Kinetics of Degradation of Glutethimide

By J. W. WESOLOWSKI*, SEYMOUR M. BLAUG, TING-FONG CHIN, and JOHN L. LACH

The hydrolysis of glutethimide (2-ethyl-2-phenyl-glutarimide) in buffered aqueous solutions in the pH range 1.5–8.0 has been studied. This region exhibited specific base catalysis. The overall rate may be expressed by the equation, rate = k' (glutethimide) at constant pH. The energy of activation, corrected for heat of ionization of water, was calculated. A mechanism of hydrolysis is proposed.

GLUTETHIMIDE is a widely used nonbarbiturate sedative hypnotic (1). It belongs to a relatively new medicinal group of compounds, the glutarimides, which exhibit CNS depressant as well as stimulant properties (2–4). A review of the literature showed that very little kinetic work has been done on glutarimides (5–8). In view of the increasing importance of this group of compounds it was felt that a quantitative investigation of the hydrolysis rate of glutethimide was warranted.

Investigations on the hydrolysis of succinimide (9) and phthalimide (10, 11) have shown that the ionization of the imide has a strong effect on the course of hydrolysis. In the acid region where the imide is undissociated, specific base catalysis has been observed. The presence of the imide anion causes a decrease in the hydrolysis rate. A complex rate expression has been derived to explain the observed effect (10).

Glutethimide hydrolyzes in weakly basic solution to 4-ethyl-4-phenyl glutaramic acid (EPG) (8, 12, 13) and to the corresponding glutaric acid in 40% potassium hydroxide (12). In the pH range studied, 1.5–8.0, it was expected that glutethimide ionization would be very small and that the predominant product of hydrolysis would be EPG.

An ion-exchange procedure was used to follow the course of glutethimide degradation. A strongly basic quaternary amine anionic-exchange resin specially purified (washed with 10% phosphoric acid and with methanol), was used to separate the glutethimide from EPG. The glutethimide was then eluted from the column by methanol and assayed spectrophotometrically at 257.5 mμ.

EXPERIMENTAL

Reagents and Apparatus—Glutethimide, recrystallized m.p. 85–86°; glutethimide NF reference standard; EPG m.p. 156–157° (prepared by refluxing glutethimide in a 10% sodium hydroxide solution for 1 hr.); sodium hydroxide, sodium chloride, C.P. Merck; monobasic, dibasic, and tribasic sodium phosphate; anhydrous methanol, reagent grade, Mallinckrodt; orthophosphoric acid, reagent grade, Allied Chemical; Anion-exchange (Cl), 50 mesh, analytical reagent grade resin[1]; Beckman DU spectrophotometer and 5-cm. cells; glass columns, 1 × 20 cm. with a glass stopcock at one end and a 100-ml. reservoir at the other end; glass columns, 2 × 45 cm. with Teflon stopcocks (Fisher); constant temperature bath with Sargent heater, circulator, and regulator, Model No. S-PN-2770; thermometer calibrated to 0.1°.

The buffers used in this study were: pH 1.5—0.4 *M* phosphoric acid; pH 2.35, 3, 4, and 5—0.4 *M* Na_2HPO_4–0.4 *M* H_3PO_4; pH 6, and 6.5—0.4 *M* Na_2HPO_4–0.4 *M* NaH_2PO_4; pH 7, 7.5 and 8—0.4 *M* Na_3PO_4–0.4 *M* NaH_2PO_4. All buffer solutions were adjusted with the aid of a Beckman model H2 pH meter. The same phosphate buffer system was used throughout the study in that the pH of this buffer is relatively independent of temperature.

Procedures—The following general procedure was used for studying the effect of pH and temperature on the rate of hydrolysis of glutethimide.

An accurately weighed glutethimide stock solution was prepared in anhydrous methanol, such that 5 ml. contained 100 mg. of glutethimide. The buffer solutions were preheated to the temperature at which the particular run was made. A 100-ml. volumetric flask was filled with 50 ml. of the preheated buffer and 5 ml. of the methanolic glutethimide stock solution was pipeted in with constant swirling. The solution was diluted with buffer to volume, mixed, and placed in the bath. After a lapse of 10 min. to allow the solution to thermally equilibrate, 5 ml. representing the zero hour sample was withdrawn, transferred onto a column of the anion-exchange resin, and 20 ml. of cold deionized water was added to the column. The sample was passed through the column at the rate of 1.5 ml./min. When the solution level reached the top of the resin, anhydrous methanol was added as the eluent and the flow rate was increased to 5–7 ml./min. One-hundred milliliters was collected in a volumetric flask and assayed spectrophotometrically at 257.5 mμ, using the eluent collected from a blank column as a blank.

Samples were removed from the volumetric flask at definite time intervals and analyzed by this procedure.

pH and Temperature Effect on the Rate of Hydrolysis—In order to study the effect of pH and

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[1] Dowex 2-4x. Obtained from J. T. Baker Chemical Co.

temperature on the rate of hydrolysis of glutethimide, the reaction was carried out at the different temperatures, in buffer solution, as follows: 50°—pH 6, 6.5, 7, 7.5, and 8.0; 60°—pH 5, 6.5, 7, 7.5, and 8.0; 65°—pH 6, 6.5, 7, 7.5, and 8.0. Since glutethimide solutions at pH's 1.5 to 5.0 show insignificant degradation after 90 hr. at 65°, the data are not included.

Effect of Ionic Strength—The effect of ionic strength on the rate of hydrolysis was determined by studying the hydrolytic rate at 65° of a solution of glutethimide in 0.4 *M* phosphate buffer at pH 7.5 and comparing the rate of hydrolysis with a solution of glutethimide of the same concentration containing 1% potassium chloride. The effect of buffer concentration on hydrolytic rate was also studied by following the hydrolysis rate at 65°–pH 7.5 in 0.2 *M* phosphate buffer.

Effect of Glutethimide Concentration—Solutions of glutethimide containing 2.217×10^{-4}, 1.915×10^{-c}, and 1.495×10^{-4} moles/L. in 0.4 *M* phosphate buffer of pH 7.5 were prepared and the rate of hydrolysis followed at 65° in the manner described.

DISCUSSION

In the pH range 5–8 studied (Figs. 1–3), the hydrolysis of glutethimide appears to be base

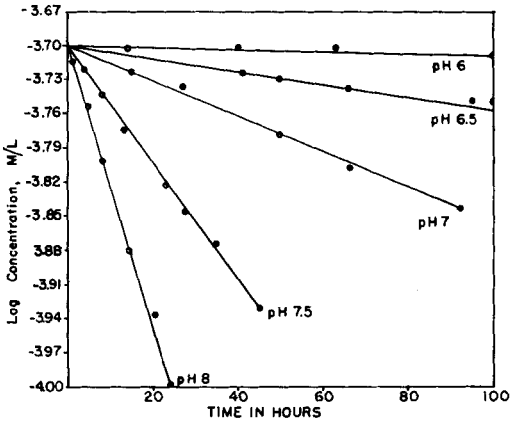


Fig. 1—Log of concentration of glutethimide against time at 50° at various pH's.

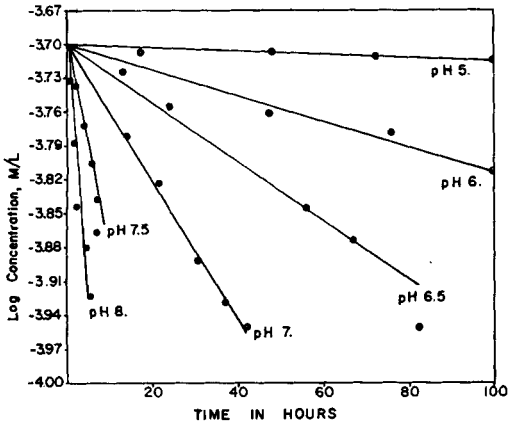


Fig. 2—Log concentration of glutethimide against time at 60° at various pH's.

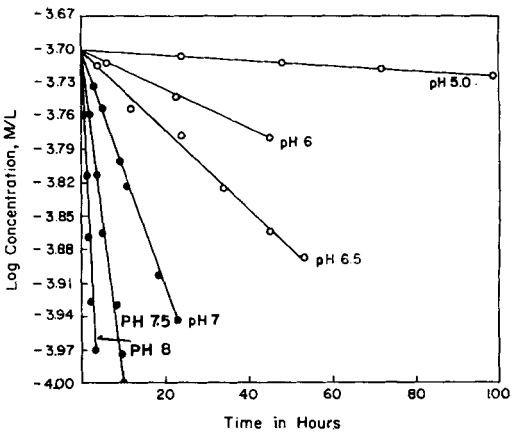


Fig. 3—Log concentration of glutethimide against time at 65° at various pH's.

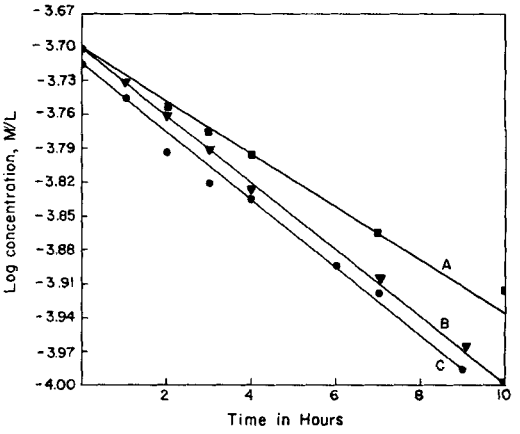


Fig. 4—Effect of buffer concentration and ionic strength on the hydrolysis rate of glutethimide at 65° in phosphate buffer at pH 7.5. Key: A, 0.4 M buffer + 1% KCl; B, 0.4 M buffer; C, 0.2 M buffer.

catalyzed. Figure 4 shows that although the phosphate buffer system was the only one used in this study, no discernible change in the hydrolytic rate was observed when the reaction was carried out in 0.2 and 0.4 *M* buffer solutions, suggesting a specific base catalysis.

It has been reported that the rate of hydrolysis of succinimide (9) and phthalimide (10, 11) is dependent upon the concentration of ionized as well as unionized form of the cyclic imide. Therefore, a decrease in the rate should be observed as the proportion of dissociated to undissociated imide increases. The imide anion is not readily attacked by the hydroxyl ion, in that greater energy would be required to overcome the electrostatic repulsive forces present in such a system.

In a previous work (10) a general second-order rate expression was reported which took into consideration the contribution of the ionized as well as the unionized species on the rate observed. Since the present work dealt with the lower pH region, k_a was considered negligible in comparison to the hydrogen ion concentration since the anionic species is insignificant in this pH range.

The hydrolysis rate is first order with respect to glutethimide since a straight line is obtained when

the logarithm of concentration is plotted against time (Figs. 1–3). This can be further verified (Fig. 5) by plotting the effect of initial glutethimide concentration on the hydrolysis rate. The plot shows the first-order dependence of the reaction with respect to glutethimide, the rate of the reaction being directly proportional to the concentration of the substrate.

The order of the reaction with respect to hydroxyl ion concentration can be determined from the relationship

$$\log k = \log C + n\text{pH}$$

A plot of log *k' versus* pH should give a straight line with a slope of *n*. Figure 6 shows that the slope, 0.897, is not in agreement with the expected value of 1.0. The deviation may be due to the analytical

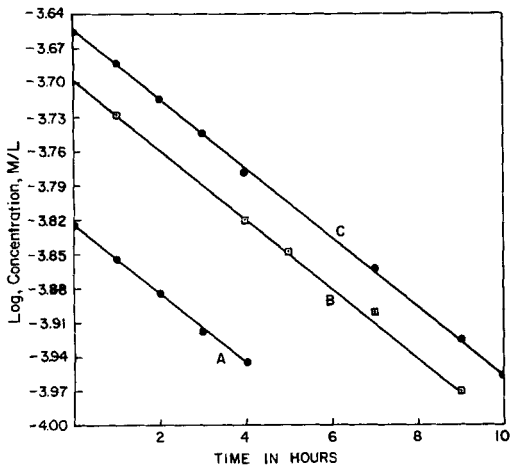


Fig. 5—Effect of initial concentration on the hydrolysis rate of glutethimide at 65°, pH 7.5. Key: A, *1.495 × 10⁻⁴ moles/L.;* B, *1.915 × 10⁻⁴ moles/L.;* C, *2.217 × 10⁻⁴ moles/L.*

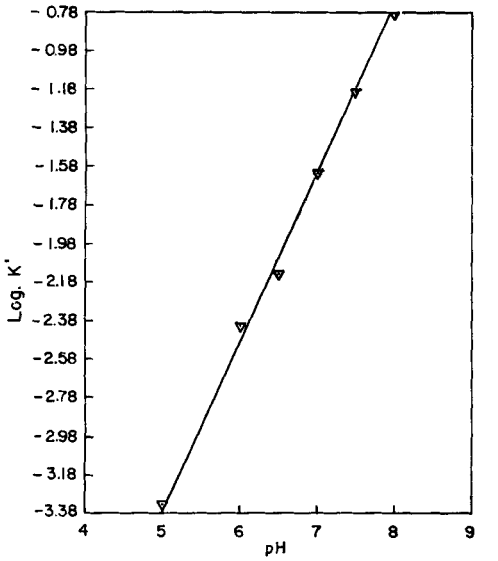


Fig. 6—Log K', the specific reaction constant against pH at 65°.

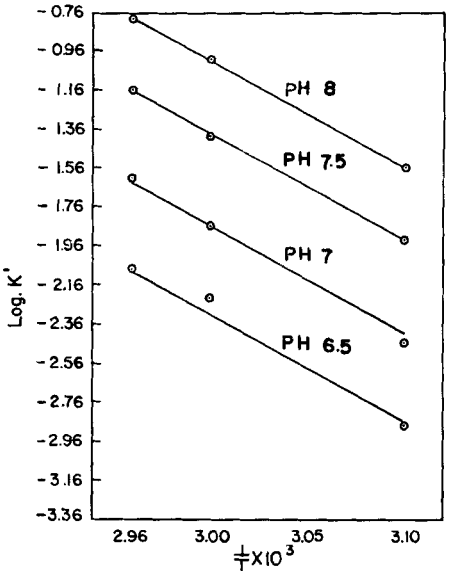


Fig. 7—Plot of log K' versus 1/T showing the temperature dependence of the rate of hydrolysis of glutethimide at various pH's.

procedure, *i.e.*, ion-exchange separation of glutethimide from EPG.

Although the ionic strength in 0.4 *M* phosphate buffers is beyond the limits of the Debye-Hückel limiting law (14), it nevertheless was desired to determine whether primary salt effects existed in the hydrolysis of glutethimide. The slight decrease in rate observed upon the addition of 1% potassium chloride (Fig. 4) cannot be unequivocally explained at this time. A plausible explanation may be that a "salting-out" effect on the slightly soluble glutethimide was brought about due to the increased electrolyte concentration.

The Arrhenius plot (Fig. 7) of log *k' versus* 1/*T* was found to give a straight line with a constant apparent energy of activation, E_a, of 25.86 Kcal. The apparent energy of activation, E_a, for the three temperatures, corrected for the ΔHi of water by the method of Harned and Hamer (15) are listed in Table I. The half-lives, $t_{1/2}$, of glutethimide at different pH were calculated for 25° by the Clausius-Clapeyron equation and are listed in Table II. A plot of log $t_{1/2}$ *versus* pH (Fig. 8) shows that below pH 5 glutethimide is very stable since at pH 1.0 and 5.0 the rate is independent of hydrogen ion concentration.

Mechanism of Hydrolysis—The hydrolysis of glutethimide probably involves a bimolecular (S_N2) mechanism (16) with direct attack by the hydroxyl ion being the rate-determining (slow step) step of hydrolysis as shown in Scheme I.

Bimolecular mechanisms have been known to be

TABLE I—ENERGY OF ACTIVATION CORRECTED FOR HEAT OF IONIZATION OF WATER[a]

50°—13.47 Kcal.
60°—13.92 Kcal.
65°—14.15 Kcal.

[a] Heats of ionization of water calculated according to Harned and Hamer (15) were found to be: 50°—12.39 Kcal., 60°—11.936 Kcal., and 65°—11.71 Kcal.

TABLE II—HALF-LIFE PERIODS, $t_{1/2}$, FOR THE HYDROLYSIS OF GLUTETHIMIDE AT DIFFERENT pH'S AND AT 25°

pH	$t_{1/2}$ (months)	log $t_{1/2}$[a]
8.0	1.02	0.008
7.5	2.43	0.386
7.0	6.82	0.840
6.5	22.10	1.345
6.0	43.40	1.637
5.0	351.00	2.546

[a] Calculated using the apparent activation energy, E_a, from the Arrhenius plot, and the specific rate constant at 25°.

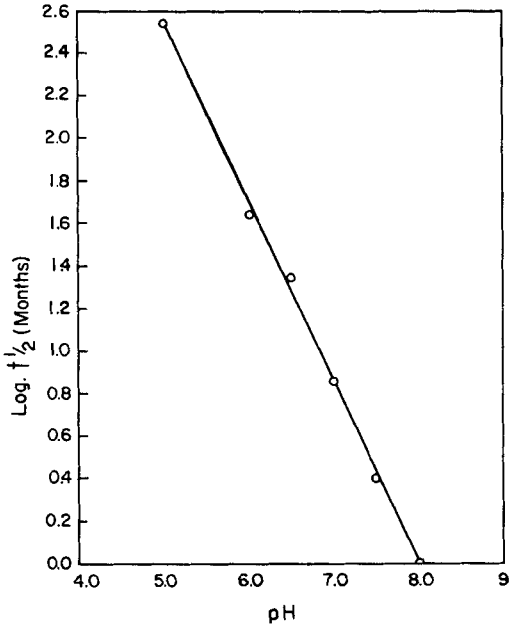
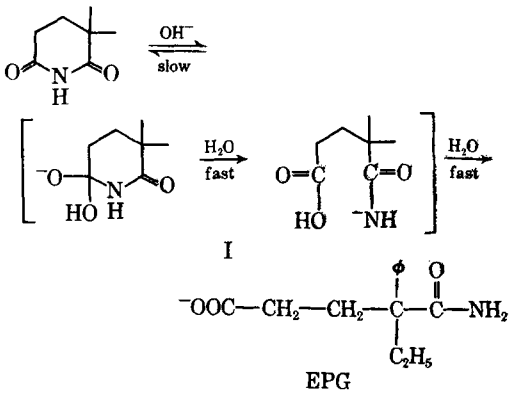


Fig. 8—Log $t_{1/2}$, the half-life of the reaction in months against pH at 25°.

subject to steric hindrance. The rear of the carbon where displacement of the C—N bond is occurring must be unhindered in order to permit facile entrance of the attacking hydroxyl group for the formation of the reaction intermediate (I). Therefore, the reaction may be expected to be sensitive to steric hindrance at the site of reaction but relatively insensitive to electronic effects.

Fischer-Hirschfelder models of glutethimide and of the postulated intermediate in the hydrolysis mechanism (I) were prepared. Steric hindrance due to the 2-ethyl-2-phenyl substituents appears to be quite effective on the 1-carbonyl carbon. The 5-carbonyl carbon is comparatively unhindered for attack by the hydroxyl ion, and 4-ethyl-4-phenyl glutaramic acid would be the main product from such an attack. Further hydrolysis to the corresponding dicarboxylic acid should occur only under adverse conditions due to the same steric retardation. This view is substantiated by a report (12) indicating that 40% potassium hydroxide is necessary for the amide cleavage.

No attempt was made to isolate the postulated degradation product, 4-ethyl-4-phenyl glutaramic acid (EPG), in the kinetic study. However, using thin-layer chromatography, EPG was found to be the degradation product of glutethimide. EPG



Scheme I

was prepared by refluxing a 5-Gm. sample of glutethimide in 50 ml. of a 10% sodium hydroxide solution for 2 hr. The solution was cooled and acidified with concentrated hydrochloric acid to a Congo Red end point. The precipitated solid was collected on a Büchner funnel and washed with deionized water. The compound recrystallized from 20% ethanol had a m.p. 156–157°.

Anal.—Calcd. for $C_{13}H_{17}NO_3$: C, 66.32; H, 7.28; N, 5.95. Found: C, 66.81; H, 7.39, N, 6.01.

A solution containing 100 mg./100 ml. of glutethimide, adjusted to pH 8.0 with phosphate buffer, was prepared. The solution was heated in a constant temperature bath adjusted to 65°. After 24 hr. the solution was cooled and a sample was spotted on a prepared thin-layer plate beside a sample taken from a solution containing ungraded glutethimide plus EPG. A methanol–acetone–triethanolamine solvent was used for development and Dragendorff's reagent for visualization. The sample taken from the glutethimide solution that had been heated for 24 hr. showed two spots which corresponded to the two spots obtained from the sample containing undegraded glutethimide and EPG.

Recent work on the hydrolysis kinetics of phthalimide and *ortho*-carboxyphthalimide (17) shows that a small region in the very low pH range exists where acid catalysis occurs at 100°. However, similar acid catalysis was unobserved in the pH ranges and temperatures employed in this study.

SUMMARY AND CONCLUSION

The degradation of glutethimide in buffer solution of pH's ranging from 5.0–8.0 at 65, 60, and 50° has been studied. The degradation of glutethimide in aqueous solutions appears to be a base-catalyzed reaction, since the contribution of ionized species to the reaction rate in the pH range studied is very small. The rate is first order with respect to the concentration of glutethimide.

The apparent energy of activation, E_a, for the degradation of glutethimide at pH 8.0 was found to be of the order of 25.86 Kcal. Corrections were made for the apparent energy of activation, E_a, for the heat of ionization of water in the temperature range studied.

The half-lives at 25° for glutethimide in aqueous solutions were determined from the calculated specific reaction constants for reactions at different pH's and at 25°. The $t_{1/2}$ of glutethimide in a

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.

REFERENCES

(1) Tripod, J., and Gross, F., *Helv. Physiol. Pharmacol. Acta*, **15**, 104(1957).
(2) Shaw, P. H., Nelson, S. E., Vass, N., Shulman, A., Anstee, J. R., and Nelson, E. R., *Nature*, **174**, 402(1954).
(3) Shulman, A., *Brit. Med. J.*, **1**, 1238(1955).
(4) Somers, T. C., *Nature*, **178**, 996(1957).
(5) Sircar, S. S. G., *J. Chem. Soc.*, **1927**, 600.
(6) *Ibid.*, **1927**, 1252.
(7) Hall, H. K. Jr., Brandt, M. K., and Mason, R. M., *J. Am. Chem. Soc.*, **80**, 6420(1958).
(8) Yamana, T., and Mizukami, Y., *Yakagaku Zasshi*, **81**, 1383(1961).
(9) Edward, J. T., and Terry, K. A., *J. Chem. Soc.*, **1957**, 3527.
(10) Tirouglet, J., and LeTrouit, E., *Compt. Rend.*, **241**, 1053(1955).
(11) Tirouflet, J., and LeTrouit, E., *Bull. Soc. Chim., France*, **1957**, 570.
(12) Salmon-Legagneur, F., and Neveu, C., *Compt. Rend.*, **234**, 1060(1952).
(13) Sheppard, H., D'Asara, B. S., and Pijmmer, A. J., *J. Am. Pharm. Assoc., Sci. Ed.*, **45**, 681(1956).
(14) Laidler, K. J., "Chemical Kinetics," McGraw-Hill Book Co., Inc., New York, N. Y., 1965, p. 229.
(15) Harned, H. S., and Hamer, W. J., *J. Am. Chem. Soc.*, **55**, 2194(1933).
(16) Gould, E. S., "Mechanism and Structure in Organic Chemistry," Holt, Rinehart and Winston, Inc., New York, N. Y., 1962, p. 251.
(17) Zerner, B., and Bender, M. L., *J. Am. Chem. Soc.*, **83**, 2267(1961).

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