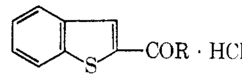
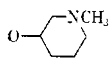
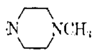
TABLE I
AMIDE AND ESTER HYDROCHLORIDES OF BENZO[b]THIOPHENE-2-CARBOXYLIC ACID



Compd	R	Purified yield, %[b]	Mp, °C	Formula	Carbon, % Calcd	Carbon, % Found	Hydrogen, % Calcd	Hydrogen, % Found	Nitrogen, % Calcd	Nitrogen, % Found
I	$OCH_2CH_2N(CH_3)_2$	45 (A)	187–188	$C_{13}H_{16}ClNO_2S$	54.60	54.43	5.66	5.91	4.91	5.19
II	$OCH_2CH_2N(C_2H_5)_2$	18 (B)	153–154	$C_{15}H_{20}ClNO_2S$	57.45	57.51	6.42	6.60	4.46	4.40
III	$OCH_2CH_2CH_2N(CH_3)_2$	65 (C)	200–201	$C_{14}H_{18}ClNO_2S$	56.10	55.76	6.05	6.30	4.67	4.70
IV	$OCH_2CH_2CH_2N(C_2H_5)_2$[a]	79 (D)	144–145	$C_{16}H_{22}ClNO_2S$	58.61	58.89	6.77	7.00	4.27	4.25
V		27 (B)	248–249	$C_{15}H_{18}ClNO_2S$	57.77	57.77	5.81	6.01	4.49	4.47
VI	$NHCH_2CH_2N(C_2H_5)_2$	43 (E)	151–152	$C_{15}H_{21}ClN_2OS$	57.58	57.65	6.77	6.77	8.95	8.72
VII		57 (F)	269–271	$C_{14}H_{17}ClN_2OS$	56.64	56.40	5.77	5.98	10.81	10.62

[a] Lit.[5] mp 144–145°. [b] Recrystallizing solvents: A, 2-propanol; B, propanol; C, absolute ethanol; D, chloroform-benzene; E, n-amyl alcohol; F, chloroform-ether.

TABLE II
PHARMACOLOGICAL SCREENING RESULTS

Compd	Local anesthetic potency duration, min[a]	HCl writhing[b] protected/tested
I	0	0/5
II	0	0/5
III	0[c]	0/5
IV	0[c]	1/5
V	5	0/5
VI	55	2/5
VII	0[c]	1/5
Lidocaine HCl	50	d

[a] 0.25 ml of 2% solution/conjunctival sac. [b] 50 mg/kg ip. [c] Produced moderate irritation. [d] ED_{50} for acetylsalicylic acid is 50 mg/kg: A. D. Rudzik and J. H. Mennear, *J. Pharm. Pharmacol.*, **17**, 326 (1965).

Compound VI, the most active, did not produce any apparent irritation, and had an LD_{50} of 170 mg/kg.[12]

The compounds were also evaluated for their potential analgetic activity by the HCl writhing[13] and the infrared hot bulb[14] tests. The HCl writhing test indicated none of the compounds to be of sufficient analgetic activity to antagonize the HCl response in greater than 50% of the test animals at a dose of 50 mg/kg. Since VI was the most active of the series, it was subjected to the infrared hot bulb test at a dose of 100 mg/kg and produced a 2.6 times increase over control; however, clonic convulsions were observed in four of the test animals. Morphine sulfate (5 mg/kg) produced a 6.8 times increase over controls, indicating it to be approximately 50 times more active than VI in this test. Pharmacological screening results are given in Table II.

Experimental Section[15]

Benzo[b]thiophene-2-carbonyl Chloride (III).—I (20 g, 0.113 mole) and 35 ml of $SOCl_2$ were heated gently for 2.5 hr. The $SOCl_2$ was azeotroped off with benzene and the resulting white solid was recrystallized from benzene–cyclohexane to give 18 g (81%) of white needles, mp 84–86°.[6]

Amides and Esters of I.—III (7.0 g, 0.036 mole) was added to 1 equiv of each of six amines or alcohols dissolved in 50 ml of dry benzene. Following the addition at room temperature, each flask became warm and white solids soon separated from flasks I–V. An oil separated from flask VI. Flasks I–V were then heated to a gentle reflux for 2 hr while flask VI was allowed to reflux overnight. The flasks were cooled and the solids collected. Physical constants of the compounds prepared are found in Table I.

1-(2-Benzo[b]thenoyl)-4-methylpiperazine Hydrochloride (VII).—III (3.0 g, 1.52 mmoles) was treated with a solution containing 140 ml of H_2O, 3 ml of N-methylpiperazine, and 11 ml of 10% NaOH. The mixture was stirred for 15 min, and the solid was collected, dried, converted to the hydrochloride salt, and recrystallized from $CHCl_3$–Et_2O to yield 2.24 g of white plates.

Acknowledgment.—We wish to acknowledge support of the U. S. Public Health Service by a fellowship, GM-24,364-02, to T. B., and by Grant GM-10366, to Indiana University. We are also indebted to the Human Health Research and Development Center, Dow Chemical Company, Indianapolis, Ind., for providing biological testing facilities.

(12) Intraperitoneally in mice.

(13) E. T. Eckhardt, F. Cheplovitz, M. Lipo, and W. M. Govier, *Proc. Soc. Exptl. Biol. Med.*, **98**, 186 (1954).

(14) F. N. Marshall, W. R. Jones, and L. C. Weaver, *ibid.*, **116**, 912 (1964).

(15) Melting points were taken on a Mel-Temp capillary melting point apparatus and are corrected. The microanalyses were performed by Midwest Microlabs, Inc., Indianapolis, Ind.

Synthesis and Preliminary Pharmacological Evaluation of a Series of N,N′-Arylidenebis(acid amides)[1]

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Synthesis and biological activity studies of N,N′-benzylidenebisnicotinamide in our laboratories revealed that this compound possessed actions reflecting CNS depression. Its marked sedative action and relative freedom from gross symptoms of toxicity gave impetus

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TABLE I

N,N′-ARYLIDENEBIS(ACID AMIDES)

$R_1CH(NHCOR_2)_2$

No.	R_1	R_2	Isolation procedure[a]	Mp, °C[b]	Lit. mp, °C[c]	% yield[d]	Recrystn[e] solvent	Formula	% calcd C	% calcd H	% calcd N	% found[f] C	% found[f] H	% found[f] N
1	C_6H_5	CH_3	A	259	250[i]	20.4	A	$C_{19}H_{18}N_2O_2$	64.04	6.85	13.59	64.40	6.86	14.38
2	C_6H_5	C_2H_5	A	242.5–243.5	220[j]	26.6	B	$C_{21}H_{22}N_2O_2$	66.62	7.75	11.96	66.78	7.76	11.71
3	C_6H_5	2-C_3H_7	A	237–237.5	...	13.7	B	$C_{25}H_{32}N_2O_2$	68.65	8.46	10.69	68.81	8.51	10.62
4	C_6H_5	C_5H_4N[g]	A	239	...	3.8	C	$C_{19}H_{16}N_4O_2$	68.64	4.86	16.87	69.03	5.02	17.19
5	C_6H_5	C_6H_5	A	241–242	225[k]	21.4	B	$C_{21}H_{18}N_2O_2$	76.33	5.50	8.48	76.00	5.50	8.77
6	$C_6H_5CH{=}CH$	CH_3	A	251	234[l]	3.4	C	$C_{23}H_{18}N_2O_2$	67.20	6.95	12.07	67.11	7.12	11.46
7	$C_6H_5CH{=}CH$	C_2H_5	A	236	220–221[m]	8.1	E	$C_{25}H_{26}N_2O_2$	69.18	7.75	10.77	68.84	7.53	10.81
8	$C_6H_5CH{=}CH$	2-C_3H_7	A	233.5–234	...	11.0	D	$C_{27}H_{30}N_2O_2$	70.78	8.39	9.72	70.81	8.23	9.60
9	$C_6H_5CH{=}CH$	C_5H_4N	B	240–240.5	...	8.6	B, C	$C_{25}H_{18}N_4O_2$	70.36	5.07	15.64	70.53	5.20	15.97
10	$C_6H_5CH{=}CH$	C_6H_5	C	250	250[n]	4.0	B	$C_{29}H_{26}N_2O_2$	77.49	5.66	7.87	77.37	5.35	8.00
11	4-$CH_3OC_6H_4$	CH_3	A	238	230–231[o]	15.4	E	$C_{21}H_{22}N_2O_4$	60.98	6.83	11.86	61.37	6.88	11.90
12	4-$CH_3OC_6H_4$	C_2H_5	A	239	228[p]	19.0	B	$C_{24}H_{28}N_2O_4$	63.59	7.63	10.61	63.50	7.72	10.58
13	4-$CH_3OC_6H_4$	2-C_3H_7	A	253	...	13.9	B	$C_{26}H_{34}N_2O_4$	65.71	8.28	9.59	65.84	8.52	10.21
14	4-$CH_3OC_6H_4$	C_5H_4N	A	242–242.5	...	2.9	E	$C_{20}H_{18}N_4O_4$	66.27	5.01	15.47	66.11	5.01	15.45
15	4-$CH_3OC_6H_4$	C_6H_5	A	238–239	223–224[q]	7.1	B	$C_{32}H_{30}N_2O_4$	73.30	5.60	7.78	73.15	5.60	7.99
16	3,4-$CH_2O_2C_6H_3$[h]	CH_3	A	253	237–238[r]	5.7	B, E	$C_{21}H_{18}N_2O_4$	57.57	5.64	11.20	57.85	5.81	11.11
17	3,4-$CH_2O_2C_6H_3$	C_2H_5	A	240.5–241	225[s]	12.3	B	$C_{24}H_{28}N_2O_4$	60.40	6.52	10.07	60.16	6.56	9.92
18	3,4-$CH_2O_2C_6H_3$	2-C_3H_7	D	238–239	...	3.5	E	$C_{26}H_{32}N_2O_4$	62.71	7.24	9.15	62.89	7.15	9.10
19	3,4-$CH_2O_2C_6H_3$	C_5H_4N	D	243–243.5	...	3.8	A	$C_{20}H_{16}N_4O_4$	63.80	4.29	14.90	63.80	4.63	15.11
20	3,4-$CH_2O_2C_6H_3$	C_6H_5	A	244	222[l]	11.4	B	$C_{22}H_{18}N_2O_4$	70.56	4.85	7.49	70.30	4.97	7.51
21	4-$(CH_3)_2NC_6H_4$	CH_3	A	269.5–270	...	0.9	D	$C_{23}H_{28}N_4O_2$	62.61	7.69	16.86	63.28	7.16	16.86
22	4-$(CH_3)_2NC_6H_4$	C_2H_5	D	242–242.5	...	4.0	E	$C_{25}H_{32}N_4O_2$	64.93	8.36	15.16	65.17	8.16	15.29
23	4-$(CH_3)_2NC_6H_4$	2-C_3H_7	A	246–246.5	...	7.6	B	$C_{27}H_{37}N_4O_2$	66.83	8.92	13.77	67.21	8.68	13.80
24	4-$(CH_3)_2NC_6H_4$	C_5H_4N	A	235.5–236	...	1.5	E, B	$C_{29}H_{24}N_6O_2$	67.16	5.64	18.67	67.16	5.40	18.32
25	4-$(CH_3)_2NC_6H_4$	C_6H_5	E	234	223–224[u]	1.6	B	$C_{28}H_{28}N_4O_2$	73.95	6.21	11.26	73.62	5.99	11.45

[a] See text. [b] Triplicate melting points (uncorrected) taken on a Fisher-Johns calibrated apparatus (calibration ±0.5° at all points). [c] The literature reference in this column is the highest recorded for that compound. [d] Yields are reported for the final analyzed product. [e] A = 30% ethanol, B = 95% ethanol, C = distilled water, D = 75% ethanol, E = 50% ethanol. [f] Analyses performed by Drs. G. Weiler and J. B. Strauss, Microanalytical Laboratory, Oxford, England. Mean values recorded from duplicate samples except for compounds **7, 10, 18, 24,** and **25** (single sample analyzed). [g] C_5H_4N = pyridyl. [h] 3,4-$CH_2O_2C_6H_3$ = 3,4-methylenedioxyphenyl. [i] D. Shemin and R. M. Herbst, *J. Am. Chem. Soc.*, **60,** 1954 (1938), reported mp 250°; G. S. Bhatnagar and K. C. Pandya, *Proc. Indian Acad. Sci.*, **24,** 487 (1946), reported mp 245°; M. C. Paulson and J. M. Merserau, *Trans. Illinois State Acad. Sci.*, **47,** 94 (1955), reported mp 239.5°; W. A. Noyes and D. B. Forman, *J. Am. Chem. Soc.*, **55,** 3493 (1933), reported mp 238°; A. Spasov and I. K. Ivanov, *Godishnik Sofiiskiya Univ., Fiz.-Mat. Fak., Kniga 2,* **38,** 85 (1941/42); *Chem. Abstr.*, **42,** 2585*e* (1948), reported mp 236–237°. [j] Bhatnagar and Pandya[i] reported mp 220°. [k] E. Hoffman and V. Meyer, *Ber.*, **25,** 209 (1892), reported mp 225°; Spasov and Ivanov[i] reported mp 224°; Bhatnagar and Pandya[i] reported mp 217.5°; E. Roth, *Ann. Chem.*, **154,** 72 (1870), reported mp 197°. [l] Paulson and Merserau[i] reported mp 234°; R. K. Mehra and K. C. Pandya, *Proc. Indian Acad. Sci.*, **7,** 376 (1938), reported mp 234°. [m] Mehra and Pandya[l] reported mp 220–221°. [n] Mehra and Pandya[l] reported mp 250°. [o] R. K. Mehra and K. C. Pandya, *Proc. Indian Acad. Sci.*, **10A,** 285 (1939), reported mp 230–231°; Paulson and Merserau[i] reported mp 224°. [p] Mehra and Pandya[o] reported mp 228°. [q] Mehra and Pandya[o] reported mp 223–224°. [r] K. C. Pandya and P. G. Varghese, *Proc. Indian Acad. Sci.*, **14A,** 18 (1941), reported mp 237–238°; Paulson and Merserau[i] reported mp 227–228 dec. [s] Pandya and Varghese[r] reported mp 225°. [t] Pandya and Varghese[r] reported mp 222°. [u] J. Bojanovic, V. Vandjel, M. Mihailovic, and G. Stefanovic, *Glasnik Khem. Drushtva Beogcad,* **20,** 267 (1955); *Chem. Abstr.*, **52,** 16350*f* (1958), reported mp 223–224°.

to the investigation of N,N′-arylidenebis(acid amides), $R_1CH(NHCOR_2)_2$, as potentially useful sedative or hypnotic drugs. A series of 25 such compounds was synthesized and examined for toxicity and sedative action. Syntheses of 13 of the compounds have been reported in the literature; however, uncertainties as to purity of reported product and complete absence of pharmacological data justified their reexamination. Twelve members of the series are new compounds.

Experimental Section

The N,N′-arylidenebis(acid amides) were synthesized by heating an aromatic aldehyde with an acid amide. The amide (0.2 mole) and the aldehyde (0.1 mole) were mixed and then placed in a bath previously heated to 120–130°. After 2 hr the flask was removed and allowed to stand at room temperature. The drying procedure for all the compounds was uniform, but the methods for isolation and crystallization of the product varied for the different compounds.

Procedure A.—After cooling the contents of the flask, the product, which was a solid in all cases, was crystallized from an appropriate solvent. The crystalline product filtered from the solvent was allowed to air dry and then was dried further under an infrared lamp.

Procedure B.—Water was added to the original solvent to "shock-out" the product. The product was then suction filtered and washed with ethanol.

Procedure C.—While near boiling, the solution containing product was suction filtered, and the solution was placed in the freezer to effect crystallization.

Procedure D was the same as procedure A, except Norit was added during the recrystallization process to aid in the removal of color.

Procedure E.—The cooled solid product was broken into small pieces, and the pieces were removed from the reaction flask, mixed with 200 ml of ethanol, and stirred under reflux for 1 hr. The contents of the flask, while still hot, were suction filtered, and the solution was immediately placed in a freezer overnight. The flask was then removed and allowed to stand at room temperature for several hours. The product was suction filtered, washed with ethanol, and dried as previously described. After isolation, the products were recrystallized and dried to constant melting point. The compounds, yields, melting points, recrystallization solvents, formulas, and elemental analyses are presented in Table I.

Pharmacology.—The initial biological evaluation involved a comprehensive study of gross behavior of mice under the influence of N,N′-benzylidenebisnicotinamide (**4**). The results of this study determined the nature of biological testing that would be most appropriate for other members of the series.

Behavior Pattern Screen.—Groups of three female albino mice (I.C.R. strain) weighing 18–23 g were used at each dose level of the compound. Mice in this and subsequent experiments were allowed free access to food and water prior to drug administration. The compound was injected intraperitoneally as a 4% suspension in an aqueous vehicle containing 2% acacia. The animals were injected at dose levels of 100, 200, 400, and 800 mg/kg and observed for 5 hr. The 400- and 800-mg/kg animals were checked

TABLE II

RESULTS OF BEHAVIOR PATTERN SCREEN IN MICE OF N,N′-BENZYLIDENEBISNICOTINAMIDE

Dose, mg/kg ip	Gross observations
100	1, 2, and 3 hr postinjection (pi): slight diminution of irritability response to sound and touch, diminishment of general motor activity; 3 hr pi: some attenuation of escape response, hind-quarter stretch noted in one animal 40 min pi.
200	1, 2, and 3 hr pi: decrease in irritability response to sound and touch and marked decrease in general motor activity; 2 hr pi and thereafter: marked loss of escape response and some increase in status center time.
400	1, 2, and 3 hr pi: marked decrease in general motor activity, decrease in irritability response to sound and touch, attenuation of ability to maintain position on rotating rod; 2 hr pi: increase in status center, partial loss of escape response, and minor decrease in positioning sense; 27 hr pi: escape response still attenuated.
800	1, 2, and 3 hr pi: decrease in irritability response to sound and touch, marked decrease in general motor activity, slight attenuation of gait, definite decrease in grasping ability, minor decrease in positioning sense, and a marked loss of escape response; 27 hr pi: still a loss of escape response, slight decrease in positioning sense, and a decrease in grasping ability.

also at 27 hr. Control animals received 2% acacia vehicle alone. Type and rate of respiration, abnormal tail position, piloerection, tremors, convulsions, loss of righting reflex, salivation, lacrimation, abnormal gait, hind-quarter stretch, writhing, general motor activity, and spontaneous behavior were all monitored. The mice were also rated for irritability response (sound and touch), status center, pinna and corneal reflexes, escape response, positioning sense, and grasping response. The animals were allowed access to water after 1 hr postinjection and to food after 3 hr postinjection. Results are summarized in Table II.

Acute Toxicity Determination.—Groups of six or ten female albino mice (I.C.R. strain) weighing 18–22 g were used for each dose determination. The compounds were injected intraperitoneally as 4% suspensions in 4% acacia vehicle. The mice were allowed access to water after 1 hr postinjection and to food after 3 hr postinjection. The animals were observed for 24 hr. The mice were then sacrificed and their peritoneal cavities were examined for evidence of tissue injury and unabsorbed compound.

The experimental data were used to calculate the "line of best fit" (linear regression) employing log dose-probit metameters. The LD_{50} and its standard error were calculated. The maximum dose administered was 1500 mg/kg. Results are recorded in Table III.

Sodium Hexobarbital "Sleeping Time" Determination. Method 1.—Groups of ten female albino mice (I.C.R. strain) weighing 18–22 g were employed for each compound at each dose and for each control. The compounds were injected as above in a dose of 1000 mg/kg. The aqueous acacia vehicle and hexobarbital served as the control. Sodium hexobarbital[3] as a freshly prepared aqueous solution was administered at 100 mg/kg. The compound was administered into the left lower quadrant and followed immediately by the injection of sodium hexobarbital into the right lower quadrant. Results are recorded in Table III.

Method 2.—The same procedure as in method 1 was used with the following modifications. (1) The compounds were administered 3 hr preceding the administration of the hexobarbital, 100 mg/kg. (2) The compounds were administered at doses of 50, 100, 200, and 400 mg/kg, ten animals/dose. Results are recorded in Table III.

Method 3.—The same procedure as in method 1 was used with the following modifications. (1) The compounds were administered at 1000 mg/kg 3 hr preceding the administration of the barbiturate, ten animals/dose. (2) The hexobarbital was administered at 116 mg/kg. Results are recorded in Table III.

(3) Eli Lilly and Co., Indianapolis, Ind., 250 mg, diluted with water for injection (U.S.P.).

TABLE III

TOXICITY AND EFFECT OF N,N′-ARYLIDENEBIS(ACID AMIDES) ON HEXOBARBITAL-INDUCED SLEEP

No.	LD_{50} ± SE, mg/kg ip	Dose, mg/kg (method)	Sleeping time, mean ± SE, min: Compd and hexobarbital	Hexobarbital control	Act.[a] Ratio
1	1268 ± 63	1000 (1)	32.1 ± 3.9	34.6 ± 3.6	...
1		50 (2)	35.7 ± 3.1	43.5 ± 4.0	...
1		100 (2)	36.8 ± 3.3	43.5 ± 4.0	...
1		200 (2)	58.0 ± 1.7	43.5 ± 4.0	...
1		400 (2)	72.3 ± 6.5	43.5 ± 4.0	166.2
2	>1500	1000 (1)	48.4 ± 7.2	34.6 ± 3.6	...
3	>1500	1000 (1)	33.0 ± 4.1	25.7 ± 3.0	...
4	>1500	1000 (1)	61.2 ± 5.5	23.4 ± 1.9	261.5
4		50 (2)	36.4 ± 2.2	23.2 ± 2.6	156.9
4		100 (2)	47.4 ± 6.4	23.2 ± 2.6	204.3
4		200 (2)	52.0 ± 3.6	23.2 ± 2.6	224.1
4		400 (2)	48.9 ± 2.1	23.2 ± 2.6	210.8
4		1000 (3)	314.1 ± 30.7	56.0 ± 6.0	560.9
5	>1500	1000 (1)	38.4 ± 2.7	34.6 ± 3.6	...
6	1323 ± 145	1000 (1)	84.9 ± 5.7	33.3 ± 4.0	255.0
7	>1500	1000 (1)	79.2 ± 9.6	33.3 ± 4.0	237.8
8	>1500	1000 (1)	39.8 ± 4.2	34.6 ± 3.6	...
9	>1500	1000 (1)	70.0 ± 6.2	49.1 ± 3.1	111.0
10	>1500	1000 (1)	45.6 ± 6.0	33.0 ± 4.0	...
11	>1500	1000 (1)	20.2 ± 3.8	23.4 ± 1.9	...
12	>1500	1000 (1)	31.4 ± 4.1	23.4 ± 1.9	...
12		1000 (3)	377.0 ± 32.8	56.0 ± 6.0	673.2
13	>1500	1000 (1)	38.9 ± 4.0	34.6 ± 3.6	...
14	>1500	1000 (1)	38.8 ± 4.8	34.6 ± 3.6	...
15	>1500	1000 (1)	37.5 ± 6.9	34.6 ± 3.6	...
16	>1500	1000 (1)	36.6 ± 2.2	25.7 ± 3.0	142.4
17	>1500	1000 (1)	40.4 ± 5.3	23.4 ± 1.9	172.6
18	>1500	1000 (1)	54.4 ± 8.6	25.7 ± 3.0	211.7
19	>1500	1000 (1)	38.5 ± 4.5	27.7 ± 3.0	149.8
19		1000 (3)	139.0 ± 17.9	56.0 ± 6.0	248.2
20	>1500	1000 (1)	25.5 ± 2.0	25.7 ± 3.0	...
21	>1500	1000 (1)	63.7 ± 7.7	49.7 ± 4.0	...
22	>1500	1000 (1)	21.8 ± 1.8	25.7 ± 3.0	...
23	>1500	1000 (1)	64.5 ± 5.6	49.1 ± 3.1	131.3
24	>1500	1000 (1)	75.2 ± 5.6	49.1 ± 3.1	154.1
25	>1500	1000 (1)	47.1 ± 3.3	28.8 ± 2.4	...

[a] The mean sleeping time of the ten mice of the hexobarbital and acacia control was assigned an activity value of 100. The determination of significant difference in sleeping time between the mean controls and the mean compounds was established by use of Student's t test, double tailed, 95% confidence level. If a significant difference existed, the mean value for the control was divided into the mean value for the compound and multiplied by 100 to give the activity ratio.

Discussion

The primary effect observed in the testing of these compounds was CNS depressant activity. From Table III it can be seen that the N,N′-arylidenebis(acid amides) exhibited only slight toxicity. All of the compounds possess LD_{50}'s greater than 1250 mg/kg. Examination of the peritoneal cavities of the test animals used in the toxicity studies revealed that incomplete absorption was a contributory factor. No compound residue was found in the peritoneal cavities of mice having been injected with **1**, **11**, and **21**. The remainder of the compounds were found to be incompletely absorbed. No gross change nor inflammation of visceral organs or peritoneum was found in any of the animals.

The potentiation (or increase) in sleeping time (see Table III) observed when the compounds were administered in the hexobarbital test indicates that these compounds do possess significant ability to accentuate the action of CNS depressants. Compounds **1** and **4** possess activity at dose levels less than 1000 mg/kg. Significant activity was seen with **4** at 50 mg/kg.

The effect of interim between drug and hexobarbital administration was seen with **1**. At 1000 mg/kg (method 1) no significant activity was seen, but significant activity was seen at 400 mg/kg (method 2). Similarly, **12** did not possess significant activity with method 1, but did display an enhancement effect with method 3. In the case of **12** a part of the effect was due to increased increment of hexobarbital administered, but the increase cannot be entirely attributed to this (see **19**, methods 1 and 3). The apparent difference in activity is almost certainly due to slow absorption of the compound, slow attainment of the requisite concentration at the site of action, or biotransformation of the compound to the active form.

When administered simultaneously with the hexobarbital, the preliminary evaluations indicate that the N,N′-piperonylidenebis(acid amides) and the N,N′-arylidenebisnicotinamides are the most active in regard to their CNS depressant action.

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TABLE I

EHRLICH ASCITES SCREENING DATA[a]

Compd	Dose, mg/kg	Mortality	Ascites vol, ml	T/C[b]	TPCV	T/C[b]	Bone marrow[c]
8	200.0	0	3.6	1.71			
	100.0	0	2.4	1.14			
	50.0	0	1.7	0.81			
	50.0	0	3.8	1.90			
	25.0	0	2.6	1.30			
9	30.0	0	3.0	0.70			N
	15.0	0	2.9	0.64			
10	10.0	0			1.12	2.33	N
11	20.0	0			0.02	0.04	N
	10.0	0			0.17	0.3	N
	5.0	0			0.52	1.08	
	2.5	0			0.39	0.81	
12	40.0	0			0.02	0.04	
	20.0	0			0	0	
	10.0	0			0	0	
	5.0	0			0	0	
	160.0[d]	10			...	...	
	80.0	10			...	...	
	40.0	3			0.04	0.03	↓ ↓ ↓
	20.0	1			0.04	0.03	↓ ↓
	10.0	0			0.05	0.04	↓
	5.0	0			0.05	0.04	N
	2.5	0			0.04	0.03	
	1.3	0			0.57	0.48	
	0.6	0			1.50	1.28	
	0.3	0			1.32	1.12	
13	20.0	0			0.76	1.58	N

[a] See text for a description of total packed-cell volume (TPCV) and therapeutic index (TI). [b] T/C = treated/control animals. [c] Degree of depression: ↓ ↓ ↓, strong; ↓ ↓, moderate; ↓, slight; N, negative. [d] The therapeutic index was 21. *Cancer Chemotherapy Rept.*, **17**, 56 (1962), gives LD_{10} = 1.5 mg/kg, ED_{90} = 0.096 mg/kg, and TI = 16 for $CH_3N(CH_2CH_2Cl)_2 \cdot HCl$ (HN_2).

Effect of Organic Compounds on Reproductive Processes. VI. Alkylating Agents Derived from Various Diamines

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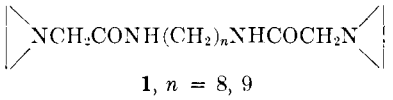
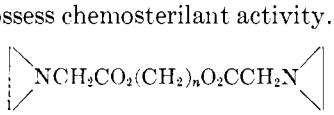
A program of synthesis of various alkylating agents has enabled us to define some of the chemical parameters required for chemosterilant activity in the housefly (*Musca domestica* L.).[1-3] Previous work has demonstrated activity in the series of N,N′-bis(aziridinylacetyl)-α,ω-polymethylenediamines (**1**). Optimum ac-



1, n = 8, 9

tivity in this series was found in compounds **1**. The series of esters (**2** and **3**) corresponding to the bisamides (**1**) did not possess chemosterilant activity.[2]



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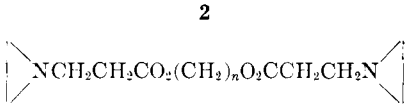
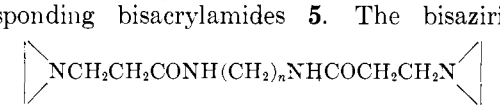


3

n = 6, 8, 10

We have now prepared a series of N,N′-bis(aziridinylpropionyl)-α,ω-polymethylenediamines (**4**), in which the two nitrogen functions of compounds **1** have been separated by an additional methylene group. Compounds **4** were prepared by the addition of aziridine to the corresponding bisacrylamides **5**. The bisaziridinyl-

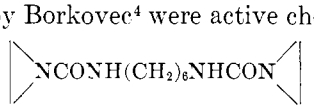


4, n = 6, 8, 10, 12

$CH_2{=}CHCONH(CH_2)_nNHCOCH{=}CH_2$

5

propionamides (**4**) and bisacrylamides (**5**) were inactive as inhibitors of reproduction in the housefly. This result appears to further define the requirement that the alkylating group must be α to the carbonyl in this series. However, several very active napthalene biscarbamoylaziridines and one aliphatic carboxamide (**6**) prepared by Borkovec[4] were active chemosterilants.



6

The rationale for testing the aziridine compounds and other alkylating agents as insect chemosterilants has been reported by Borkovec, who suggested a similarity of rapid cellular division in reproductive and cancerous systems.[5] An impetus was thus given to the investigation of a number of "carcinostatic" alkylating agents as potential insect chemosterilants. It seemed reasonable to reverse this rationale and screen the compounds prepared on this program against a tumor system. The use of the lower homologs of the bis-

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(2) W. A. Skinner, J. Hayford, T. E. Shellenberger, and W. T. Colwell, *ibid.*, **9**, 605 (1966).

(3) W. A. Skinner, M. Cory, T. E. Shellenberger, and J. I. DeGraw, *ibid.*, **10**, 102 (1967).

(4) A. B. Borkovec and C. W. Wood, *ibid.*, **8**, 545 (1965).

(5) A. B. Borkovec, *Science*, **137**, 1034 (1962).