydrazides of maleic acid display anti-inflammatory action in agar tests.

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