Pyrimidine Derivatives VIII

5-Arylazopyrimidines and Their Inhibitory Effects Against Transplantable Mouse Tumors

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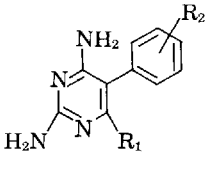
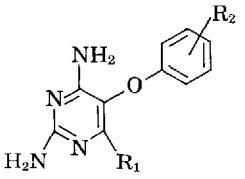
Twenty-five substituted 5-arylazopyrimidine derivatives have been prepared as potential "small molecule" antagonists of folic acid *via* reaction of 5-unsubstituted pyrimidines with appropriate diazotized aniline derivatives. The antineoplastic activity of 19 of these compounds has been examined *in vivo* by means of a primary screening program utilizing five transplantable mouse tumors. At nontoxic dosages, several of these agents showed effective tumor growth inhibition, as evidenced by reduction in mean tumor size. Histopathological examination of mice treated with 2,4-diamino-6-hydroxy-5-phenylazopyrimidine revealed intestinal irritation and evidence of nephrotoxicity, as well as incomplete absorption of the drug.

THE EFFECTIVENESS of aminopterin (4-aminopteroylglutamic acid) against acute leukemia in children (1) has led to the synthesis of a variety of folic acid antagonists as potential tumor inhibitory substances. Included among these are the so-called "small molecule" antifolics (*i.e.*, compounds less closely related to the structure of folic acid but retaining antifolic activity),[1] which are represented by the following examples: the 2,4-diamino-5-arylpyrimidines (I) (2), the 2,4-diamino-5-aryloxypyrimidines (II) (3), the 2,4-diamino-5-benzylpyrimidines (III) (4), and the 2,4-diamino-5-aryl-*as*-triazines (IV) (5), all synthesized by Hitchings and his collaborators, and the 4,6-diamino-1-aryl-1,2-dihydro-*s*-triazines (V) (6), prepared in these laboratories. These antimetabolites all approximate the 2,4-diamino-5-arylpyrimidine structure. A more complete comparison of these "small molecule" antifolic structures has already appeared elsewhere (7). Various substituted 5-arylazopyrimidines (VI), which structurally resemble the aforementioned compounds, have been prepared by us for evaluation as potential antitumor agents. Preliminary communications on the synthesis (8) and microbiological activity (9) of these compounds have appeared; the latter report was published simultaneously, by mutual arrangement, with that of Timmis and co-workers (10), whose independent studies paralleled our own. At about the same time, Tanaka and his colleagues also described the biological activity of some 5-arylazopyrimidines (11), and various reports have since appeared concerning the antifolic and tumor inhibitory activity of this type of compound (12–15). The authors now describe the synthesis of some hitherto unreported derivatives of this series and summarize their experimental antitumor properties.



I



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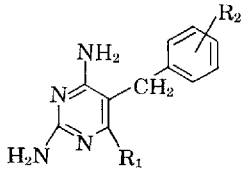

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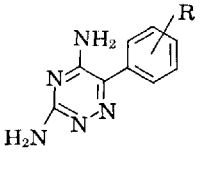

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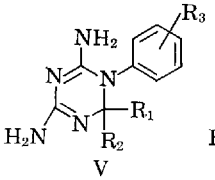

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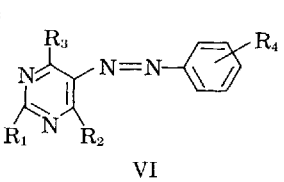

VI

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Previous paper: Israel, M., Protopapa, H. K., and Modest, E. J., *J. Pharm. Sci.*, **54**, 1626(1965).

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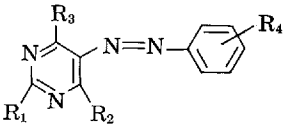
[1] For a general discussion of folic acid antagonists see Jukes, T. H., and Broquist, H. P., in "Metabolic Inhibitors," vol. I, Hochster, R. M., and Quastel, J. H., eds., Academic Press Inc., New York, N. Y., 1963, pp. 501–529.

DISCUSSION

The 5-arylazopyrimidines prepared during this investigation (Tables I and II) were obtained by reaction of a 5-unsubstituted pyrimidine with an appropriate diazotized aniline derivative. Compounds *1–14* represent a series of derivatives of 2,4-diamino-6-hydroxypyrimidine with modification in the arylazo substituent (R_4). In this series, the coupling reaction was accomplished at pH 5.0–5.5 in 10% sodium acetate, following essentially the conditions of Benson, Hartzel, and Savell (16); the yields varied from 50–100%. With other pyrimidines, no general pH optimum was found for diazo coupling. Some pyrimidines (*e.g.*, 2,4-diamino-6-chloropyrimidine) coupled best at a slightly acid pH; others (*e.g.*, 2,4-diamino-6-mercaptopyrimidine) required an alkaline pH for coupling. In some instances (*e.g.*, 2,4-diaminopyrimidine), coupling did not occur at any pH tried. In two instances (compounds *19* and *21*), the coupling reaction was most efficient when the pH was kept constant during the entire course of the reaction by means of a buffered system. It appears, therefore, that optimal conditions for diazo coupling represent a compromise between the solubility of the pyrimidine and its relative reactivity toward electrophilic substitution, on the one hand, and the concentration of the diazonium salt and its rate of decomposition on the other.

The crude products were purified initially by thorough washing with alcohol, water, and ether, and then, if they contained hydroxy or mercapto substituents, by precipitation from alkaline solution by the addition of dilute acid; material purified to this stage was sufficiently pure for biological evaluation. Analytically pure samples were obtained by one of three general methods: sublimation, recrystallization, or reprecipitation. The method of choice, wherever applicable, was high vacuum sublimation; this technique afforded analytically pure, anhydrous material directly. Second choice was recrystallization. Arylazopyrimidines are, in general, insoluble in most common organic solvents. However, a limited few were crystallizable from hydrophilic solvents such as ethanol or 2-ethoxyethanol. The third choice was repeated precipitation either from basic solution by the addition of acid or from acetone solution by the addition of water. This procedure usually returned hydrates of variable composition which retained

TABLE I.—5-ARYLAZOPYRIMIDINES (VI)



Compd.	R_1	R_2	R_3	R_4	Method of Prepn.[a]	Yield, %[b]	Purification[c]	M.p., °C.	Color
1[d,e]	NH_2	OH	NH_2	...	*A*	90	S (250–275)	>300	Yellow
2	NH_2	OH	NH_2	3′—Cl	*A*	95	A	>300	Yellow-orange
3[e]	NH_2	OH	NH_2	4′—Cl	*A*	65	C	>300	Yellow
4	NH_2	OH	NH_2	3′,4′—Cl_2	*A*	80	S (280–300)	273–274	Yellow
5	NH_2	OH	NH_2	2′—Br	*A*	85	P	>300	Orange
6[e]	NH_2	OH	NH_2	4′—NO_2	*A*	88	P	>300	Red
7[f,g]	NH_2	OH	NH_2	4′—SO_3Na	*E*	95	W[h]	>300	Yellow
8	NH_2	OH	NH_2	2′—CH_3	*A*	60	S (285)	>300	Yellow
9	NH_2	OH	NH_2	4′—CH_3	*A*	80	S (300)	>300	Yellow
10	NH_2	OH	NH_2	2′,6′—$(CH_3)_2$	*A*	86	A	257–258	Yellow-orange
11	NH_2	OH	NH_2	2′—OCH_3	*A*, *C*	65	S (290)	>300	Yellow
12[e]	NH_2	OH	NH_2	4′—OCH_3	*A*, *C*	85	P	>300	Yellow
13	NH_2	OH	NH_2	2′,3′—Benzo	*C*	80	P	>300	Red-orange
14	NH_2	OH	NH_2	3′,4′—Benzo	*C*	70	P	>300	Red-orange
15[i]	NH_2	Cl	NH_2	...	*D*	72	A	242–243	Yellow
16[j]	NH_2	Cl	NH_2	4′—Cl	*D*	68	C-W	271–272	Yellow
17[j]	NH_2	NH_2	NH_2	...	*A*, *D*	90	C-W	262–263	Yellow
18[k]	NH_2	NH_2	NH_2	4′—Cl	*A*, *D*	87	C-W	253.5–255	Yellow
19[i]	NH_2	SH	NH_2	...	*F*	67	E	257–259 dec.	Pale orange
20	NH_2	SH	NH_2	4′—Cl	*B*	65	P	>300	Red
21[l]	NH_2	SCH_3	NH_2	...	*G*	39	A	180.5–182	Yellow
22[e]	OH	NH_2	NH_2	...	*B*	25	P	>300	Pale yellow
23	SCH_3	NH_2	NH_2	...	*A*	73	S (245)	235 dec.	Yellow
24	OH	NH_2	OH	...	*A*	52	P	>300	Yellow
25	OH	OH	OH	...	*A*	63	A	288–289	Yellow

[a] See under *Experimental*. [b] Percentage yield figures for compounds *1–14*, *19*, *20*, *22*, *24*, and *25* are based upon material which had been once purified by precipitation at pH 4–5 from an alkaline solution; yields for the remaining compounds are based on product purified according to the method indicated under *Purification*. [c] Purification procedure used to obtain the analytical sample. Crystallization solvents: A, 95% ethanol; C, cellosolve (2-ethoxyethanol); C-W, cellosolve–water; E, ether; W, water; S, sublimation in high vacuum (sublimation temperature in °C. in parentheses); P, precipitation at pH 4–5 from alkaline solution. [d] Previously reported as a hydrate (16). [e] Cited in Parker, R. P., and Webb, J. S., U. S. pat. 2,543,333 (Feb. 27, 1951); however, no analytical values are given. [f] The sesquihydrate is obtained on drying at 85° for 48 hr. *in vacuo*; drying at 140° for 72 hr. afforded the hemihydrate. [g] Free acid previously reported by Hartzel, L. W., and Benson, F. R., *J. Am. Chem. Soc.*, **76**, 2263(1954). [h] Containing 5% sodium chloride. [i] *Reference 19*. [j] Also prepared by Timmis *et al.* (10). [k] Reported (10) m.p. 262°. [l] *Reference 21*.

TABLE II.—ANALYTICAL DATA FOR NEW COMPOUNDS LISTED IN TABLE I

Compd.	Formula	Anal., % Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
1	$C_{10}H_{10}N_6O$	52.17	4.38	...	52.25	4.6	...
2	$C_{10}H_9ClN_6O$	45.38	3.43	31.76	45.70	3.38	31.56
3	$C_{10}H_9ClN_6O$	45.38	3.43	31.76	45.71	3.59	31.80
4	$C_{10}H_8Cl_2N_6O$	40.15	2.70	28.10	39.97	2.85	27.80
5	$C_{10}H_9BrN_6O$	38.85	2.93	...	39.38	2.62	...
6	$C_{10}H_9N_7O_3$	43.64	3.30	35.63	43.50	3.50	35.20
7[a]	$C_{10}H_9N_6NaO_4S.1.5\ H_2O$	33.43	3.37	9.12[b]	33.39	3.43	9.27[b]
8	$C_{11}H_{12}N_6O$	54.09	4.95	34.41	53.89	5.20	34.20
9	$C_{11}H_{12}N_6O$	54.09	4.95	...	53.86	5.10	...
10	$C_{12}H_{14}N_6O$	55.80	5.46	32.54	55.83	5.62	32.75
11	$C_{11}H_{12}N_6O_2$	50.76	4.65	32.30	50.31	4.78	32.40
12	$C_{11}H_{12}N_6O_2$	50.76	4.65	32.30	50.46	4.90	31.90
13	$C_{14}H_{12}N_6O$	59.99	4.32	...	59.73	4.20	...
14	$C_{14}H_{12}N_6O$	59.99	4.32	...	59.55	4.50	...
18	$C_{10}H_{10}ClN_7$	45.54	3.82	37.19	45.56	3.99	36.88
20	$C_{10}H_9ClN_6S$	42.78	3.23	29.94	42.62	3.17	29.80
22	$C_{10}H_{10}N_6O$	52.17	4.38	...	51.70	4.09	...
23	$C_{11}H_{12}N_6S$	50.75	4.65	32.29	50.86	4.82	31.90
24	$C_{10}H_9N_5O_2$	51.94	3.92	...	51.72	4.21	...
25	$C_{10}H_8N_4O_3$	51.73	3.47	24.13	51.88	3.62	24.42

[a] Analysis for hemihydrate.—Calcd. for C, 35.19; H, 2.95. Found: C, 35.12; H, 3.15. [b] Per cent sulfur.

TABLE III.—INHIBITORY ACTIVITY OF NONTOXIC DOSAGES OF 5-ARYLAZOPYRIMIDINES AGAINST TRANSPLANTABLE MOUSE TUMORS[a]

Compd.	L1210 Dose	L1210 S, %	P1534 Dose	P1534 S, %	P1534 I, %	C1498 Dose	C1498 S, %	C1498 I, %	DBRB Dose	DBRB S, %	DBRB I, %	S180 Dose	S180 S, %	S180 I, %
1	80	+5	80	−8	+60 (*11*)[c]	20	+33[b]	+72 (*12*)[c]	80	+5	+60 (*8*)[c]	80	+11	+21 (*10*)
2	500	+5	500[d]	−29	+61 (*11*)[c]	125	+4	+45 (*12*)	125	−4	+38 (*13*)	500	−15	+53 (*12*)[c]
3	500	0	...	...	...	500	−11	+38 (*12*)	...	...	...	...	...	...
4	125	+15	...	...	...	125[d]	−27	+61 (*12*)[c]	...	...	...	...	...	...
7	125	−5	6.25	0	+52 (*11*)[c]	100	+3	+100 (*11*)[c]	25	−7	+9 (*11*)	6.25	+4	+14 (*7*)
8	125	+3	5	−14	+26 (*11*)	20	−1	+4 (*14*)	5	−3	−9 (*11*)	20	−4	+12 (*13*)
9	125	−2	31.25	−19	+59 (*11*)[c]	320	−9	+21 (*14*)	1000	+13	+72 (*8*)[c]	500[d]	−26	+17 (*13*)
10	500	−3	...	...	...	...	...	...	...	...	...	...	...	...
11	125	−5	31.25	−12	+1 (*11*)	7.8	−8	−5 (*10*)	7.8	+22[b]	−5 (*11*)	7.8	+12	+12 (*10*)
12	500	−11	...	...	...	125	−16	+32 (*12*)	...	...	...	125	−24	+47 (*17*)
13	31.25	+6	...	...	...	...	...	...	...	...	...	...	...	...
14	500	+3	...	...	...	31.25	+3	+50 (*12*)[c]	...	...	...	...	...	...
15	80	0	...	...	...	100	−22	+91 (*12*)[c]	...	...	...	...	...	...
16	125	+29[b]	...	...	...	125	−16	+60 (*14*)[c]	...	...	...	125	−24	+27 (*13*)
17	6.25	−3	8	−8	+32 (*9*)	8	+15	+11 (*12*)	0.5	−22	+8 (*8*)	8	+34[b]	+30 (*10*)
18	31.25	−3	31.25	−2	+34 (*9*)	31.25	−10	+23 (*14*)	31.25	−17	+76 (*8*)[c]	31.25	−3	+8 (*13*)
20	31.25	+3	7.8	−4	+34 (*11*)	31.25	−7	+41 (*12*)	31.25	+6	+15 (*11*)	7.8	−2	−5 (*10*)
23	500	+14	31.25	−2	+26 (*11*)	500	−10	+68 (*12*)[c]	500	+23[b]	+25 (*11*)	125	+17	+33 (*13*)
24	31.25	−2	125	−24	+11 (*13*)	125	−8	+85 (*14*)[c]	31.25	+23[b]	+19 (*15*)	...	...	...

[a] Transplantable mouse tumors employed: L1210 ascitic lymphatic leukemia in the BDF/1 hybrid; P1534 lymphatic leukemia in the DBA/2 inbred strain; C1498 myelogenous leukemia in the C57BL/6 inbred strain; DBRB mammary adenocarcinoma in the DBA/1 inbred strain; S180 spindle cell sarcoma in the CAF/F1 hybrid. Dosages are in mg./Kg./day. A suspension of the agent in 10% polysorbate 80 was administered i.p. once daily starting with the first post-tumor inoculation day and continuing until the death of the last animal. *S*, per cent change in mean survival of treated mice compared with controls; *I*, per cent change in mean tumor size of treated mice compared with controls. Numbers in parentheses indicate day of tumor measurement. [b] Significant survival increase ($S > +20\%$). [c] Significant tumor inhibition ($I > +50\%$). [d] Toxic dose ($S < -25\%$).

water tenaciously; extreme drying conditions were then necessary to obtain anhydrous material.

Nineteen of the 25 compounds reported here were examined for antineoplastic activity against transplantable mouse tumors following the standard assay procedures employed at this foundation (17). (Table III.) All 19 were evaluated against the L1210 ascitic lymphatic leukemia in the BDF/1 mouse and against as many other primary screen tumors as availability of sample would permit. These include the following mouse tumors: P1534 lymphatic leukemia in the DBA/2 inbred strain, C1498 myelogenous leukemia in the C57BL/6 inbred strain, DBRB mammary carcinoma in the DBA/1 inbred strain, and S180 spindle cell sarcoma in the CAF/1 hybrid. The agent was administered intraperitoneally as a suspension in 10% polysorbate 80[2] once daily starting with the first post-tumor inoculation day; injections were continued until the death of the last experimental animal.

At nontoxic dosages,[3] only one agent (compound *16*) showed significant activity against the L1210 leukemia as evidenced by an increase in mean survival time. A number of agents effected moderate to marked tumor inhibition in the other assay systems, but few showed a concomitant increase in mean survival time. These data are summarized in Table III.

[2] Marketed as Tween 80 by Atlas Chemical Industries, Inc., Wilmington, Del.

[3] A dose level was regarded as toxic if the mean survival time of mice administered this dose was 25% or below the mean survival time of control mice.

Tanaka and his associates have claimed that positions 2, 4, and 6 of the pyrimidine ring of VI should all be substituted with amino groups for maximum activity against mouse tumors (14). However, the authors' results (Table III) indicate that, although the 2,4,6-triamino derivatives are active (compounds *17* and *18*), the 2,4-diamino-6-hydroxy derivatives (compounds *1*, *7*, and *9*) and the 2,4-diamino-6-chloro derivatives (compounds *15* and *16*) are more active. A more accurate but nevertheless tentative generalization might be that the 2,4-diamino-5-arylazopyrimidine configuration is required for experimental antitumor activity.

When compound *1* was administered at 20, 40, and 80 mg./Kg./day × 20 i.p. to mice bearing the P1534 leukemia, and compound *24* at 31.25, 62.5, and 125 mg./Kg./day × 12 i.p. to mice bearing the C1498 leukemia, gross inspection at autopsy performed 1 day later in these groups of mice revealed incomplete absorption of compound from abdominal cavities. In addition, compound *1* at the 2 higher doses, and compound *24* at all 3 doses produced intestinal irritation, as manifested by adhesions between surfaces of peritoneal viscera (liver, spleen, and kidney), and capsular thickening. Compound *24*, at the 2 higher dose levels, showed histological evidence of nephrotoxicity, with hyaline casts in dilated proximal and distal convoluted tubules, and degenerating renal epithelium.

In an acute oral toxicity experiment, compound *1* was administered to normal BAF/1 mice by gavage at single doses of 500, 750, and 1000 mg./Kg. The mice were sacrificed 4 days later and, on autopsy, the agent was still evident in the stomachs of the treated animals. The kidneys were pale and enlarged; histology indicated that pigment casts were present in both collecting and convoluted tubules. Additional casts of an amorphous, pink-staining material with dilatation of convoluted tubules pointed to nephrotoxicity.

As mentioned earlier, the compounds have also been examined for antimetabolic activity in microbiological systems and have been found to exhibit antifolic and antipurine properties (9). These results and a detailed analysis of the structure-activity correlations will be the subject of the next paper in this series.

EXPERIMENTAL[4]

Starting Materials.—The aromatic amines were Eastman Kodak white label grade and were used without purification, except for aniline, which was redistilled from zinc prior to use.

The pyrimidines were obtained as follows: (*a*) 2,4-diamino-6-hydroxypyrimidine, 2,4-diamino-6-chloropyrimidine,[5] 2,4-diamino-6-mercaptopyrimidine,[5] and 4-amino-2,6-dihydroxypyrimidine by procedures recently described (19); (*b*) 2,4,6-triaminopyrimidine by the method of Traube (20) from malononitrile and guanidine free base; (*c*) 2,4-diamino-6-methylthiopyrimidine by methods developed in these laboratories (21); (*d*) 4,6-diamino-2-hydroxypyrimidine by hydrolysis of 4,6-diamino-2-mercaptopyrimidine with chloroacetic acid following the procedure of Bendich, Tinker, and Brown (22); (*e*) 4,6-diamino-2-methylthiopyrimidine from Dougherty Chemical Co., Richmond Hill, N. Y., and barbituric acid from Matheson Coleman and Bell, East Rutherford, N. J.

Preparation of 5-Arylazopyrimidines.—The compounds listed in Table I were prepared by the following methods. Although many of the arylazopyrimidines can be made by more than one of the procedures indicated in Table I, the method specified for each compound is the optimal method found during this study. In particular, compounds *7* (*Method E*), *15* and *16* (*Method D*), *19* (*Method F*), and *21* (*Method G*) are best prepared by the specific procedures indicated. Analytical data for the new compounds prepared are given in Table II.

Method A.—This is essentially the method of Benson, Hartzel, and Savell (16). The diazotized aromatic amine in hydrochloric acid solution was added to a cold, stirred solution of an equimolar amount of the pyrimidine in 10% sodium acetate; the pH at the end of the reaction was 5–6. For alkali-soluble products, the work-up was modified to include an initial purification step: the crude product was dissolved in warm 1 *N* sodium hydroxide, the solution filtered free of tarry or insoluble impurities, and the product precipitated at pH 5 by the addition of acetic acid.

Method B.—This procedure differs from *Method A* in that the diazonium intermediate was added to a suspension of the pyrimidine (0.05 mole) in 450 ml. of 10% sodium acetate. The mixture was stirred for 1 hr. at room temperature and the product collected by suction filtration, washed, and dried.

Method C.—In this modification, 0.1 mole of the aromatic amine in 100 ml. of 6 *N* hydrochloric acid was diazotized and coupled with 2,4-diamino-6-hydroxypyrimidine (0.1 mole) dissolved in 500 ml. of 10% sodium carbonate.

Method D.—A diazotized amine solution, prepared in the usual manner and freed of excess nitrous acid by the addition of urea, was added to a cold, stirred solution of the pyrimidine in 3 *N* acetic acid. Immediately after admixture, solid sodium hydroxide was added to adjust the pH to 4; the pH was then brought to 5.5 by the addition of a large quantity of solid sodium acetate. The reaction mixture was stirred for at least 3 hr., the temperature gradually rising to room temperature. The product was collected, washed with water, and dried.

Method E.—A suspension of 0.2 mole of diazotized sulfanilic acid (23) was added at room temperature to a solution of 0.2 mole of 2,4-diamino-6-hydroxypyrimidine in 160 ml. of 1 *N* sodium hydroxide. A bright yellow precipitate formed instantaneously and the resulting suspension was stirred for 15 min. The final pH was 5.0. The product was collected, washed sparingly with cold water, and dried for 17 hr. at 60° *in vacuo*. Isolation of the sodium salt of compound *7* was accomplished by addition of an equal volume of absolute ethanol to a solution of 20 Gm. of the crude material in 600 ml. of 1% aqueous sodium bicarbonate.

[4] Melting points were taken by the capillary method at a rate of heating of 2°/min. in a modified Wagner-Meyer melting point apparatus (18) and are uncorrected. Drying of analytical samples was carried out at 70–100° for 17 hr. *in vacuo* over phosphorus pentoxide. Analyses were performed by Dr. Stephen Nagy and his associates, Microchemical Laboratories, Massachusetts Institute of Technology, Cambridge, Mass., and by the Scandinavian Microanalytical Laboratory, Herlev, Denmark.

[5] Larger quantities of this material were obtained through the courtesy of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Bethesda, Md., and were prepared by Aldrich Chemical Co., Milwaukee, Wis., according to the procedures outlined in *Reference 19*.

Method F.—The coupling reaction was achieved at a constant pH of 9.6 in an ammonium hydroxide–ammonium chloride buffer following the procedure recently described (19).

Method G.—Coupling was accomplished at constant pH of 5.0 in a citric acid–disodium phosphate buffer; this procedure is described in *Reference 21.*

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Effects of Pentobarbital, Acetylsalicylic Acid, and Reserpine on Blood Pressure and Survival of Rats Subjected to Experimental Stress

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Sodium pentobarbital, 20 mg./Kg. *per os* daily, and acetylsalicylic acid (ASA), 100 mg./Kg. *per os* daily, failed to prevent the development of hypertension in rats subjected to experimental stress. ASA enhanced the lethal effects of the stressors and potentiated the effects of the stress conditions on gastric mucosa. Reserpine phosphate, 0.1 mg./Kg. (base) i.p. daily, administered after the animals had been subjected to the stress conditions for 6 weeks, did lower the blood pressure to control levels.

THE DEVELOPMENT of hypertension in animals exposed to experimental stress has been reported by numerous investigators (1–5). Buckley *et al.* (5) found that reserpine phosphate, 0.1 mg./Kg. i.p., and chlorpromazine hydrochloride, 4 mg./Kg. i.p., administered 1 hr. prior to subjecting rats to a 4-hr. variable stress program not only failed to decrease the pressor effects induced by the stressors over a 27-week period but also appeared to potentiate the lethal effects of the stressors. This present study was undertaken to investigate the effects of pentobarbital and acetylsalicylic acid (ASA) on animals subjected to chronic variable stress programs, and the effects of reserpine phosphate[1] administered to the experimental animals after physiological effects of stress exposure were evident.

EXPERIMENTAL

The stress chambers utilized were semisoundproof rooms designed by the Industrial Acoustics Co., New York, N. Y. (5). The stress program consisted of (*a*) flashing 150-w. spotlights (installed in each corner of the cage) which were on for $^1/_4$ sec. and off for $^3/_4$ sec. (in alternate pairs); (*b*) audiogenic stimulation at 5-min. intervals for 0.5-min. periods produced by amplifying a tape recording of noxious sound so that the intensity was approximately 100 decibels at the center of the cage; and (*c*) oscillation at the rate of 140/min. A simple conditioned avoidance response program utilizing automatic pole climbing units (4) was also utilized in the stress program. The cycle was initiated every 2.75 min. and consisted of a low tone for 15 sec., followed by the delivery of an electric shock (3 ma.) to the grid

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[1] Kindly supplied by Dr. William E. Wagner, Ciba Pharmaceutical Co., Summit, N. J.