105° and maintained at this point for 15 hr. The residue, left after distilling the solvent under reduced pressure, was stirred well with ice water and the unreacted starting materials (1.9 g) were filtered off rapidly. The filtrate was adjusted to pH 8.5 (cooling) and the product was extracted with chloroform, washed with water, and dried (Na_2SO_4). Filtration and removal of the chloroform left a thick oil which solidified on rubbing with petroleum ether (bp 30–60°). Recrystallizations from chloroform–hexane (Norit) yielded 1.99 g (29%) of V as white crystals, mp 106–108°. Two additional recrystallizations from chloroform–petroleum ether (bp 30–60°) gave an analytical sample of V as white, needle-shaped crystals: mp 107–109°; infrared ($CHCl_3$), 2.96 and 3.10 (NH), and 5.83 μ (C=O); ultraviolet maximum (95% ethanol), 2.04 mμ (log ϵ 4.43), showing a bathochromic shift (10^{-3} *N* alcoholic KOH) to 2.37 mμ (log ϵ 3.28).

Anal. Calcd for $C_{15}H_{20}N_2O_2$: C, 69.20; H, 7.74; N, 10.76; mol wt, 260.3. Found: C, 69.06; H, 7.82; N, 10.69; mol wt, 261.8.

Antiviral Compounds. XIII. Aminoacethydrazones of Aromatic α-Ketoaldehydes

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In a previous paper we described the synthesis of water-soluble derivatives obtained by condensing α-ketoaldehydes and Girard reagent.[1] Since several aromatic α-ketoaldehydes have been shown to exhibit antiviral activity,[2–4] we have prepared a series of new phenylglyoxal N,N-disubstituted aminoacethydrazones in order to study their antiviral activity (Table I).

All compounds were tested on embryonated eggs infected with vaccinia virus and A-PR8 virus. They were found inactive against vaccinia virus; the phenylglyoxal derivatives were also inactive against A-PR8 virus. Some derivatives of biphenylglyoxal (**4** and **5**) of *p*-phenylthiophenylglyoxal (**9**), and all derivatives of *p*-phenoxyphenylglyoxal exhibited virucidal activity against A-PR8 virus.

No activity was observed up to a concentration of 50 μg/ml when the compounds were tested for bacteriostatic activity on the following microorganisms: *Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Bacillus subtilis, Staphylococcus aureus, Streptococcus pyogenes, Mycobacterium tuberculosis, Trychophyton*, and *Candida*.

The compounds were also screened for acute toxicity in mice, for smooth muscle relaxing activity, for effects on blood pressure and respiration, for coronary vasodilatation, and for antiarrhythmic, antitussive, anticonvulsant, and antiinflammatory activity. A low anticonvulsant activity was shown by **3–6**; compounds **4**, **8–10**, and **13** exhibited antiinflammatory activity on formalin edema, but were ineffective as analgesics. The data on acute toxicity, antiviral, anticonvulsant, and antiinflammatory activities are summarized in Table II.

Experimental Section[5]

The α-ketoaldehydes were prepared from the corresponding α,α-dichloroacetophenones.[6]

N-Pyrrolidinoacethydrazide.—A mixture of ethyl N-pyrrolidinoacetate (15.7 g, 0.1 mole), hydrazine hydrate (5 g, 0.1 mole), and 20 ml of ethanol was refluxed for 4 hr. The solvent was evaporated and the crude oil was distilled at 105–110° (0.2 mm), yield 11 g (77%).

Anal. Calcd for $C_6H_{13}N_3O$: N, 29.35. Found: N, 29.49.

Treatment of an ethanol solution of the free base with HCl gave the hydrochloride salt, which was recrystallized from ethanol, mp 203–205° dec.

Anal. Calcd for $C_6H_{13}N_3O \cdot 2HCl$: C, 33.34; H, 6.99; Cl, 32.82; N, 19.44. Found: C, 33.11; H, 6.96; Cl, 32.34; N, 19.63.

The N-piperidino-, N-morpholino-, and N-diethylaminoacethydrazides were prepared by the same procedure.[7]

Phenylglyoxal N,N-Disubstituted Aminoacethydrazones. General Procedure.—A mixture of the α-ketoaldehyde (0.01 mole) and the corresponding N,N-disubstituted aminoacethydrazide (0.01 mole) in 10 ml of methanol or ethanol was stirred at 20° for 8 hr. After cooling, the products were filtered and recrystallized. The yields, melting points, solvents of crystallization, and analytical data are summarized in Table I.

Biological Testing. Maximal Tolerated Dose (MTD) in the Embryonated Egg.—The compounds were dissolved in saline solution buffered at pH 7.2, containing 500 IU of penicillin G and 0.5 mg of streptomycin/ml. Descending doses of each compound dissolved in 0.1 ml were inoculated into the allantoic sac. Each dose was injected in three embryonated 9-day old eggs. The highest dose which did not provoke mortality within 3 days was defined as the MTD.

Antiviral Methods.—Embryonated 9-day-old leghorn hen eggs and influenza A virus [allantoic fluid containing 10^8–10^9 EID_{50} (median egg-infecting dose) of egg-adapted PR-8 strain] were used. Vaccinia mouse neurotropic virus [(ATCC) CAM (chorioallantoic membrane) homogenized and purified by centrifugation containing 10^6–10^7 ELD_{50} (egg lethal dose) of egg-adapted WR strain] was used.

A. Virucidal Tests.—For each dose, 0.5 MTD dissolved in 10 ml of buffered saline solution was added to 10^2, 10^3, or 10^4 EID_{95} and the three solutions were kept in water baths at 37° for 1 hr. Then the allantoic sacs of 5 eggs (for each dose) were inoculated with 0.1 ml of one of the incubated solutions.

B. Virustatic Tests.—For each dose, the allantoic sacs of five eggs were inoculated with 0.1 ml of allantoic fluid containing either 1, 10, or 100 EID_{95} of virus, and the eggs were stored 1 hr at 37°. Then the allantoic sac was inoculated with 0.1 ml of buffered saline solution containing 0.5 MTD of each compound.

C. Evaluation of the Activity.—For influenza virus, the eggs were stored at 35° for 48 hr, then at 4° for 12 hr, and finally tested for the presence of hemoagglutinin. For vaccinia virus, the eggs were stored at 37° for 7 days and the mortality of chick embryos was recorded.

Antimicrobial and Antifungal Methods.—The compounds were diluted in 1:2 ratios in Difco nutrient agar inoculated with *E. coli* 100, *P. aeruginosa* H2, *P. vulgaris*, *B. subtilis*, *S. aureus* S.G. 511, and in Difco brain heart infusion agar inoculated with *Str. pyogenes humanus* A88, and the results were read after incubation for 18 hr at 35–37°. By the same procedure the compounds were tested in Kirchner–Hermann medium + 10% beef serum inoculated with *M. tuberculosis* 37 Ra, and the results were read after incubation for 10, 17, and 24 days at 35–37°. The compounds were tested also in Sabouraud broth inoculated with *Trichophyton mentagrophytes* 1236 (the results were read after incubation for 4 days at 26°), and with *Candida albicans* 28 (the results were read after incubation for 18 hr at 35–37°).

Pharmacological Methods.—For all tests NMRI albino mice and Wistar albino rats were used. The *acute toxicity* of each compound was determined by administering it intraperitoneally to mice in descending doses. Mortality was recorded over 24 hr and indicative LD_{50} values were estimated.

Smooth muscle relaxing activity was tested *in vitro* by Magnus' method[8] on the small intestine of a guinea pig stimulated by

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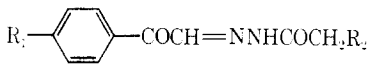
TABLE I



No.	R_1	R_2	Yield, %	Mp, °C	Solvent of crystn[a]	Formula	Calcd, % C	Calcd, % H	Calcd, % N	Calcd, % Cl	Found, % C	Found, % H	Found, % N	Found, % Cl
1	H	N-Pyrrolidino	40	212–213	E	$C_{13}H_{17}N_3O_2 \cdot HCl$	56.85	6.13	14.21	11.99	57.09	6.22	14.27	11.83
2	H	N-Piperidino	50	214–215	E–Et	$C_{14}H_{19}N_3O_2 \cdot HCl$	58.15	6.50	13.56	11.41	58.15	6.65	13.30	11.63
3	H	N-Morpholino	72	138	I	$C_{13}H_{17}N_3O_3$	61.08	6.22	15.26		61.17	6.15	14.95	
4	C_6H_5	N-Diethylamino	60	130	E	$C_{21}H_{25}N_3O_2$	71.19	6.87	12.45		71.12	6.50	12.21	
5	C_6H_5	N-Pyrrolidino	71	159–160	E–M	$C_{20}H_{21}N_3O_2$	71.62	6.31	12.53		71.57	6.40	12.83	
6	C_6H_5	N-Piperidino	71	170–171	E	$C_{21}H_{23}N_3O_2$	72.18	6.63	12.03		71.97	6.77	11.93	
7	C_6H_5	N-Morpholino	69	194	M	$C_{20}H_{21}N_3O_3$	68.36	6.02	11.96		68.64	6.20	11.75	
8	C_6H_5S	N-Pyrrolidino	68	140	E	$C_{20}H_{21}N_3O_2S$	65.38	5.76	11.45	8.69[b]	65.35	6.04	11.58	8.78
9	C_6H_5S	N-Piperidino	68	161	E	$C_{21}H_{23}N_3O_2S$	66.12	6.08	11.03	8.38[b]	65.99	6.15	10.90	8.37
10	C_6H_5S	N-Morpholino	70	144	M	$C_{20}H_{21}N_3O_3S$	62.65	5.52	10.96	8.36[b]	62.87	5.49	10.68	8.27
11	C_6H_5O	N-Pyrrolidino	65	128	E	$C_{20}H_{21}N_3O_3$	68.36	6.02	11.96		68.78	6.29	12.30	
				189	E	$C_{20}H_{21}N_3O_3 \cdot HCl$	61.93	5.71	10.83	9.14	61.90	5.62	10.93	9.13
12	C_6H_5O	N-Piperidino	45	130	E	$C_{21}H_{23}N_3O_3$	69.02	6.34	11.50		68.86	6.35	11.73	
				170–171	M–Et	$C_{21}H_{23}N_3O_3 \cdot HCl$	62.75	6.02	10.45	8.82	62.63	6.31	10.55	9.05
13	C_6H_5O	N-Morpholino	60	134	E	$C_{20}H_{21}N_3O_4$	65.38	5.76	11.11		65.35	5.72	11.72	
				208–209	E	$C_{20}H_{21}N_3O_4 \cdot HCl$	59.17	5.46	10.11	8.78	59.72	5.65	10.18	8.82

[a] E = ethanol, Et = ethyl ether, M = methanol. [b] Analysis for sulfur.

TABLE II

EMBRYONATED EGGS

No.	MTD,[a] moles/egg	A-PR8 virus[b] Virucidal activity	A-PR8 virus[b] Virustatic activity	LD_{50}, mg/kg ip	Anticonvulsant activity,[c,d] mg/kg ip	Anticonvulsant activity,[c,d] mg/kg Orally	Antiinflammatory activity,[d] mg/kg
1	10	0	0	200[e]	0	0	0
2	20	0	0	150[e]	0	0	0
3	>10	0	0	1000	200	0	0
4	20	1	0	3000	300	300	100
5	10	1	0	2000	0	300	0
6	20	0	0	3000	0	300	0
7	10	0	0	2000	0	0	0
8	20	0	0	3000	0	0	100
9	20	>2	0	>3000	0	0	100
10	20	0	0	>3000	0	0	100
11	25	>2	0	150[e]	0	0	0
12	2.5	1	0	200[e]	0	0	0
13	20	>2	0	600[e]	0	0	100

[a] Maximal tolerated dose. [b] The numbers represent the difference between log EID_{95} of control and log EID_{50} of treated. [c] Dose protecting 70% of animals. [e] The hydrochloride salt was used. [d] 0 = no effect.

0.025 μg/ml of histamine dihydrochloride, on the small intestine of a mouse stimulated by 0.15 μg/ml of acetylcholine, and on the seminal vesicle of a rat stimulated by 1.8 μg/ml of epinephrine hydrochloride according to Leitch, *et al.*[9]

Effects on Blood Pressure and on Respiration.—The compounds were tested intravenously on rats anesthetized with urethan. Blood pressure was recorded by means of a mercury manometer connected to a cannulated carotid artery; the pneumotachogram was recorded from the cannulated trachea.

Antiarrhythmic Activity.—All compounds were tested intravenously on rats anesthetized with pentobarbital sodium, and their ability to prevent cardiac arrhythmias induced by $CaCl_2$ was determined.

Coronary vasodilator activity was determined by perfusing isolated rabbit heart by Langendorff's method as modified by Setnikar.[10] Antitussive activity was tested administering the substance to mice in which cough was provoked by inhalation of nebulized H_2SO_4. For anticonvulsant activity, the compounds were given orally and intraperitoneally to groups of 10 mice and, after 60 and 30 min, respectively, the animals were subjected to electroshock. To evaluate antiinflammatory activity, the compounds were injected subcutaneously and their effects on rat's paw edema induced by local injection of a 3% formalin solution and measured by Courvoisier and Duerots's method[11] was tested. Active compounds provoked a statistically significant diminution of edema over 3 hr and were then tested also by the Randall and Selitto's method.[12]

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Potential Antimicrobial Agents. Bromo Compounds of Eugenol

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The knowledge that the bactericidal and antiseptic activity of phenols is enhanced by the introduction of halogen atoms[2] prompted us to prepare some new bromo compounds of eugenol (I).

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