buffered solution of pH 5 was calculated to be 28.3 years at 25°. The use of water-soluble hydrochloride glutethimide is recommended due to high solubility and high stability.

A mechanism is proposed which involves direct attack by a hydroxyl ion on the unhindred carbonyl of the glutethimide, followed by cleavage of the ring to 4-ethyl-4-phenyl glutaramic acid.

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Keyphrases

Glutethimide degradation
Hydrolysis, gluthethimide—factors affecting
Half-life, glutethimide—variable pH
Degradation product—glutethimide
Anionic exchange resin—separation
TLC—identity

Some Pharmacological and Toxicological Properties of Several Phthalimidoaldehydes

By A. M. BURKMAN*, G. L. RINGHAM†, and M. H. WEINSWIG

Four phthalimidoaldehyde derivatives were prepared and several parameters of pharmacological activity were examined: acute toxicity, coordination deficit, ability to alter barbiturate-induced "sleep" in mice, provoke lacrimation and chromodacryorrhea in rats, and influence contractility of excised guinea pig ileum. Gamma-phthalimidobutyraldehyde and α-phthalimido-β-methylbutyraldehyde, the more toxic members of the group, significantly prolonged hexobarbital sleep time. The most intense lacrimatory effects were produced by α-phthalimido-β-methylbutyraldehyde while the most pronounced contractile response in isolated gut was provoked by phthalimidoacetaldehyde. The two latter responses were blocked by atropine premedication. None of the compounds produced chromodacryorrhea.

ALTHOUGH A variety of aldehyde derivatives and acyclic ketones have been examined for hypnotic activity since the introduction of chloral hydrate by Liebreich in 1869 (1), few remain prominent today as therapeutic agents. Most of these substances proved to be less desirable in terms of toxicity or effectiveness than other available hypnotics (2, 3) although chloral hydrate itself still occupies a preeminent position as a highly regarded and widely used soporific (4). In the search for and evaluation of aldehydic substances having potential hypnotic activity, attention was directed toward a group of phthalimidoaldehydes whose biological activity had never been investigated. Although the compounds to be described in this communication are known substances, that is, their syntheses have been reported (5–7), no pharmacological information is available from the literature. It was our intent to prepare and examine several phthalimidoaldehyde derivatives for gross CNS depressant activity. The nature of the responses displayed by animals during an initial toxicity screen would then dictate other pharmacological tests to be pursued.

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METHODS

Synthesis—The phthalimidoaldehydes were all prepared using the Rosenmund reduction procedure

(8). This involved, in all cases, a three-step sequence of reactions in which (*a*) a phthalimido acid was formed by interaction of phthalic anhydride and one of four amino acids, (*b*) conversion of the acid to the acid chloride, and (*c*) reduction of the acid chloride to the aldehyde. Glycine, β-alanine, γ-aminobutyric acid, and valine were used as amino acid reactants and thus the following compounds were formed: phthalimidoacetaldehyde, β-phthalimidopropionaldehyde, γ-phthalimidobutyraldehyde, α-phthalimido-β-methylbutyraldehyde.

Preparation of Drugs for Biological Testing—The compounds, which were all sparingly soluble in water, were administered to animals as an aqueous suspension or, in the case of α-phthalimido-β-methylbutyraldehyde, an emulsion. The vehicle contained 0.25% methylcellulose, 1,500 cps. For the *in vitro* experiments, the drugs were dissolved in polyethylene glycol 200.

Acute Toxicity—Median lethal doses and their 95% confidence intervals were determined in female albino mice[1] using the intraperitoneal route of administration. For each drug three doses, six mice per dose, were used to estimate the position of the dose-lethality line (9).

Coordination Deficit—The method of Dunham and Miya (10) was employed to examine the drugs' ability to alter coordinated motor activity in mice. Animals were placed on a 2.5-cm. diameter horizontal rod rotating at a constant rate of 5 r.p.m. Mice that remained on the rod for 3 min. on each of two consecutive trials were selected for subsequent experimentation. Approximately 96% of the mice drawn from a stock colony met this criterion. Groups of six experimental mice were injected i.p. with doses of the compounds ranging from 50 to 400 mg./Kg. and reexamined for coordination deficit at intervals of 15 min. from injection until 3 hr. had elapsed and at 30-min. intervals thereafter until 6 hr. had elapsed. Animals that failed to remain on the rod *more* than once out of two consecutive trials were judged to suffer from a drug-induced neurological deficit. Twenty-four mice were used for each drug.

Potentiation of Barbiturate Sleep Time—For each of the phthalimidoaldehydes a fixed dose, equivalent to 1.06 mmoles/Kg., was administered to groups of mice. This dose, held constant to facilitate a comparison among drugs, represented no more than one-third the LD_{50} of the most toxic compound. Phthalimidoacetaldehyde was injected into seven groups of six mice. An eighth group of six, which served as a control, received only saline. Sodium hexobarbital, 120 mg./Kg., i.p., was administered to the groups at intervals of 0, 15, 30, 60, 90, 120, and 180 min. from aldehyde injection. "Sleep time" was recorded as that period during which the righting reflex was abolished. An animal was not credited with recovery until it was able to right itself twice within 15 sec. The results were subjected to an analysis of variance and Dunnett's test (11). This protocol was repeated for each aldehyde.

Lacrimation and Chromodacryorrhea—The method described by Malone *et al.* (12), was employed to detect and quantify muscarinic-like activity. Two female albino rats were used to test each of two dose levels of phthalimidoaldehyde. Saline was employed as a "blank" control and methacholine chloride, 10 mg./Kg., as a "positive" control. Lacrimation and chromodacryorrhea scores were assigned at 15-min. intervals for a period of 1 hr. following i.p. injection of the drugs. In subsequent experiments, the drugs' muscarinic activity was similarly evaluated in rats pretreated with atropine sulfate, 1 mg./Kg., i.p.

Contractility of Guinea Pig Ileum—Segments of terminal ileum were excised, suspended in aerated Tyrode's solution (13), and subjected to varying concentrations of the aldehydes which had been dissolved in appropriate amounts of polyethylene glycol 200. Smooth muscle activity was recorded electromechanically *via* an isotonic myograph. The glycol vehicle alone was included among the drugs tested. A single gut segment was used to evaluate the peripheral activity of *each* drug. The segments were initially challenged with two or three small doses of acetylcholine (ACH) chloride (0.001–0.004 mcg./ml. of bath) until stable, reproducible contractile responses were obtained. After the stabilization period, a dose of 0.004 mcg./ml. evoked a "standard" response (for each segment) against which aldehyde responses were subsequently compared. During the course of the experiment gut segments were periodically challenged with acetylcholine Cl, 0.002 mcg./ml. of bath, and atropine sulfate, 0.02 mcg./ml. of bath.

RESULTS AND DISCUSSION

Synthesis—The four phthalimidoaldehydes were successfully prepared in good yields and their melting points, along with those described in the literature, are summarized in Table I. The α-phthalimido-β-methylbutyraldehyde obtained as a viscous oil could not be crystallized. Similar difficulties were evidently encountered by others (7).

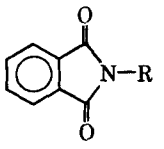
Biological Activity—Table II lists the 24-hr. LD_{50}'s of the four compounds along with their 95% confidence intervals. Phthalimidoacetaldehyde (PIA) and α-phthalimido-β-methylbutyraldehyde (APIM) were the least and most toxic compounds, respectively. The dose-lethality lines for β-phthalimidopropionaldehyde (BPIP) and γ-phthalimidobutyraldehyde (GPIB) were virtually superimpossible and thus their LD_{50}'s did not differ significantly. The dose-lethality lines for all four compounds, having a combined slope of approximately 16.8 probits/log unit, did not deviate significantly from parallelism.

Although PIA was least toxic in terms of lethal dose requirements, it was the only member of the series that elicited clonic convulsions in mice at high doses in the lethal range. Animals usually died in the midst of a convulsive seizure or during a period of extreme excitability which often followed convulsions. No other compound possessed convulsive activity. On the contrary, animals receiving drugs other than PIA usually lapsed into a gradually progressive depression terminating in death or slow recovery. Animals that survived remained markedly depressed for about 24 hr.

At sublethal concentrations each of the drugs, including PIA, was observed to suppress locomotor activity lasting for several hours without loss of the righting reflex. This apparent depressant activity was not reflected in any recognizable coordination deficit. When subjected to doses up to 400 mg./

[1] In this and all subsequent mouse experiments, female albino mice (Harlan ICR strain), 18–22 Gm. were employed.

TABLE I—PHTHALIMIDOALDEHYDES PREPARED FOR STUDY



Compd.	Code	R	Mol. Wt.	Experimental % Yield	Experimental M.P.[a]	Literature % Yield	Literature M.P.[a]
Phthalimidoacetaldehyde	PIA	—CH_2—CHO	189	87	110–112°	70	112–114°
β-Phthalimido-propionaldehyde	BPIP	—$(CH_2)_2$—CHO	203	88	114–116°	65	115–117°
γ-Phthalimido-butyraldehyde	GPIB	—$(CH_2)_3$—CHO	217	17	71–73°	91	72–73°
α-Phthalimido-β-methylbutyraldehyde	APIM	—CH(CHO)—CH(CH_3)(CH_3)	231	...	oil	...	oil

[a] Uncorrected.

TABLE II—ACUTE MEDIAN LETHAL DOSES OF PHTHALIMIDOALDEHYDES IN MICE

Compd.	Intraperitoneal LD_{50} and 95% Confidence Interval mg./Kg.	mmoles/Kg.
PIA	1725 (1531–1932)	9.1 (8.1–10.2
BPIP	1055 (942–1182)	5.2 (4.6–5.8)
GPIB	1055 (942–1182)	4.9 (4.3–5.5)
APIM	800 (721–888)	3.4 (3.0–3.7)

Kg., mice continued to successfully maintain their position on the rotating rod. At no time during the 6-hr. observation period did they fail the test.

In order to assess the subtler aspects of the drugs' CNS depressant action, the compounds' ability to alter hexobarbital-induced depression was investigated. The results are presented in Table III. Analysis revealed that using a fixed dose of 1.06 mmoles/Kg. of each drug only GPIB and APIM produced significant prolongation of sleep time. The dose employed was purposefully chosen so as not to exceed one-third the LD_{50} of the most toxic derivative (APIM). In spite of its relative insolubility significant hexobarbital potentiation was observed for GPIB even when the substance was administered at the same time as hexobarbital (0 premedication time). Although potentiating action could be demonstrated even when the hexobarbital injection was delayed for 120 min., GPIB's influences were apparently dissipated by 180 min. A significant but much less impressive potentiating effect was seen following APIM administration. Significant prolongation of hexobarbital sleep time could be demonstrated only after 60 min. had elapsed between APIM premedication and hexobarbital administration. This suggests that GPIB may be not only intrinsically more active than APIM, but more efficiently absorbed as well. The potentiating activities of GPIB and APIM are not, of course, necessarily reflections of direct CNS action. Their ability to influence hexobarbital sleep could be due to a wholly peripheral effect (*e.g.*, inhibition of barbiturate metabolizing enzymes). Furthermore, failure of the phthalimidoaldehydes to influence rotating rod performance clearly demonstrates that the compounds lack potent depressant activity. This, coupled with the fact that the depression of spontaneous locomotor activity may have been due to peritoneal irritation, leads to the inescapable suspicion that these compounds' central actions are weak at best.

At comparatively high doses the phthalimidoaldehydes were frequently observed to produce lacrimation, salivation, and flushing of the paw pads and ears. These observations prompted experiments designed to evaluate the drugs' cholinomimetic action *in vivo* and *in vitro*. Chromodacryorrhea, the secretion of porphyrin pigments by the Harderian gland (14) first described by Freud (15), was characteristically evoked by muscarinic stimulants. Although the response was readily provoked by methacholine, it was not elicited by any of the aldehydes (Table IV).

All compounds, except GPIB, increased lacrimatory responses and premedication of the animals with atropine successfully abolished these effects. APIM was responsible for the most intense lacrimation at a dose level of 2.1 mmoles/Kg. These cholinergic-like effects are not manifested until fairly high doses are attained and at these concentrations all animals were distinctly sedated.

TABLE III—INFLUENCE OF PHTHALIMIDOALDEHYDES ON HEXOBARBITAL SLEEP TIME IN MICE[a]

Compd.[b]	Control	Premedication Time, Min. 0	15	30	60	90	120	180
PIA	74 ± 10[c]	96 ± 14	85 ± 23	107 ± 12	92 ± 11	74 ± 13	71 ± 7	77 ± 7
BPIP	78 ± 13	64 ± 13	100 ± 24	97 ± 9	89 ± 10	83 ± 12	73 ± 9	57 ± 8
GPIB	51 ± 7	120 ± 22[d]	169 ± 22[d]	134 ± 19[d]	159 ± 24[d]	126 ± 20[d]	131 ± 6[d]	101 ± 16
APIM	72 ± 9	108 ± 15	111 ± 18	104 ± 14	141 ± 22[d]	109 ± 14	96 ± 11	87 ± 11

[a] Sodium hexobarbital, 120 mg./Kg., i.p. [b] Each compound was administered in fixed molar equivalent quantities (1.06 mmoles/Kg.). [c] Mean sleep time in min. ± standard error (6–10 mice/determination). [d] Significant at 5% level (1-tailed).

TABLE IV—LACRIMATION AND CHROMODACRYORRHEA RESPONSES

Compd. and Dose	Response[a]	Time from Injection, min. 15	30	45	60
Saline control	L	4.3[b]	3.3	2.3	2.8
	C	0	0	0	0
Methacholine Cl, 10 mg./Kg.	L	13.8	8.0	3.0	3.3
	C	5.8	0	0	0
Methacholine, Cl 10 mg./Kg. and	L	4.0	2.3	...	...
Atropine SO_4, 1 mg./Kg.	C	0	0	...	...
PIA, 400 mg./Kg. (2.1 mmoles)	L	8.8	5.0	5.5	3.0
	C	0	0	0	0
800 mg./Kg. (4.2 mmoles)	L	20.0	11.3	5.5	5.5
	C	0	0	0	0
PIA, 800 mg./Kg. and	L	10.5	7.5	...	...
Atropine SO_4, 1 mg./Kg.	C	0	0	...	...
BPIP, 300 mg./Kg. (1.4 mmoles)	L	6.3	6.0	3.3	1.5
	C	0	0	0	0
600 mg./Kg. (2.8 mmoles)	L	10.0	6.3	3.3	2.8
	C	0	0	0	0
BPIP, 600 mg./Kg. and	L	2.0	4.0	...	...
Atropine SO_4, 1 mg./Kg.	C	0	0	...	...
GPIB, 300 mg./Kg. (1.4 mmoles)	L	3.0	4.0	2.5	2.5
	C	0	0	0	0
600 mg./Kg. (2.8 mmoles)	L	3.8	3.8	2.8	3.0
	C	0	0	0	0
APIM, 300 mg./Kg. (1.3 mmoles)	L	9.3	2.3	3.8	2.5
	C	0	0	0	0
500 mg./Kg. (2.1 mmoles)	L	14.0	7.0	4.8	3.3
	C	0	0	0	0
APIM, 500 mg./Kg. and	L	6.8	6.3	...	...
Atropine SO_4, 1 mg./Kg.	C	0	0	...	...

[a] L-lacrimation; C-chromodacryorrhea. [b] Scores are in millimeters, the mean values derived from two albino rats; the higher the score the greater the intensity of response.

TABLE V—INFLUENCE OF PHTHALIMIDOALDEHYDES ON ISOLATED GUINEA PIG ILEUM

Ileum Segment[a]	ACH Response, mm.[b]	Drug	Dose, mmoles/ml. bath	Drug Response mm.	% ACH	CD50[c], mmoles/ml. bath
1	12	PIA	1.0×10^{-3}	14	1.17	
			8.0×10^{-4}	10	0.84	5.8×10^{-4}
			5.0×10^{-4}	4	0.33	
2	10	BPIP	1.0×10^{-3}	6	0.60	
			8.0×10^{-4}	4	0.40	8.9×10^{-4}
			5.0×10^{-4}	0.5	0.05	
3	16	GPIB	1.2×10^{-3}	10	0.62	
			1.0×10^{-3}	7.5	0.47	1.0×10^{-3}
			8.0×10^{-4}	5	0.32	
			5.0×10^{-4}	0	0	
4	10	APIM	1.0×10^{-3}	−20	−2.00	
			5.0×10^{-4}	−9.5	−0.95	...[d]

[a] Adjacent segments, each approximately 1 cm. in length, were cut from a 5-cm. section of terminal ileum. [b] The "standard" response evoked by acetylcholine chloride, 0.004 mcg./ml. of bath; in mm. of polygraph pen deflection. [c] Contractile dose 50: the interpolated dose of aldehyde producing a contractile response one half that evoked by the "standard" dose of ACH [d] Only depression of gut tone was observed.

PIA provoked the most intense contractile response in isolated gut (Table V). BPIP and GPIB were considerably less active as spasmogens while APIM produced only a sustained relaxation of ileal musculature. Both the stimulant and depressant effects were dose-dependent and inspection revealed that the log dose-percent response lines closely approached parallelism. A comparatively long onset time (about 20–25 sec.) was associated with the stimulant response while APIM's depressant action was seen within 3–5 sec. of drug administration. Contractions could be prevented by the prior introduction of atropine into the tissue bath. Responsiveness of depressed tissues to ACH remained essentially unaltered. Control vehicle, polyethylene glycol 200, produced no observable responses in the doses used.

SUMMARY

(*a*) Four phthalimidoaldehydes were prepared and examined for biological activity: phthalimidoacetaldehyde (PIA); β-phthalimidopropionaldehyde (BPIP); γ-phthalimidobutyraldehyde (GPIB), and α-phthalimido-β-methylbutyraldehyde (APIM).

(*b*) The order of decreasing i.p. toxicity in mice was APIM, BPIP (GPIB), PIA.

(*c*) PIA, alone among the aldehydes, produced hyperactivity and convulsions in mice when administered in lethal or near-lethal concentrations. At lower doses all compounds produced distinct sedation in both rats and mice. The sedative action expressed itself primarily as a depression of spontaneous locomotor activity. Sedated mice, however, did not exhibit any coordination deficit as

determined by their ability to maintain their position on a rotating rod.

(*d*) The most pronounced prolongation of hexobarbital sleep time in mice was produced by GPIB. Less impressive, but nonetheless significant, was the sleep potentiating effect of APIM. In equimolar concentrations neither PIA nor BPIP exhibited any influence on barbiturate depression.

(*e*) At comparatively high doses, the aldehydes were frequently observed to produce lacrimation, salivation, and flushing of the paw pads and ears, observations that prompted examination of the drugs for possible cholinomimetic activities.

(*f*) The estimated order of decreasing activity as lacrimatory stimulants in rats was APIM, BPIP (PIA), GPIB. This action was blocked by atropine premedication.

(*g*) None of the aldehydes provoked chromodacryorrhea in rats.

(*h*) PIA and BPIP and GPIB evoked short-lived tonic contractions in isolated guinea pig ileum that could be blocked by atropine. APIM produced only mild gut relaxation. There appears to be little relationship between the aldehydes' spasmogenic properties and their ability to stimulate lacrimation.

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Keyphrases

Phthalimidoaldehydes—synthesis, pharmacology
LD_{50} values—phthalimidoaldehydes
Locomotor activity—depressed
Hexobarbital sleep—prolonged
Lacrimatory responses—increased
Spasmogenic activity—evaluated

Thin-Layer Chromatography and IR Spectrophotometry of Commercial Sodium Sulfobromophthalein Solutions

By F. BARBIER and G. A. DEWEERDT

Techniques and results of analysis of some commercially available sulfobromophthalein (phenoltetrabromphthalein disulfonate) solutions by means of TLC and IR spectrophotometry are reported. The IR spectra of sulfobromophthalein and sulfobromophthalein-like compounds are presented and discussed. Three of the additional fractions found could be identified as phenoltetrabromphthalein and its mono- and trisulfonate derivatives. These impurities probably are due to imperfections in the synthesis of sulfobromophthalein. With but one exception they represent less than 1 percent of the total absorbance at 578 mμ. Such low concentrations do not interfere with the clinical use of sulfobromophthalein.

DESPITE THE WIDE use and established value of sulfobromophthalein (phenoltetrabromphthalein disulfonate) in the diagnosis of liver disease, little information is available on the composition of the dye solutions for intravenous use (1–5). The purpose of this study was to analyze some sulfobromophthalein solutions, commercially available in Belgium, with the aid of thin-layer chromatography (TLC) and IR spectrophotometry.

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METHODS

Sulfobromophthalein solutions for intravenous use and marketed by the following laboratories were analyzed: Vitarine Co., lot 9364; E. Merck, lots R493, R879, R959, and T959; Hynson, Westcott and Dunning, lots P_{23}, R_{96}, T_{47}, and U_{28}; and Simes, lot 3E29.

Thin-layer Chromatography—TLC was carried