presence of some component(s) in the more water soluble subfraction F_1 possessing CNS depressant and possibly ataractic activity.

REFERENCES

(1) Lewin, L., "Uber *Pipermethysticum* (Kawa)," August Hirschwald, Berlin, Germany, 1886.
(2) Keller, F., and Klohs, M. W., *Lloydia*, **26**, 1(1963).
(3) Furgiuele, A. R., *et al.*, *J. Pharm. Sci.*, **54**, 247(1965)
(4) Furgiuele, A. R., Kinnard, W. J., and Buckley, J. P., *J. Pharmacol. Exptl. Therap.*, **137**, 356(1962).
(5) Watzman, N., *et al.*, *J. Pharm. Sci.*, **53**, 1429(1964).
(6) Dombro, R. S., and Woolley, D. W., *Biochem. Pharmacol.*, **13**, 569(1964).
(7) Krapcho, J., *et al.*, *J. Med. Chem.*, **6**, 219(1963).
(8) Burn, J. H., "Practical Pharmacology," Blackwell Scientific Publications, Oxford, England, 1952, p. 13.
(9) Bogdanski, D. F., *et al.*, *J. Pharmacol. Exptl. Therap.*, **117**, 82(1956).
(10) Aprison, M. H., *et al.*, *J. Neurochem.*, **9**, 575(1962).
(11) Udenfriend, S., Weissbach, H., and Brodie, B. B., "Methods of Biochemical Analyses," vol. 6, Glick, D., ed., Academic Press Inc., New York, N. Y., 1958, p. 105.
(12) Ariëns, E. J., personal communication.

Evaluation of Certain Hypotensive Agents VII

Tetramethylpiperidine and Benzothiadiazinate Derivatives

By WALTER B. SEVERS, WILLIAM J. KINNARD, and JOSEPH P. BUCKLEY

The hypotensive activity of four tetramethylpiperidine and four benzothiadiazinate derivatives was investigated in anesthetized rats and dogs. The active tetramethylpiperidine derivatives exerted their effects by ganglionic blockade. EX 4922, 1-hydrazinophthalazine 3,4-dihydro-6-nitro-7-sulfamoyl-1,1,3-trioxo-2H-1,2,4-benzothiadiazinate, was hypotensive in rats, cats, and dogs, the rat being the most sensitive species. The data obtained suggest that the response is due to α adrenergic blockade and a direct depressant action on vascular smooth muscle. EX 4526, 2,2,6,6-tetramethylpiperidine 3,4-dihydro-6-nitro-7-sulfamoyl-1,1,3-trioxo-2H-1,2,4-benzothiadiazinate, was also hypotensive in rats, cats, and dogs. Pre- and postganglionic conduction along sympathetic nerves was depressed, but the pressor effects to exogenous epinephrine were potentiated. A direct relaxation of vascular smooth muscle was also observed.

THE HYPOTENSIVE activity of a series of 26 compounds structurally related to the ganglionic blocking agent pempidine (1,2,2,6,6-pentamethylpiperidine) has been reported by Buckley *et al.* (1). The present report extends this series of compounds synthesized by Robertson *et al.* (2) to include evaluations of four additional compounds. The hypotensive effects of four benzothiadiazinate derivatives are also reported. The compounds have structural similarities to chlorothiazide and 7-chloro-3-methyl-1,2,4-benzothiadiazine 1,1-dioxide (diazoxide) (3), a nondiuretic benzothiadiazine which lowers arterial pressure by a direct action on vascular smooth muscle (4). The structures of the compounds investigated are found in Table I.

EXPERIMENTAL

Hypotensive Activity in Rats.—Hypotensive activity was evaluated using the method described by Bickerton *et al.* (5). Normotensive Wistar rats, anesthetized with urethan, 1.25 Gm./Kg. i.p., were prepared for blood pressure recording from a carotid artery onto a slowly revolving kymograph *via* a mercury manometer. Compounds were administered into an exposed femoral vein and solutions prepared so that the dose could be administered in a volume of 1.0 ml./Kg. Normal saline was used as a solvent for EX 4272, EX 4629, EX 4827, and EX 4916. A 50% solution of dimethylacetamide (DMAC) was used as the solvent for EX 4348, EX 4526, EX 4826, and EX 4922.

Hypotensive Activity in Dogs.—The experimental compounds were further investigated for hypotensive activity in dogs anesthetized with sodium pentobarbital, 35 mg./Kg. i.v. Blood pressure was recorded from a femoral artery onto a slowly moving kymograph. Pressor responses to a 10-sec. bilateral carotid occlusion (BCO), epinephrine, 1–2 mcg./Kg., norepinephrine, 1–2 mcg./Kg., and angiotensin II, 1 mcg./Kg., were obtained prior to and after administration of an experimental compound into an exposed femoral vein.

Cat Nictitating Membrane Preparation.—The cat nictitating membrane–superior cervical ganglia preparation was utilized to assess the effects of the more active compounds on ganglionic transmission. Cats of either sex were anesthetized with sodium pentobarbital, 35 mg./Kg. i.p. Blood pressure was recorded from a femoral artery onto a Grass polygraph using a Statham (P23AC) transducer. Platinum stimulating electrodes were placed under the pre- and postganglionic fibers of the superior cervical nerve and contractions of the nictitating membrane recorded *via* a Grass FT03 force displacement transducer. The nerves were bathed in warm mineral oil to prevent drying. Responses of the nictitating membrane to submaximal stimulation of the pre- and postganglionic nerves and to 5–10 mcg. of epinephrine i.v. were obtained prior to and following intravenous administration of EX 4348, EX 4526, EX 4629, EX 4826, EX 4916, or EX 4922.

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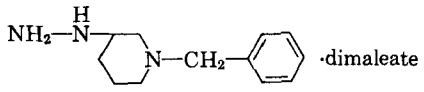
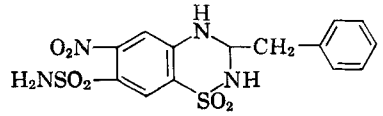
TABLE I.—STRUCTURE OF COMPOUNDS INVESTIGATED

EX 4272



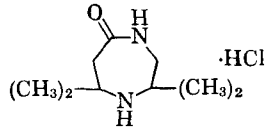
1-Benzyl-3-hydrazinopiperidine dimaleate

EX 4348



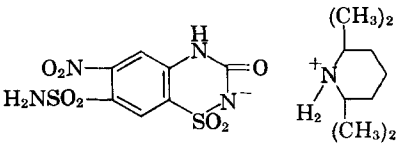
3-Benzyl-3,4-dihydro-6-nitro-7-sulfamoyl-2H-1,2,4-benzothiadiazine-1,1-dioxide

EX 4629



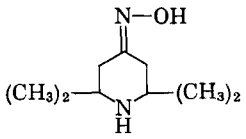
2,2,7,7-Tetramethyl-1,4-diazacycloheptan-5-one HCl

EX 4526



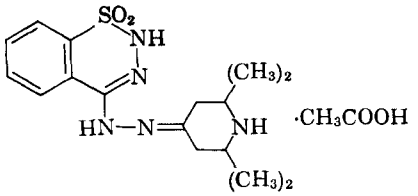
2,2,6,6-Tetramethylpiperidine 3,4-dihydro-6-nitro-7-sulfamoyl-1,1,3-trioxo-2H-1,2,4-benzothiadiazinate

EX 4826



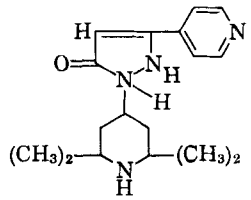
2,2,6,6-Tetramethyl-4-piperidone oxime

EX 4916



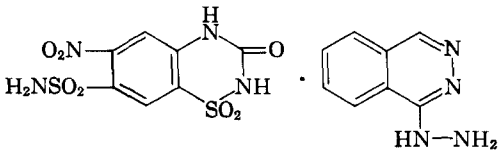
2,2,6,6-Tetramethyl-4-piperidone-1,1-dioxo-2H-1,2,3-benzothiadiazin-4-ylhydrazone acetate

EX 4827



1-(2′,2′,6′,6′-Tetramethyl-4′-piperidyl)-3-(4-pyridyl)-5-pyrazolone

EX 4922



1-Hydrazinophthalazine 3,4-dihydro-6-nitro-7-sulfamoyl-1,1,3-trioxo-2H-1,2,4-benzothiadiazinate

Denervated Hindlimb of the Dog.—EX 4526 and EX 4922 were tested for direct vasodilatory effects using a denervated, autoperfused dog hindlimb preparation. Dogs were anesthetized with sodium pentobarbital, 35 mg./Kg. i.v. The sciatic and femoral nerve trunks of the leg to be perfused were severed. A wire tourniquet was passed under the femoral artery and vein of this limb and tightened with a Schiffrin wire tightener. One hundred to 125 ml. of a 6% dextran (mol. wt. = 73,000) in normal saline solution was administered by intravenous infusion, and the animal heparinized with 1000 units/Kg. Blood was withdrawn from a cannulated carotid artery and passed into a reservoir maintained at 40°. The reservoir outflow passed through a Sigmamotor pump to a catheter inserted into the femoral artery of the leg to be perfused. Blood pressure was recorded on a polygraph using a Statham transducer from the contralateral femoral artery. and perfusion pressure recorded from a T tube placed between the pump outflow and the perfusing catheter. Perfusion pressure was adjusted to approximate systemic pressure. A C clamp on the carotid outflow tubing was used to maintain a fairly constant reservoir level. Clonninger and Green (6) have reported that the denervation procedure results in a limb free of autonomic innervation.

Effects of EX 4922 on Pithed Cats.—Cats of either sex were anesthetized with sodium pentobarbital, 35 mg./Kg. i.p. Blood pressure was recorded from a femoral artery onto a Grass polygraph; the trachea was cannulated and intermittent positive pressure respiration initiated and maintained throughout the experiment. The skin and musculature overlying the area between C-1 and the occipital bone were retracted, and a pithing rod, approximately 0.5 cm. in diameter, was inserted from this point down the length of the spinal column. Both vagi were then sectioned. Epinephrine, 5 to 10 mcg. i.v., was administered prior to and following the intravenous injection of EX 4922.

Effects of EX 4526 and EX 4922 on Cardiac Output and Femoral Blood Flow.—Dogs, anesthetized and prepared for blood pressure recording as previously described, were maintained on positive pressure respiration while the chest was entered at the

TABLE II.—HYPOTENSIVE ACTIVITY OF THE EXPERIMENTAL COMPOUNDS IN NORMOTENSIVE RATS

Compd.	Animals, No.	Dose, mg./Kg.	% Drop in Blood Pressure ± S. D.	Mean Time to Return to Predrug Levels (min.) and Range	Rating and Comments
EX 4272	6	40.0	0	0	Inactive
EX 4629	6	40.0	0	0	Inactive
EX 4826	6	40.0	27.5 ± 5.4	68 (12–140)	++
EX 4827	6	40.0	27.7[a]	6 (4–7)	Inactive
EX 4526	6	10.0	41.7 ± 6.0	200+	++++
EX 4916	6	40.0	35.5 ± 14.9	39 (10–120)	+
EX 4922	6	2.5	48.7 ± 4.6	200+	++++

[a] A secondary rise in blood pressure occurred in two animals.

TABLE III.—EFFECTS OF THE EXPERIMENTAL COMPOUNDS ON THE BLOOD PRESSURE AND SEVERAL PRESSOR RESPONSES IN THE ANESTHETIZED DOG

Compd.	Animals, No.	Dose, mg./Kg.	% Drop in Blood Pressure	Time to Return to Predrug Levels, min.	% of Control Responses BCO[a]	Epi[b]	NE[c]	Ang[d]
EX 4272	1	25.0	0	0	52	113	140	140
EX 4629	1	15.0	56	80	0	269	156	124
EX 4629	1	20.0	71	90	0	137	89	62
EX 4826	2	10.0	49	120+	18	221	144	139
EX 4827	1	20.0	27	9	74	112	92	66
EX 4348	2	10.0	25	8	68	37	60	60
EX 4526	2	7.5	41	110+	0	178	156	149
EX 4916	2	10.0	61	60+	0	156	125	127
EX 4922	1	1.0	17	80	94	55	140	108
EX 4922	1	2.5	11	80	100	67	79	65
EX 4922	2	5.0	23	120+	25	...[e]	62	71

[a] BCO, bilateral carotid occlusion. [b] Epi, epinephrine. [c] NE, norepinephrine. [d] Ang, angiotenin II. [e] Initial pressor followed by a more prolonged reversal.

TABLE IV.—EFFECTS OF CERTAIN EXPERIMENTAL COMPOUNDS ON THE CAT NICTITATING MEMBRANE–SUPERIOR CERVICAL GANGLIA PREPARATION

Compd.	Animals, No.	Dose, mg./Kg.	% Drop in Blood Pressure	Time to Return to Predrug Levels, min.	% of Control Epi Response B.P.[a]	N.M.[b]	% of Control Response Pre-G[c]	Post-G[d]
EX 4629	3	10.0	45	23	127	98	29	86
EX 4826	2	10.0	48	90	158	100	0	74
EX 4826	1	15.0	60	50	143	89	0	106
EX 4348	2	10.0	19	50	62	100	99	102
EX 4526	4	7.5	34	180	157	103	0	21
EX 4916	1	10.0	50	30	236	108	30	50
EX 4916	1	15.0	58	83	150	100	40	80
EX 4922	2	5.0	30	90	58	98	90	106

[a] B.P., blood pressure. [b] N.M., nictitating membrane. [c] Pre-G, preganglionic stimulation. [d] Post-G, postganglionic stimulation.

fourth intercostal space at the thoracic level and an electromagnetic flow-probe (14 mm. I.D.) placed around the ascending aorta. The lungs were overinflated, the chest sutured, and the animal permitted to breathe voluntarily. A 3-mm. I.D. flow-probe was fastened around a femoral artery and cardiac output and femoral flow obtained with a Medicon electromagnetic flowmeter. Cardiac output, femoral blood flow, and femoral blood pressure were recorded on a polygraph.

RESULTS

Hypotensive Activity in Rats.—EX 4922 and EX 4526 produced marked prolonged hypotension in the anesthetized rat. Both compounds have the same benzothiadiazinate moiety; EX 4922 is combined with a hydrazine derivative and EX 4526 with 2,2,6,6-tetramethylpiperidine, an entity which in itself is a potent ganglionic blocking structure (1). EX 4826 and EX 4916 produced moderate hypotensive responses with variable duration. Three pempidine derivatives, EX 4272, EX 4629, and EX 4827, were essentially devoid of hypotensive activity in rats. EX 4348 could not be evaluated by this method as a slow continuous fall in blood pressure occurred over several hours, never stabilizing or returning to control levels. As a 50% DMAC solution was used as a solvent for four compounds, six rats were tested for possible solvent effects. Except for a transient hypotensive response, 1 ml./Kg. of 50% DMAC did not alter blood pressure. (See Table II.)

Hypotensive Activity in Dogs.—The effects of the compounds in anesthetized dogs are summarized in Table III. EX 4526, EX 4629, EX 4826, and EX 4916 all produced marked hypotensive responses in dogs with a concomitant attenuation of bilateral carotid occlusion response and potentiation of the pressor effects of the catecholamines and angiotensin. The marked depressor response observed in dogs following EX 4629, EX 4826, and EX 4916 was not observed in the rat. The three compounds

TABLE V.—EFFECTS OF EX 4526 AND EX 4922 ON THE PERFUSION PRESSURE TO THE DENERVATED HINDLIMB OF THE DOG

Expt.	Compd.	Rt.[a]	Dose, mg./Kg.	% Drop in Perfusion Pressure	Recovery, min.	% of Control Epi[b] Response
1	EX 4526	i.a.	7.5	20	20	89
2	EX 4526	i.v.	7.5	13	15	92
3	EX 4922	i.v.	5.0	17	20	50
4	EX 4922	i.v.	5.0	19	12	58

[a] Compounds injected into a femoral vein (i.v.) or directly into the femoral artery inflow tubing (i.a.). [b] Five micrograms of epinephrine administered i.a. to the perfused limb.

TABLE VI.—EFFECTS OF EX 4526, 7.5 mg./Kg. i.v., AND EX 4922, 5 mg./Kg. i.v., ON CARDIAC OUTPUT AND FEMORAL BLOOD FLOW IN THE ANESTHETIZED DOG

Expt.	Compd.	% Drop in Blood Pressure	% of Control Cardiac Output	% of Control Femoral Blood Flow	Duration, min. B.P.	Duration, min. C.O.	Duration, min. F.B.F.
1	EX 4922	33	83	112	80	100+	100+
2	EX 4922	37	79	113	120+	120+	120+
3	EX 4526	37	67	117	120+	120+	120+
4	EX 4526	40	63	111	120+	120+	120+

exhibited a similar recovery pattern in dogs, in that approximately 60% recovery occurred within 15–30 min. EX 4922, 5 mg./Kg., inhibited norepinephrine, angiotensin, and bilateral carotid occlusion pressor responses, while epinephrine produced an initial pressor effect followed by a prolonged depressor effect. EX 4348 produced only transient hypotensive effects and inhibited all pressor responses.

Cat Nictitating Membrane Preparation.—The results of these experiments are summarized in Table IV. EX 4629 and EX 4826 markedly inhibited transmission through the sympathetic ganglia. EX 4922 did not inhibit contractions produced by stimulation of either the pre- or postganglionic nerves but consistently inhibited the pressor response to exogenous epinephrine. EX 4916 inhibited contractions produced by stimulation of either nerve. The preganglionic response appeared to be depressed more than the postganglionic. EX 4526 blocked the preganglionic-induced response completely and depressed the postganglionic contractions by 79%, suggesting a dual action: (*a*) ganglionic blockade and (*b*) a more distal inhibition of sympathetic function.

Denervated Hindlimb of the Dog.—The effects of EX 4526 and EX 4922 on the perfusion pressure to the denervated hindlimb of dogs are summarized in Table V. Both compounds produced a moderate direct vasodilatory action lasting not more than 20 min. EX 4922 markedly inhibited the vasoconstrictor response of 5 mcg. of epinephrine administered into the arterial inflow of the perfused leg.

Effects of EX 4922 on Pithed Cats.—The data obtained in this study agree with the results obtained in the hindlimb perfusion studies. EX 4922 in three cats produced a mean drop in blood pressure of 28.3 ± 2.4% (S.E.) with a duration of 20.7 ± 3.5 min. (S.E.). The pressor response to exogenous epinephrine was 50.7% of normal (S.E. 1.5). The mean control blood pressure of the three pithed cats was 62 ± 2.4 mm Hg (S.E.).

Effects of EX 4526 and EX 4922 on Cardiac Output and Femoral Blood Flow.—The effects of EX 4526 and EX 4922 on cardiac output and femoral blood flow are summarized in Table VI. Both compounds produced a decrease in cardiac output with a concomitant increase in femoral blood flow. EX 4526 produced a greater depression of cardiac output, but femoral blood flow alterations were similar to those produced by EX 4922.

DISCUSSION

EX 4827, 1-(2′,2′,6′,6′-tetramethyl-4′-piperidyl)-3-(4-pyridyl)-5-pyrazolone, and EX 4272, 1-benzyl-3-hydrazinopiperidine dimaleate, did not induce hypotensive effects in anesthetized rats and dogs.

EX 4826, 2,2,6,6-tetramethyl-4-piperidone oxime, produced moderate hypotension in rats with a highly variable duration of action but produced marked hypotensive effects in dogs and cats.

The fourth pempidine related structure studied, EX 4629, 2,2,7,7-tetramethyl-1,4-diazacycloheptan-5-one HCl, like EX 4826, was relatively inactive in anesthetized rats but produced marked hypotensive effects in cats and dogs. Data obtained in the current study indicate that both EX 4826 and EX 4629 are potent ganglionic blocking agents. EX 4629 has structural similarities to EX 4707, 1,2,2,7,7-pentamethyl - 4 - (β - chloroethyl) - 1,4 - diazacycloheptane 2HCl. EX 4707 was found to be hypotensive in rats as well as dogs and cats and has been reported to produce ganglionic blockade (1). Comparing EX 4707 and EX 4629, the data show that an extra nitrogen can be incorporated into the piperidine ring without decreasing the ganglionic blocking activity.

EX 4348, 3-benzyl-3,4-dihydro-6-nitro-7-sulfamoyl-2H-1,2,4-benzothiadiazine 1,1-dioxide, could not be evaluated for hypotensive activity in rats as the compound produced a prolonged very gradual fall in blood pressure, which never stabilized or returned to control values and appeared to be due to a toxic effect of the compound. EX 4348 was nonhypotensive in dogs but was capable of depressing the vascular response to bilateral carotid occlusion, epinephrine, norepinephrine, and angiotensin. Mild hypotensive effects were observed in cats without alteration of sympathetic nerve function.

EX 4916, 2,2,6,6-tetramethyl-4-piperidone 1,1-dioxo - 2H - 1,2,3 - benzothiadiazin - 4 - ylhydrazone acetate, produced moderate depressor effects in anesthetized rats; but the response was highly variable with respect to duration of action. The compound produced marked hypotension in dogs and

cats. However, approximately 60% recovery from the depressor response occurred within 15–30 min. The response to bilateral carotid occlusion was blocked and the epinephrine, norepinephrine, and angiotensin effects potentiated. Transmission through sympathetic ganglia was inhibited but not blocked; and postganglionic transmission also appeared depressed, although not to the same degree as preganglionic transmission. At least part of the hypotensive effect produced by this compound appeared to be due to interference with sympathetic nervous system activity.

EX 4922, 1-hydrazinophthalazine 3,4-dihydro-6-nitro - 7 - sulfamoyl - 1,1,3 - trioxo - 2H - 1,2,4-benzothiadiazinate, produced hypotensive effects in rats, cats, and dogs, with the rat being the most sensitive of the species tested. The compound did not appreciably alter the response of the nictitating membrane to pre- and postganglionic stimulation but did markedly depress the pressor effect of exogenous epinephrine in the cat. EX 4922, 5 mg./Kg. i.v., to dogs attenuated the pressor effect of norepinephrine and reversed the pressor effect of epinephrine to a depressor action. A moderate direct vasodilatory effect was observed in dogs and cats. The data suggest that this compound produces depressor activity by blocking α adrenergic receptors and by a direct depressant action on vascular smooth muscle.

EX 4526, 2,2,6,6-tetramethylpiperidine 3,4-dihydro - 6 - nitro - 7 - sulfamoyl - 1,1,3 - trioxo - 2H-1,2,4-benzothiadiazinate, produced hypotensive effects in rats, cats, and dogs. The compound exerted a depressant action on the sympathetic nervous system at the level of the ganglia and at a more distal locus. The α adrenergic receptors were not inhibited when exogenous epinephrine was administered after EX 4526. The compound also produced a moderate direct vasodilatory effect and marked depression in cardiac output. The data suggest that the following factors may be involved in the hypotensive response elicited by EX 4526: (*a*) a reduction in cardiac output, (*b*) a moderate direct vasodilation, (*c*) an interruption of sympathetic function distal to the ganglia, and (*d*) possible ganglionic blockade.

SUMMARY

The hypotensive activities of four pempidine derivatives and four benzothiadiazine structures related to chlorothiazide and diazoxide are reported. The possible mechanisms of action of the more potent compounds are discussed.

REFERENCES

(1) Buckley, J. P., *et al.*, *J. Pharm. Sci.*, **53**, 24(1964).
(2) Robertson, J. E., Biel, J. H., and DiPierro, F., *J. Med. Chem.*, **6**, 381(1963).
(3) Rubin, A. A., *et al.*, *Science*, **133**, 2067(1961).
(4) Rubin, A. A., *et al.*, *J. Pharmacol. Exptl. Therap.*, **136**, 344(1962).
(5) Bickerton, R. K., *et al.*, *J. Am. Pharm. Assoc., Sci. Ed.*, **49**, 183(1960).
(6) Clonninger, G. L., and Green, H. D., *Am. J. Physiol.*, **181**, 258(1955).

Single-Step Stability Studies

By S. P. ERIKSEN and H. STELMACH

By the use of a reciprocal heating machine, kinetic studies under nonisothermal conditions have been made. These single-step concentration-time-temperature studies permit a complete investigation of those parameters important for stability prediction to be made on one sample, in one run, regardless of whether previous screening studies have been made. The hydrolysis of two esters (ethyl acetate and *p*-nitrophenol acetate) has been followed to demonstrate the suitability of the theory and the method for determining both the energy of activation and the rate constant at any temperature. The potential of a single-step study method such as the one proposed, for investigations where large numbers of temperature sensitivity studies must be made, as for formula stability predictions, will be apparent, but other advantages over classical kinetic methods, such as number of samples and the volume of controlled temperature space required, increase its potential still further.

THE METHOD of exaggerating temperatures in order to accelerate degradation and thus mathematically to predict the shelf-life stability of formulations of pharmaceutical interest has been used considerably in the past 10 years. The literature is replete with examples of both its use and its usefulness (1). The basic procedure, that of determining the concentration independent rate constant at several temperatures, calculating the activation energy and then predicting the rate at shelf temperature, has been changed or improved remarkably little since the idea was first presented, either in the pharmaceutical or the chemical literature. A survey of the pertinent literature indicates that the improvements in the classical method have been limited to the introduction of differential thermal

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