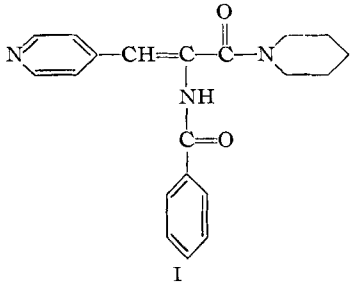
Pharmacological Studies on Some New Azlactone Derivatives

By G. ALAN ROBISON and F. W. SCHUELER

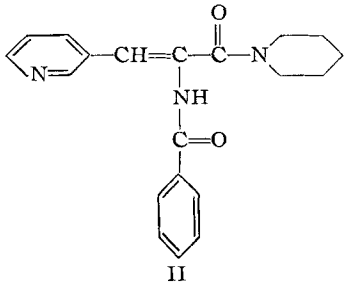
Several members of a new series of azlactone derivatives (substituted acrylic acid amides), which by themselves exhibit convulsant properties, were found to be capable of prolonging the duration of hexobarbital-induced hypnosis in mice. Experiments are described which indicate that this action is secondary to inhibition of the biotransformation of hexobarbital in the liver. One member of the series was found to be a long-acting depressant capable of potentiating the action of strychnine.

THE CHEMICAL and physical properties of the azlactones [5(4)-oxazolones] have been well studied (1–4), but there have been relatively few investigations of the pharmacological activity of either the azlactones themselves or (with the exception of the α-amino acids) their many possible derivatives. Schueler and Hanna (5) studied the cardiac activity of a series of azlactones and related products, and Cronheim, *et al.* (6), recently reported on the interesting sedative properties of trimethoxybenzoyl-glycine-diethylamide, which could be thought of as the derivative of a saturated azlactone (4).

In testing several new series of these derivatives for pharmacological activity, our attention was initially drawn to a series of amides. Of particular interest was the finding that α-benzoyl-amino-β-(4-pyridyl) acrylic acid piperidide (I) was a central stimulant, whereas its 3-pyridyl congener (II) acted to depress the CNS. This relationship does not exist in the case of their respective diethylamide analogues, which are both convulsants.



I



II

On studying the central activity of these agents further, the discovery was made that I (and both the diethylamides) would, despite their convulsant properties, markedly prolong the duration of hexobarbital-induced hypnosis in mice, whereas II was relatively inactive in this respect.

Studies were subsequently initiated to determine the mechanism of action of this effect. The results of these studies, together with the results of studies designed to characterize the nature of the central activity of the compounds, are reported herein.

EXPERIMENTAL

Preparation of Materials.—The azlactones used as intermediates were prepared by reacting the appropriate acyl glycine with the appropriate aldehyde in the presence of acetic anhydride and sodium acetate, as previously described (2), although heating is not required in the case of the pyridinecarboxaldehydes. Commercially unavailable acyl glycines were prepared by the regular Schotten-Baumann reaction. The majority of the azlactones were recrystallized from 95% ethanol.

The amides were prepared by the method B of Barnes, *et al.* (1), and were recrystallized from benzene-hexane. Those amides not previously reported in the literature are shown in Table I. The yields varied from about 40 to 80%.

Preliminary Screening.—The compounds were administered to Swiss albino mice in doses up to at least 500 mg./Kg. (orally, in suspension with 0.5% carboxymethylcellulose), and the gross effects observed. With reference to Table I, compounds 5 to 10 were administered parenterally. LD_{50}'s were determined for some of the compounds (for mice by i. p. injection), either by a simplified method of probits (7) or by the method of moving averages (8). Rectal temperatures (mice) were measured by means of a YSI model 43 tele-thermometer.

Drugs used in attempting to antagonize the convulsant effect of I (compound No. 5 in Table I)

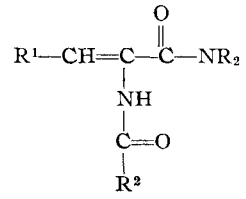
Received October 3, 1960 from the Department of Pharmacology, Tulane University, School of Medicine, New Orleans, La.

Accepted for publication October 25, 1960.

Abstracted from a thesis presented by G. A. Robison to the Graduate School of Tulane University in partial fulfillment of the requirements for the degree of Master of Science (1960). Mr. Robison is grateful for a fellowship which he received from the Schering Corporation.

This work was supported in part by a research grant (CY-3037) from the U. S. P. H. S.

TABLE I.—SUBSTITUTED ACRYLIC ACID AMIDES



No.	R^1	R^2	NR_2	M. P., °C.[a]	Formula		Analyses[b] C, %	H, %	N, %
1	Phenyl	tmp[c]	Piperidino	decompn. 180	$C_{24}H_{28}N_2O_5$	Calcd.	67.90	6.60	6.60
						Found	68.31	6.81	6.50
2	tmp	tmp	Piperidino	decompn. 198	$C_{27}H_{34}N_2O_8$	Calcd.	63.00	6.60	5.44
						Found	62.73	6.55	5.79
3	tmp	Phenyl	Piperidino	160–162	$C_{24}H_{28}N_2O_5$	Calcd.	67.90	6.60	6.60
						Found	66.94	6.40	6.53
4	tmp	Phenyl	Diethylamino	196–197	$C_{23}H_{28}N_2O_5$	Calcd.	67.00	6.80	6.80
						Found	66.27	6.52	7.03
5	4-Pyridyl	Phenyl	Piperidino	195–196	$C_{20}H_{21}N_3O_2$	Calcd.	71.65	6.26	12.50
						Found	71.25	6.11	12.29
6	4-Pyridyl	Phenyl	Diethylamino	197–198	$C_{19}H_{21}N_3O_2$	Calcd.	70.50	6.50	13.00
						Found	69.83	6.46	12.56
7	4-Pyridyl	Methyl	Piperidino	148–149	$C_{15}H_{19}N_3O_2$	Calcd.	66.00	6.95	15.39
						Found	66.18	6.88	15.61
8	4-Pyridyl	tmp	Piperidino	decompn. 169	$C_{23}H_{27}N_3O_5$	Calcd.	65.00	6.35	9.88
						Found	65.48	6.57	9.69
9	3-Pyridyl	Phenyl	Piperidino	187–189	$C_{20}H_{21}N_3O_2$	Calcd.	71.65	6.26	12.50
						Found	71.61	6.26	12.73
10	4-Pyridyl	Phenyl	Diethylamino	186–187	$C_{19}H_{21}N_3O_2$	Calcd.	70.50	6.50	13.00
						Found	70.62	6.50	13.03

[a] Melting points are uncorrected. [b] Analyses are by Alfred Bernhardt, Max Planck Institute, Mulheim, W. Germany. [c] tmp = 3,4,5-Trimethoxyphenyl.

included pyridoxine HCl, hexobarbital, zoxazolamine, and trimethadione. Drugs used in combination with II (compound No. 9 in Table I) included, in addition to its 4-pyridyl congener (I), strychnine and pentylenetetrazol.

Hexobarbital Sleeping Time.—This was measured as the duration of loss of righting reflex, the mice being considered asleep until they could right themselves three times in one minute. Controls were always run along with the experimental group(s), and the significance of the difference between the mean sleeping times of the control and experimental group(s) was determined by means of Student's "*t*" test. Compound No. 5 (I) was used as an additional control in all experiments. The various amides used to pretreat the mice were either given orally, in suspension with 0.5% carboxymethylcellulose, or, when only the pyridyl derivatives were being compared, parenterally (s. c. or i. p.) as the hydro chloride or tartrate salts. Hexobarbital was always administered intraperitoneally as the sodium salt, using a dose of either 110 mg./Kg. or 150 mg./Kg. Results are expressed in terms of per cent prolongation of the mean control sleeping time.

Hexobarbital Metabolism.—For the determination of the effect of compound No. 5 on total body hexobarbital concentration, the experimental mice were pretreated with 25 mg./Kg. of the drug, subcutaneously, followed in thirty minutes by the intraperitoneal administration of hexobarbital (200 mg./Kg). One hour after the administration of the hexobarbital, the total body concentration (mg./Kg.) was determined, either by the method of Brodie, *et al.* (9, 10), or by the one used by Fujimoto, *et al.* (11). Results by either method are in close agreement.

Isolated rat liver perfusions were performed to localize the effect of Tu-702 on hexobarbital metabolism at the organ level. The livers were obtained from male Sprague-Dawley or Long-Evans rats weighing 400–550 Gm. They were perfused (100 ml. albumin-Locke solution plus 50 ml. freshly drawn whole rat blood) in a recirculating system similar to that of Plaa and Hine (12). The perfused liver was given approximately one hour to equilibrate with its new environment and then the disappearance of added hexobarbital from the system was studied in the presence and absence of compound No. 5. The rate of disappearance of added hexobarbital from the system was followed by determining the hexobarbital concentration in aliquots of the perfusate taken at ten, twenty, forty, eighty, and one hundred and twenty minutes, and by determination of the terminal concentration of hexobarbital in the liver at one hundred and twenty minutes.[1]

RESULTS

Of the compounds listed in Table I only those having a pyridyl moiety attached to the β-carbon caused grossly observable effects in mice (in doses up to 500 mg./Kg). Compounds 5, 6, and 10 were convulsants, superficially resembling pentylenetetra-

[1] The authors wish to express their gratitude to Dr. Gabriel L. Plaa for his invaluable assistance in carrying out the liver perfusions.

zol in this respect. Of the anticonvulsants tested, trimethadione was by far the most effective (Table II). Zoxazolamine and hexobarbital were less effective in that they protected only after doses that caused obvious depression. Pyridoxine was completely ineffective.

The depressant effect of compound 9, the only member of the series that was found to have this effect, was of long duration. In doses less than those required to cause loss of the righting reflex, its most prominent effect in mice was the production of a state of catalepsy. After a dose of 400 mg./Kg. the mice would in many cases "sleep" (lose their righting reflex) for longer than twelve hours. A surprising observation was that pretreatment with this compound lowered the threshold to convulsions induced by either its 4-pyridyl congener (compound 5) or strychnine, as shown in Table III. Pentylenetetrazol, on the other hand, was not potentiated by the compound.

Neither the dose of the 4-pyridyl congener (compound 5) nor of the strychnine sulfate were sufficient to induce convulsions in the control mice, but they became very potent convulsant doses after pretreatment with the depressant. An interesting observation was that after pretreatment with doses sufficient to cause loss of the righting reflex, the subsequent administration of 1.5 mg./Kg. of strychnine sulfate would not necessarily induce convulsions, but was invariably lethal (death being due to respiratory failure). Like its depressant effect, the ability of the depressant to potentiate the action of its 4-pyridyl congener and of strychnine was of long duration.

TABLE II.—PROTECTION BY TRIMETHADIONE AGAINST CONVULSIONS INDUCED BY α-BENZOYLAMINO-β-(4-PYRIDYL) ACRYLIC ACID PIPERIDIDE[a]

	Ratio of Number Convulsing to Number Treated	Ratio of Number Dying to Number Treated
Control (no pretreatment)	10/10	9/10
Pretreated with trimethadione, 500 mg./Kg. (i. p.), 15 min. before convulsant	0/10	0/10

[a] Swiss albino mice, 100 mg./Kg., s. c. injection.

TABLE III.—EFFECT OF PRETREATMENT WITH α-BENZOYLAMINO-β-(3-PYRIDYL) ACRYLIC ACID PIPERIDIDE[a] ON LETHALITY OF VARIOUS CONVULSANTS

	Ratio of Number of Mice Dying to Number Treated with the Corresponding Convulsant	
	Controls (no Pretreatment)	Pretreated
Compound 5 (Table I), 50 mg./Kg., i. p.	0/10	10/10
Strychnine sulfate, 1.5 mg./Kg., i. p.	0/10	10/10
Pentylenetetrazol, 100 mg./Kg., i. p.	9/10	4/10

[a] A 200 mg./Kg. dose, s. c., thirty minutes before administration of the convulsant.

The effect on the duration of hexobarbital-induced hypnosis in mice varied depending on the conditions of the experiment but, using a dose of hexobarbital of 110 mg./Kg. and a pretreatment time of forty minutes, 50 mg./Kg. of the 4-pyridyl piperidide caused a prolongation in sleeping time of 996% (i. e., 24.1 ± 0.9 minutes for the controls *vs.* 263.8 ± 17.5 minutes after pretreatment). Taking the per cent prolongation after the 4-pyridyl piperidide in each experiment as unity, the relative potencies of the other derivatives tested are listed in Table IV. Also shown in this Table is the significance of the difference between the effect of each of the compounds and that of the 4-pyridyl piperidide. Shown, in addition, is the estimated LD_{50} for each of the compounds (based on 24 to 48 mice per assay).

TABLE IV.—PROLONGATION OF HEXOBARBITAL-INDUCED HYPNOSIS IN MICE

No. (from Table I)	Potency Relative to Compound 5[a]	P	LD_{50}, mg./Kg.
5	1.00	..	74
6	1.06	>0.05	132
7	0.43	<0.05	>1600
8	0.52	<0.05	>800
9	0.19	<0.01	476
10	0.89	>0.05	126

[a] Doses equimolar to doses used of compound 5.

In all cases the dose used of each of the derivatives was equimolar with the dose used of the 4-pyridyl piperidide. Under these conditions the compounds listed in Table I but not shown in Table IV caused no significant prolongation of the control sleeping time.

Table V shows the effect of compound 5 on hexobarbital body levels in mice. It will be seen from these data that in one hour the rate of disappearance is reduced by almost one-half by pretreatment with this drug.

TABLE V.—EFFECT OF α-BENZOYLAMINO-(4-PYRIDYL) ACRYLIC ACID PIPERIDIDE ON HEXOBARBITAL BODY LEVELS

	Hexobarbital, mg./Kg.		Disappearance, %
	Initial	After 60 min.[a]	
Controls (6 mice)	200	80.3 ± 3.5	59.9
Pretreated (6 mice)	200	133.7 ± 15.3	33.2

[a] ± Standard error.

The results of the liver perfusion experiments are shown by means of decay curves (Fig. 1), hexobarbital concentration being expressed as a percentage of the concentration initially present (approx. 650 mcg./ml.). While these curves each represent the results of a single experiment, the results of duplicate experiments were in excellent agreement with those illustrated. The control curves were calculated to follow most closely a second-order rate reaction, with a half-life (based on the initial concentration) of about forty minutes.

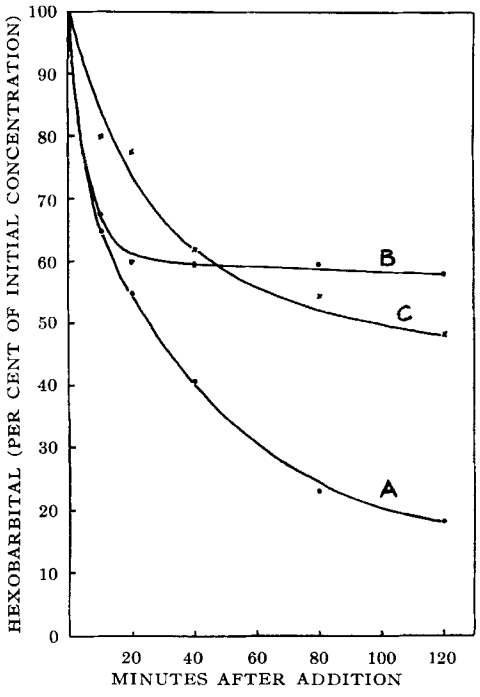


Fig. 1.—Effect of α-benzoylamino-β-(4-pyridyl) acrylic acid amide (I) on rate of disappearance of hexobarbital from rat liver perfusate. *A*, control; *B*, 25 mg. of I added fifteen minutes before hexobarbital; *C*, 25 mg. I added thirty minutes before hexobarbital.

DISCUSSION

The relationship between structure and activity in this series of compounds represents something of a paradox. The situation in the case of the structures (i. e., I being a convulsant and II a depressant) does not exist in the case of their diethylamide analogues, the transition from diethylamino to piperidino having in fact a different effect depending on the position of the nitrogen in the pyridyl ring. In the 4-pyridyl series this change, represented by the transition from compound 6 to compound 5, results in a quantitative change only (an increase in toxicity, as shown by the LD_{50}'s in Table IV). The same change in the 3-pyridyl series, represented by the transition from compound 10 to compound 9, corresponds to an opposite change in acute toxicity and, in addition, there is a qualitative change from stimulant to depressant.

Based on gross observations, the convulsants in this series appear to be of the same type as pentylenetetrazol, a suggestion supported by the antagonistic effect of trimethadione (13). Pentylenetetrazol has a stimulating effect on excitatory synapses within the central nervous system (14).

The depressant effect of α-benzoylamino-β-(3-pyridyl) acrylic acid piperidide is poorly understood, but its ability to potentiate strychnine is interesting. Unlike pentylenetetrazol, strychnine is a convulsant by virtue of its ability to depress inhibitory synapses within the CNS (14, 15). One class of depressants that potentiate strychnine are the erythrina alkaloids, which are capable of blocking transmission between motor-axon collaterals and Renshaw cells (16). Whether a similar mechanism of action can be invoked in the case of the 3-pyridyl piperidide remains to be seen. The fact that after pretreatment with this compound strychnine is lethal without necessarily causing convulsions suggests that strychnine's ability to inhibit transmission at excitatory synapses (15) may be of importance in explaining some of its gross effects.

Substitution of either methyl or trimethoxyphenyl (as in compounds 7 and 8, respectively) for the phenyl moiety of compound 5 results in a drastic reduction in central activity, although the ability to prolong the duration of action of hexobarbital is retained to a considerable degree, as shown by the data in Table IV. The prolongation of hexobarbital sleeping time is probably secondary to inhibition of the biotransformation of hexobarbital in the liver, which is the principal means by which the action of hexobarbital in the body is terminated (17).

REFERENCES

(1) Barnes, D. K., Campaigne, E., and Shriner, R. L., *J. Am. Chem. Soc.*, **70**, 1769(1948).
(2) Schueler, F. W., and Wang, S. C., *ibid.*, **72**, 2220 (1950).
(3) Schueler, F. W., and Hanna, C., *ibid.*, **74**, 5542(1952).
(4) Cornforth, J. W., in "Heterocyclic Compounds," Vol. 5, John Wiley & Sons, New York, N. Y., 1957, pp. 336–372.
(5) Schueler, F. W., and Hanna, C., *Arch. intern. pharmacodynamie*, **86**, 91(1951).
(6) Cronheim, G., Gourzis, J. T., and Toekes, I. M., *Science*, **128**, 1570(1958).
(7) Miller, E. C., and Tainter, M. L., *Proc. Soc. Exptl. Biol. Med.*, **57**, 261(1944).
(8) Weil, C. S., *Biometrics*, **8**, 249(1952).
(9) Brodie, B. B., Burns, J. J., Mark, L. C., Lief, P. A., Bernstein, E., and Papper, E. M., *J. Pharmacol. Exptl. Therap.*, **109**, 26(1953).
(10) Axelrod, J., Reichenthal, J., and Brodie, B. B., *ibid.*, **112**, 49(1954).
(11) Fujimoto, J. M., Pearce, K. B., and Plaa, G. L., *ibid.*, **129**, 139(1960).
(12) Plaa, G. L., and Hine, C. H., *A. M. A. Arch. Ind. Health*, **21**, 114(1960).
(13) Everett, G. M., and Richards, R. K., *J. Pharmacol. Exptl. Therap.*, **81**, 402(1944).
(14) Purpura, D. P., and Grundfest, H., *J. Neurophysiol.*, **20**, 494(1957).
(15) Bradley, K., Easton, D. M., and Eccles, J. C., *J. Physiol.*, **122**, 474(1953).
(16) Longo, V. G., Martin, W. R., and Unna, K. R., *J. Pharmacol. Exptl. Therap.*, **129**, 61(1960).
(17) Brodie, B. B., Maickel, R. P., and Jondorf, W. R., *Federation Proc.*, **17**, 1163(1958).