tration. The overall shape of the pH–rate profile obtained for tecomine is not uncommon among degradation patterns of weak bases involving either oxidation or hydrolysis; similar profiles were obtained in the oxidative degradation of morphine (8) and in the hydrolysis of homatropine (9). From the study of the effect of pH on the rate of tecomine degradation, it can be deduced that acid medium, below pH 4, is appropriate for obtaining a stable tecomine preparation.

Effect of Temperature on Tecomine Degradation—The rate of tecomine degradation was determined at pH 10.4 and temperatures of 40, 70, and 80° (Figs. 3–5). Figure 7 represents the Arrhenius plot from which the value of k at 25° was found to be 1.995×10^{-3} hr. and $t_{1/2}$ = 14 days at pH 10.4. The energy of activation and the frequency factor were calculated and found to be 26.67 kcal./mole and 1.099×10^{13}/min., respectively.

Effect of Antioxidants on Tecomine Degradation—The rate of tecomine degradation in alkaline medium was significantly decreased in the presence of 0.1% sodium sulfite (Fig. 4); furthermore, the presence of antioxidant delayed the onset of degradation for almost 2 weeks at pH 10.4 and 40°. The results obtained increase the probability of oxidation being the mechanism responsible for degradation.

CONCLUSION

The results of the stability study performed on tecomine point to an oxidation reaction, accompanied by the formation of highly conjugated colored oxidation products, as the most probable degradation route. The decrease in the degradation rate in the presence of antioxidant, the appearance of colored degradation products, and the presence in tecomine of a carbonyl group susceptible to enolization all point to the probability of oxidation. The changes observed in the UV spectrum of tecomine during degradation are in accordance with the postulated degradation route. Theoretically, the peak at 227 nm. resulting from the presence in tecomine of a conjugated system may be subject to a bathochromic shift if further conjugation is added to the structure. Or it may disappear without the appearance of additional peaks if the original conjugation is destroyed. In the present study, the disappearance of the peak at 227 nm. during degradation was accompanied by the occurrence of absorption in the visible range, thus favoring the formation of highly conjugated colored oxidation products.

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ACKNOWLEDGMENTS AND ADDRESSES

Received November 4, 1970, from the *Pharmaceutics Department, Faculty of Pharmacy, Alexandria University, U.A.R.*

Accepted for publication March 17, 1971.

Kinetics of Hydrolysis of Barbituric Acid Derivatives

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Abstract □ The neutral and alkaline hydrolyses of barbituric acid and some of its substituted derivatives were followed spectrophotometrically. The rate–pH profiles for all of the 5,5-disubstituted barbiturates were similar. A different profile was observed for barbituric acid because of its comparatively low pKa_1'. The rate–pH profile of metharbital showed no curvature at high pH values, since the 1-methyl substituent prevents the second possible proton dissociation. All profiles could be explained by hydroxyl-ion attack on the undissociated and monoanion forms of the barbiturate, whereas the dianions are unreactive. The reactivity of the barbiturates was correlated with the Newman rule of six as a measure of steric influence on hydrolysis. A previously unmentioned, reversible system between the barbiturate and its ring-opened malonuric acid derivative was observed and may be used to postulate that the fraction decomposing *via* 1,6 ring opening is a function of pH rather than a direct consequence of a unique degradation pathway for the ionic form of the barbiturate. Ionic strength effects in the alkaline hydrolyses of amobarbital and phenobarbital increase the rate of barbiturate degradation by the attack of hydroxyl ion on the monoanion and/or its kinetic equivalent. The Arrhenius parameters for all compounds were determined.

Keyphrases □ Barbituric acid and 5,5-disubstituted barbiturates—hydrolysis kinetics, pH effect □ Hydrolysis kinetics, parameters—barbituric acid and derivatives □ UV spectrophotometry—monitoring, barbituric acid hydrolysis

Interest in the kinetics of barbiturate solvolysis arose from studies on halouracils and halouridines which showed that barbituric acid and ribosylbarbituric acid, respectively, were formed as hydrolytic intermediates in strong alkali (1, 2). Although the decomposition of barbiturate salts in aqueous solution or in the presence of alkali from room to autoclaving temperatures was reported in a descriptive manner (3–12), systematic kinetic approaches to the hydrolysis of barbiturates were undertaken in only a few instances (13–20). These studies include the excellent work of Eriksson and coworkers (16–19) on the hydrolysis of 1,5,5-trisubstituted barbiturates and the only published report on thiobarbiturate solvolysis by Goto *et al.* (20). Nevertheless, quantitative kinetic data pertaining to the stability of many pharmaceutically useful barbiturates are still lacking. The literature indicates that many questions regarding the alkaline hydrolysis of barbituric acid derivatives, such as mechanism, ionic strength effects, rate–pH profiles,

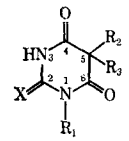


Table I—Compounds Used in This Study

Number	Compound	Substituents R1	R2	R3	X	λmax. (nm.) of Monoanion at pH 10.1	Source
1	Barbituric acid	H	H	H	O	256	Eastman Organic Chemicals
2	Amobarbital	H	$-CH_2-CH_3$	$-CH_2-CH_2-CH(CH_3)_2$	O	239	Eli Lilly and Co.
3	Barbital	H	$-CH_2-CH_3$	$-CH_2-CH_3$	O	238	Retort Pharmaceutical Co.
4	Butabarbital	H	$-CH_2-CH_3$	$-CH(CH_3)-CH_2-CH_3$	O	239	McNeil Laboratories
5	Butethal	H	$-CH_2-CH_3$	$-CH_2-CH_2-CH_2-CH_3$	O	238	Abbott Laboratories
6	Cyclobarbital	H	$-CH_2-CH_3$		O	233	Sterling Winthrop Research Institute
7	Pentobarbital	H	$-CH_2-CH_3$	$-CH(CH_3)-CH_2-CH_2-CH_3$	O	239	Abbott Laboratories
8	Phenobarbital	H	$-CH_2-CH_3$		O	240	Mallinckrodt Chemical Co.
9	Vinbarbital	H	$-CH_2-CH_3$	$-C(CH_3)=CH-CH_2-CH_3$	O	236	Merck Sharp & Dohme Laboratories
10	Secobarbital	H	$-CH_2-CH=CH_2$	$-CH(CH_3)-CH_2-CH_2-CH_3$	O	239	Eli Lilly and Co.
11	Thiamylal	H	$-CH_2-CH=CH_2$	$-CH(CH_3)-CH_2-CH_2-CH_3$	S	303	Parke, Davis & Co.
12	Metharbital	CH_3	$-CH_2-CH_3$	$-CH_2-CH_3$	O	245	Abbott Laboratories

and structure–hydrolytic activity relationships, have not been completely answered. These present studies on therapeutically useful barbiturates (Table I) were begun with the expectation that a systematic quantitative approach to the problem would help answer some of these questions.

EXPERIMENTAL

Materials—The barbiturates studied were supplied or purchased from the manufacturers listed in Table I and were used without further purification. Compounds 1, 5, 9, and 12 were obtained as the free acids; the others were available as the sodium salts. Diethylmalonuric acid (m.p. 159–161° dec.; after solidifying, m.p. 206–207°) and diethylacetylurea (m.p. 208–209°) were obtained by hydrolysis of barbital according to the method of Aspelund and Skoglund (21). Silica gel GF_{254}[1] and cellulose powder MN 300 UV_{254}[2] were used for TLC experiments. All other materials employed in this study were of analytical reagent grade.

Kinetic Procedures—Appropriate quantities of the barbiturates to produce final concentrations of 10^{-2} *M* were weighed into volumetric flasks and diluted to volume with distilled water. From these stock solutions, aliquots were taken and diluted with alkali of appropriate concentration or with a buffer solution of known pH to produce, in general, a final barbiturate concentration of about 10^{-4} *M*. The solutions of alkali and buffer were equilibrated at the temperature of the study prior to initiation of the reaction.

The reaction flasks were maintained in constant-temperature oil baths, controlled within 0.1°, at selected temperatures between 60 and 80°. Samples were withdrawn at suitable time intervals and cooled to room temperature. Spectrophotometric readings at the absorption maximum ($\lambda_{max.}$) were taken on the Beckman model DU-2 spectrophotometer, or the entire spectrum was recorded on the Cary model 15 spectrophotometer. Matched spectrophotometric cells (10 mm.) were used for all measurements, and a slit width of 0.10 mm. was employed. Since the $\lambda_{max.}$ values of the barbiturates vary with pH, the appropriate values for each compound were experimentally determined in each alkali and buffer solution prior to reaction. Table I lists some pertinent $\lambda_{max.}$ values. Samples in the lower pH buffer solutions, where the barbiturates exist predominantly in the nonchromophoric unionized form, were adjusted to about pH 11.5 by the addition of an appropriate volume of sodium hydroxide solution immediately before spectrophotometric measurements.

Alkali solutions were prepared by dilution of a standardized sodium hydroxide solution[3] with distilled water. The effect of ionic strength on the reaction rate in alkaline solutions was checked by adding varying amounts of potassium chloride to the reaction flasks. All reactions run in buffer solutions were maintained at a constant ionic strength of 0.15, where possible.

pH Measurements—The pH values of the sodium hydroxide solutions at the temperature of study were calculated from activity coefficient data in the literature (22). The pH values of the buffer solutions were measured directly at the temperature of study on a Photovolt pH meter equipped with high-temperature electrodes. The pH meter was standardized with standard buffer solutions at the same temperature.

TLC—Thin-layer chromatograms were made by coating 20 × 20-cm. glass plates with a 250-μ layer of either silica gel GF_{254} or cellulose MN 300. The chromatograms were usually spotted with 10–20 μl. of sample (10^{-2} *M*) and developed in closed tanks for the time required for the solvent front to travel 15 cm. from the starting line. After drying, the plates were viewed under a

[1] E. Merck AG, Darmstadt, Germany.
[2] Machery, Nagel & Co., Düren, Germany.
[3] Acculute, Anachemia Chemicals Ltd., Champlain, N. Y.

Table II—Apparent First-Order Rate Constants, 10^6k in sec.$^{-1}$, for Hydrolysis of Some Barbiturates at 80.0°

pH	Buffers [H_2PO_4]	Buffers [HPO_4^{-2}]	Barbital	Phenobarbital	Amobarbital	Metharbital	Thiamylal	Barbituric Acid	Vinbarbital	Butabarbital	Pentobarbital	Cyclobarbital	Secobarbital
6.01	0.100	0.0166	—	—	0.253	—	6.23	1.36	—		—	—	—
6.49	0.0582	0.0306	1.41	1.38	1.20	—	15.0	—	—	—	—	—	—
6.98	0.0250	0.0416	3.49	2.58	2.96	—	20.9	1.47	—	—		—	—
7.45	0.00894	0.0470	6.34	3.14	5.58	9.09	26.2	1.61	—	—	—	—	—
7.91	0.00187	0.0493	8.10	4.41	6.64	11.1	27.8	2.71	—	—	—	—	—
	[HCO_3^-]	[CO_3^{-2}]											
9.08	0.0460	0.0040	10.7	6.80	7.02	13.2	35.9	3.04	1.50	—	—	—	—
9.58	0.0340	0.0160	11.3	12.6	8.03	13.9	40.1	4.9	1.38	—	—	—	—
9.89	0.0226	0.0276	13.7	23.8	9.05	18.7	38.7	7.33	2.65	—	—	—	—
10.12	0.0116	0.0386	18.8	41.3	11.0	23.7	47.2	11.1	3.95	—	—	—	—
	—	[NaOH]											
10.54	—	0.010	24.7	72.0	16.4	32.1	70.7	17.9	4.18	0.697	2.28	6.46	1.40
11.20	—	0.050	101	270	72.2	100	190	64.3	14.1	2.29	2.84	9.85	3.50
11.49	—	0.100	143	363	90.5	169	257	78.4	24.1	3.90	4.77	16.5	7.21
11.75	—	0.200	208	358	142	283	355	100	34.8	10.8	7.57	21.4	—
12.05	—	0.400	261	391	211	530	400	119	40.5	14.5	11.5	33.4	—

short wavelength UV light and sprayed with various reagents to visualize the spots.

Barbituric acid degradation products were separated on cellulose TLC plates, using a solvent system consisting of *n*-butanol–acetic acid–water (6:3:1). TLC of the degradation products of thiamylal and barbital utilized silica gel plates and isopropanol–chloroform–ammonium hydroxide (25%) (9:9:2) as the solvent system. Spray reagents included: 0.1% aqueous solution of potassium permanganate for thiamylal and its degradation products; freshly prepared acidic *p*-dimethylaminobenzaldehyde (23) for barbituric acid, urea, and its derivatives; bromcresol green solution[4] for acidic compounds; and saturated aqueous solution of mercurous nitrate (24) for barbital. Identification of spots was made by comparison of R_f values and by color reactions for unknown compounds and standard substances spotted on the same plate.

RESULTS

Rate Constants and Spectral Changes—The kinetic parameters describing the hydrolysis of the barbituric acid derivatives studied were determined by following the loss of absorbance, A, of the UV chromophore as a function of time. All of the compounds studied lose their UV absorbance in the strongly alkaline and carbonate buffer regions without any significant perturbation in the chromophoric absorption band. A small residual absorbance, A_∞, was noted in some cases at the completion of the reaction. Typical spectral changes as a function of time are given for butethal in 0.4 *N* NaOH at 70° in Fig. 1.

Plots of ln $(A - A_\infty)$ at the $\lambda_{max.}$ *versus* time were reasonably linear for all studies in the alkaline pH region according to the first-order expression:

$$\ln (A - A_\infty) = \ln (A_0 - A_\infty) - kt \qquad \text{(Eq. 1)}$$

where A_0 is the absorbance at zero time, and k is the apparent first-order rate constant. The rate constants and the conditions under which they were determined are listed in Tables II and III. Several typical first-order plots for the hydrolysis of barbituric acid at various alkali concentrations and for barbital in buffers (after alkaline adjustment for spectrophotometric measurements) according to Eq. 1 are shown in Figs. 2 and 3.

Some minor spectral changes, *i.e.*, slight bathochromic shifts in the $\lambda_{max.}$, were noted for the degradation of metharbital in phosphate buffers at pH values less than 7 after approximately two half-lives. Nevertheless, the semilogarithmic plots of the data were representative of good first-order reactions, and good estimates of the apparent first-order rate constants were still obtainable from estimates of a slightly variable A_∞ for application of Eq. 1. However, when reactions were run in buffers[5], pH 6.4–8.2, significant residual absorbances which changed with time were observed after considerable decrease in the barbiturate chromophore. This anomalous behavior, which prohibited estimates of rate constants, was characteristic of all the barbiturates studied; typical spectra for the hydrolysis of metharbital in tromethamine buffer are shown in Fig. 4. As metharbital degrades in this buffer, the chromophore having a $\lambda_{max.}$ at 244 nm. decreases until it reaches an apparent asymptote. This is followed by the relatively rapid rise of a new peak, showing a $\lambda_{max.}$ at 275 nm. and a shoulder at 290 nm. No isosbestic point was observed and, thus, it can be argued that this new peak is not the direct result of the opening of the barbiturate ring; *i.e.*, there is no 1:1 correspondence between the loss of the barbiturate chromophore and the formation of the new peak. A plausible explanation is that there is a reaction between one or more of the ring-opened barbiturate hydrolysis products and the buffer to produce the new chromophore. Reactions between tromethamine and other compounds have been noted in the literature, together with a caution on the use of these buffers in studying reaction kinetics (25–27).

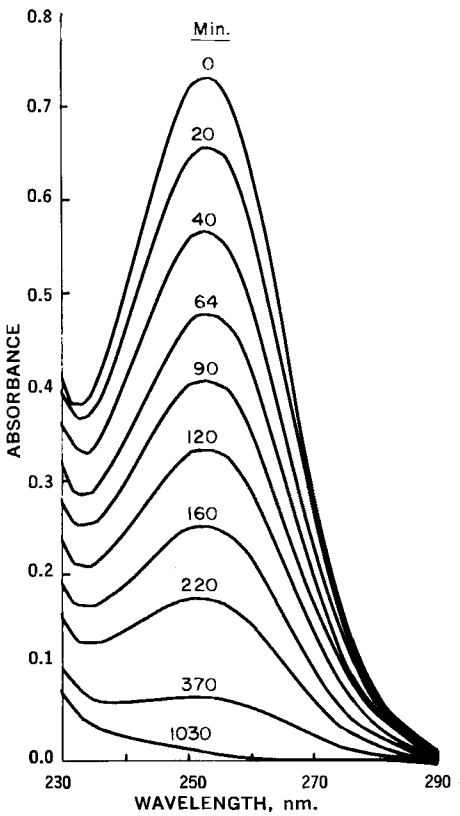


Figure 1—*Typical spectral changes for hydrolysis of 10^{-4} M butethal in 0.40 N NaOH at 70.0°. The curves are labeled as to minutes after the start of the reaction.*

[4] Applied Science Labs., Inc. TLC spray reagent according to Stahl (41).

[5] Tromethamine [TRIS, tris(hydroxymethyl)aminomethane].

Table III—Apparent First-Order Rate Constants, 10^4k in sec.$^{-1}$, and Thermodynamic Parameters for Hydrolysis of Some Barbiturates

Temperature	Barbital			Phenobarbital			Amobarbital		Butethal		Thiamylal			Metharbital		Barbituric Acid		
	0.4 *N* NaOH	pH 10.12	pH 7.37	0.4 *N* NaOH	pH 10.12	pH 7.37	0.4 *N* NaOH	pH 10.12	0.4 *N* NaOH	pH 10.12	0.4 *N* NaOH	pH 10.12	pH 7.37	0.4 *N* NaOH	pH 10.12	0.4 *N* NaOH	pH 10.12	pH 7.37
80.0°	261	18.8	6.34	391	41.3	3.14	211	11.0	199	14.3	400	47.2	26.2	530	23.7	119	11.1	1.61
75.0°	180	12.3	3.83	311	27.6	1.67	130	7.81	137	9.98	324	33.2	16.5	405	18.7	76.9	6.86	0.84
70.0°	141	8.01	2.48	237	19.1	1.41	—	5.98	109	5.93	195[a]	22.8	11.5	326	12.8	49.9	4.48	0.47
60.0°	54.0	3.15	—	115	8.17	—	4.58	2.22	51.8	2.08	—	10.4	4.25	193	5.06	19.6	1.38	0.15
ΔH_a[c], kcal./mole	18.2	20.8	22.4	14.4	18.8	19.1	17.4	18.6	15.4	22.8	19.2	16.2	17.7	21.0	17.7	18.4	21.0	243	27.4
ln P	17.8	18.8	20.0	12.8	16.47	14.5	16.3	15.2	13.5	21.4	15.0	15.4	15.3	19.4	9.08	15.6	20.9	23.2	25.7
ΔS_a[b,d], cal./°K-mole	−23.6	−21.5	−19.1	−33.5	−25.6	−30.1	−26.5	−28.7	−32.1	−16.2	−29.0	−28.3	−28.5	−20.2	−40.8	−27.8	−17.3	−12.8	−7.76

[a] At 68°. [b] At 80°. [c] In accordance with the expression: $\ln k = \ln P - \Delta H_a/RT$. [d] In accordance with the expression: $\Delta S_a = R \ln P - [\ln (\bar{k}T/h)]$.

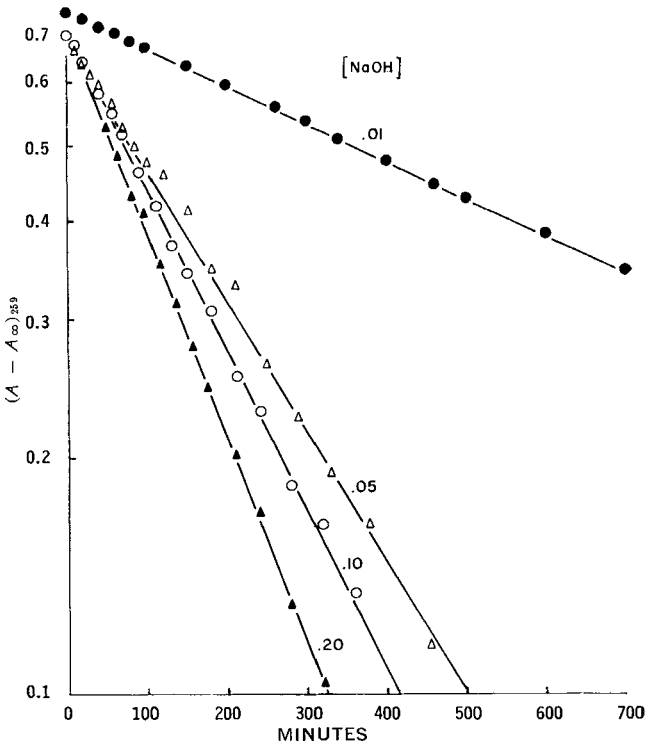


Figure 2—*Typical apparent first-order plots for hydrolysis of 10^{-4} M barbituric acid in alkali at 80.0°. The plots are labeled with the NaOH concentrations used. The absorbance values,* A, *were measured at 259 nm., and* A_∞ *is the final asymptotic absorbance.*

Significant spectral changes, other than decreases in absorbance, were observed during the hydrolysis of the thiobarbiturate, thiamylal. The decrease in absorbance with time at the $\lambda_{max.}$ (305 nm.) of this compound in sodium hydroxide solutions was accompanied by the formation of a new peak having a maximum at 235 nm. The presence of an isosbestic point at 252 nm. and the observation that the rate constant for the formation of the new peak closely matches that for the disappearance of the original chromophore are strong evidence for the occurrence of a 1:1 transformation. The spectra for the degradation of thiamylal in 0.1 *N* NaOH are shown in Fig. 5. During the hydrolysis, a low intensity, broad absorbance band appeared in the 300–400-nm. region. Higher concentrations of degraded solution allowed this absorbance band to be assigned a $\lambda_{max.}$ of 357 nm. Both new peaks, 237 and 357 nm., increased with time initially and then decreased, following apparent first-order kinetics. The spectrum of the 237-nm. chromophore is consistent with that of thiourea, one of the possible hydrolysis products of thiamylal.

Dependence of Rate on Temperature—The Arrhenius parameters for the hydrolysis of several barbiturates were calculated from the slopes and intercepts of plots of the logarithm of the apparent first-order rate constants, k, *versus* the reciprocal of the absolute temperature, T, in accordance with the expression:

$$\ln k = \ln P - \Delta H_a/RT \qquad \text{(Eq. 2)}$$

where R is 1.987 cal. deg.$^{-1}$. Typical Arrhenius plots are shown in Fig. 6. From a consideration of absolute rate theory (28),

$$k = \frac{\bar{k}T}{h} e^{\Delta S_a/R} \cdot e^{-\Delta H_a/RT} \qquad \text{(Eq. 3)}$$

and Eq. 2, the entropy of activation can be calculated by:

$$\Delta S_a = R \ln P - [\ln (\bar{k}T/h)] \qquad \text{(Eq. 4)}$$

where ΔS_a is the entropy of activation, $\bar{k}$ is the Boltzman constant, and h is the Planck constant. The k, ΔH_a, ln P, and ΔS_a values at several pH values are given in Table III.

Rate–pH Profiles—The rate–pH profiles for the hydrolysis of barbiturates were constructed from the logarithm of the apparent

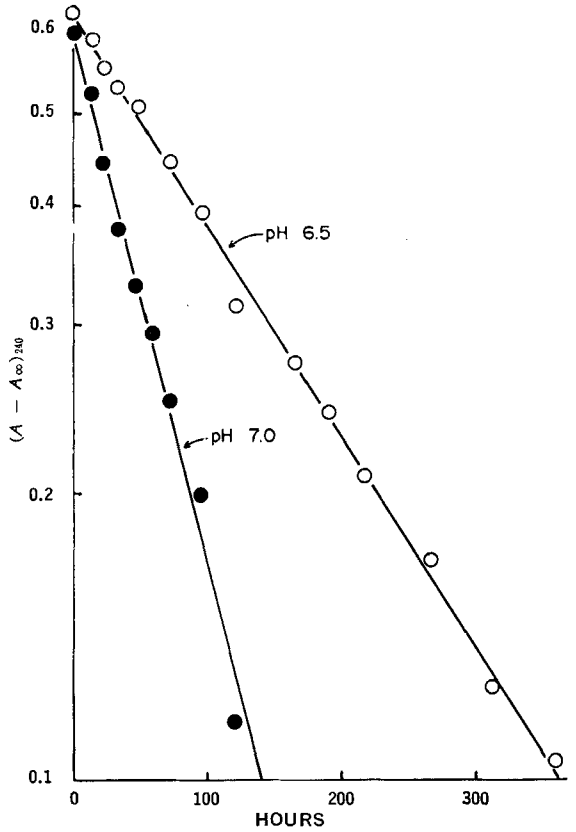


Figure 3—*Typical apparent first-order plots for hydrolysis of 10^{-4} M barbital in phosphate buffers at 80.0°. The samples were adjusted to an alkaline pH of 11.5 by diluting 1:1 with an appropriate NaOH solution immediately prior to spectrophotometric measurement.*

first-order rate constants, k, and the pH values at 80° (Table II). The profiles were similar for all of the 5,5-disubstituted barbituric acid derivatives studied (Fig. 7). The shape of these curves indicates hydroxyl-ion-catalyzed degradation of the unionized and monoionized species or the kinetically equivalent water attack on the mono- and dianionic species. Accordingly, the apparent first-order rate constant at a given pH can be defined as:

$$k = k_{OH}[OH^-]f_{BA} + k_{OH}'[OH^-]f_{BA}^- \qquad \text{(Eq. 5)}$$

or as its kinetic equivalent,

$$k = k_0 f_{BA}^- + k_0' f_{BA}^{-2} \qquad \text{(Eq. 6)}$$

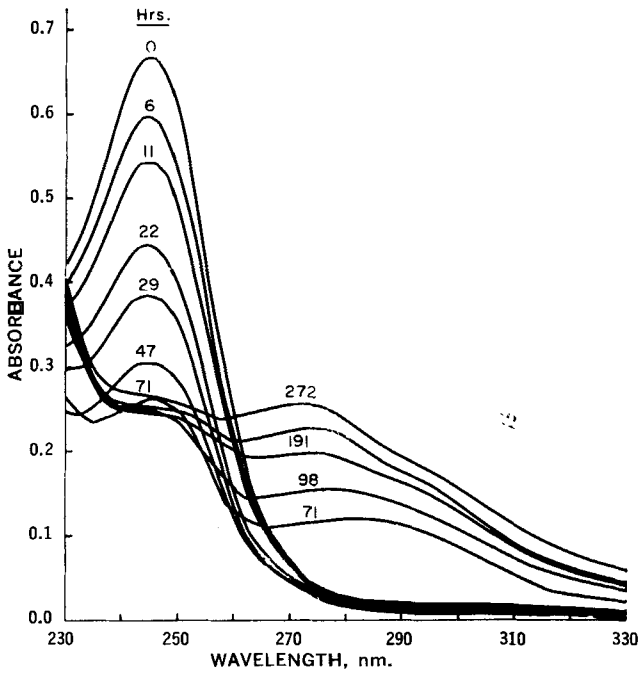


Figure 4—*Typical spectral changes for hydrolysis of 10^{-4} M metharbital in pH 8.8 tromethamine buffer at 80.0°, μ = 0.15. The curves are labeled as to minutes after the start of the reaction.*

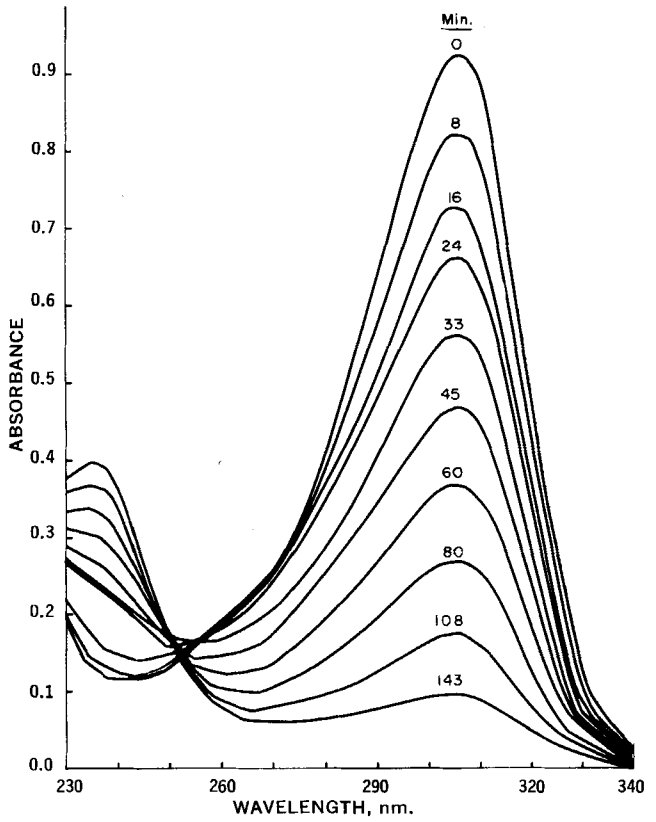


Figure 5—*Typical spectral changes for hydrolysis of 5 × 10^{-5} M thiamylal in 0.10 N NaOH at 80.0°. The curves are labeled as to minutes after the start of the reaction.*

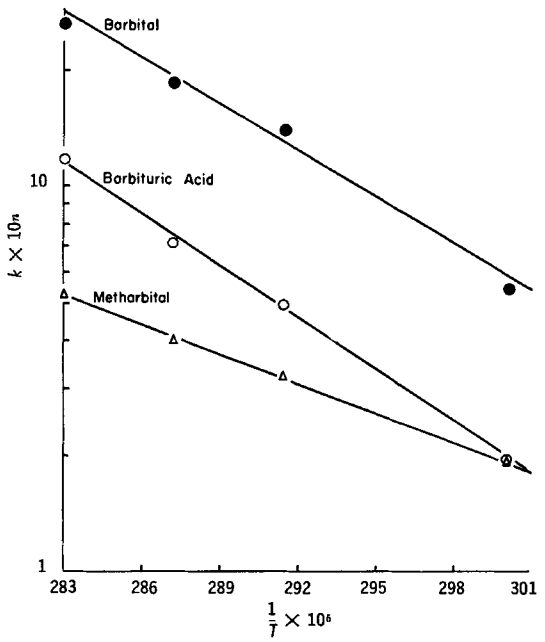


Figure 6—*Typical Arrhenius plots for apparent first-order rate constants for several barbiturates in 0.40 N NaOH; n = 4 for methorbital and 5 for barbital and barbituric acid.*

Table IV—Catalytic Rate Constants and Kinetic pKa′ from the Hydrolysis of Some Barbiturates at 80.0°

	Barbital	Amobarbital	Phenobarbital	Thiamylal	Metharbital	Barbituric acid
pKa_1'[a]	7.35	7.26	6.31	6.64	7.25	—
pKa_2'[a]	12.01	12.15	10.76	11.58	—	11.20
k_{OH}[b]	2.17	2.06	8.94	32.85	3.34	—
$10^3 k_{OH}'$[b]	2.14	1.45	35.2	6.04	270	3.39
$10^6 k_0$[c]	11.4	8.76	4.27	33.6	13.9	1.40
$10^5 k_0'$[c]	51.2	46.8	4.73	53.7	—	12.6

[a] Values reported are determined from best fit of rate–pH profile. [b] l. mole^{-1} sec.$^{-1}$. [c] sec.$^{-1}$.

where k_{OH} and k_{OH}' are the bimolecular rate constants for hydroxyl-ion attack on the undissociated (f_{BA}) and the monoanion (f_{BA^-}) forms of the barbiturate, respectively, and k_0 and k_0' are the first-order rate constants for water attack on the monoanion and dianion ($f_{BA^{-2}}$) forms, respectively.

Equation 5 can be rewritten by substituting for f_{BA} and f_{BA^-} in terms of the equilibrium constants, K_{a1} and K_{a2}, for the dissociation steps of the 5,5-disubstituted barbiturates (2):

$$k = \frac{k_{OH}K_w[H^+] + k_{OH}'K_wK_{a_1}'}{[H^+]^2 + K_{a_1}'[H^+] + K_{a_1}'K_{a_2}'} \qquad \text{(Eq. 7)}$$

The bimolecular rate constants that produced the best fits of the observed rate–pH profiles and the kinetic pKa′ values determined are given in Table IV.

The rate–pH profile obtained for thiamylal has the same shape as those for the 5,5-disubstituted barbituric acids (Fig. 7). Thus, the expressions given in Eqs. 5–7 also hold for this thiobarbiturate. The differences observed with thiamylal are in the magnitudes of the bimolecular rate constants. These data are also given in Table IV.

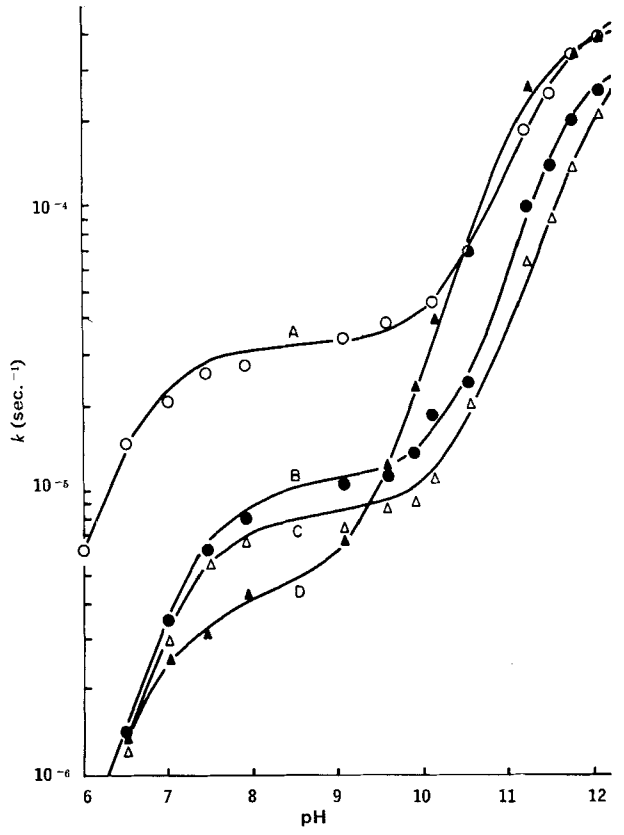


Figure 7—*Rate–pH profiles for 5,5-disubstituted barbiturates at 80.0°. The lines represent the theoretical curves drawn from Eq. 7 and the constants in Table IV. The points are the experimental values. Key: Curve A, thiamylal; Curve B, barbital; Curve C, amobarbital; and Curve D, phenobarbital.*

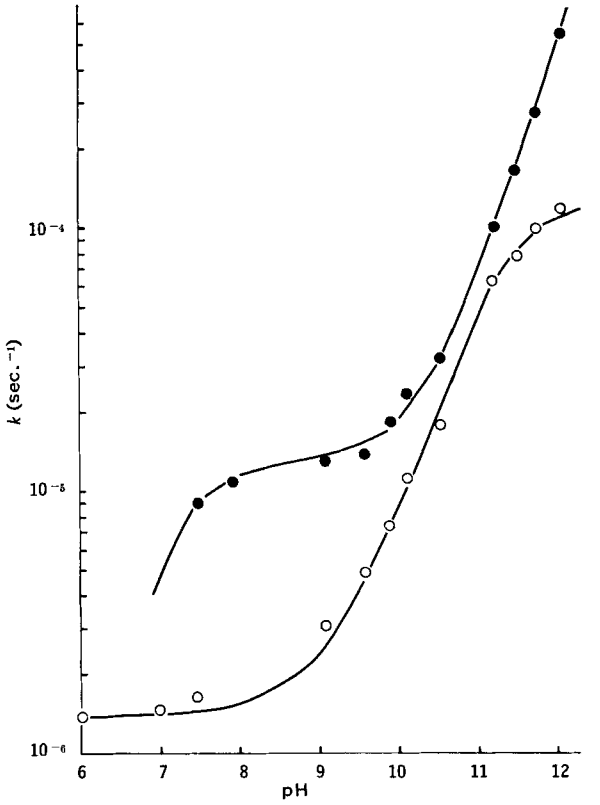


Figure 8—*Rate–pH profiles for some barbiturates. Key: ●, metharbital; and ○, barbituric acid. The metharbital curve is drawn from Eq. 8 and Table IV, and the barbituric acid curve from Eq. 10 and Table IV. The circles represent the experimentally determined values.*

Two rate–pH profiles which differ from those of the 5,5-disubstituted barbituric acids are shown in Fig. 8. The profile for the 1,5,5-trisubstituted barbiturate, metharbital, shows no curvature at the high pH values, since the 1-methyl substituent blocks the second possible dissociation; thus, hydroxyl-ion attack on the monoanion is not perturbed by the formation of a doubly negatively charged ion. An expression that fits the metharbital data is:

$$k = \frac{k_{OH}K_w + k_{OH}'[OH^-]K_a'}{[H^+] + K_a'} \qquad \text{(Eq. 8)}$$

or its kinetic equivalent:

$$k = \{k_0 + k_{OH}'[OH^-]\}\frac{K_a'}{[H^+] + K_a'} \qquad \text{(Eq. 9)}$$

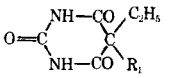
where the latter quotient is the fraction of metharbital monoanion. The catalytic constants and pKa′ estimates are given in Table IV.

The other rate–pH profile shown in Fig. 8 was obtained from studies on the hydrolysis of the unsubstituted parent barbiturate, barbituric acid. A bending of the profile at the higher pH values indicated the presence of a pKa in this region just as is found with the 5,5-disubstituted compounds. However, contrary to findings with the other barbiturates, the profile does not bend in the region of pH 7–8. This is consistent with the fact that the first pKa′ for barbituric acid is 3.9 at 20.0° (29)—well below that of the other barbiturates. One kinetically equivalent expression that fits the barbituric acid data for pH values >6 is:

$$k = \{k_0 + k_{OH}'[OH^-]\}\frac{[H^+]}{[H^+] + K_{a_2}'} \qquad \text{(Eq. 10)}$$

where the latter quotient is the fraction of barbituric acid monoanion. Values of the constants providing the best fit for this profile are also found in Table IV.

Table V—Effect of Substituents on Rate of Hydrolysis and Relationship of Order of Reactivity to the Newman Rule of Six



R_1	$k \times 10^5$[sec.$^{-1}$] 0.4 N NaOH, 80°	Total "Six Number"
C_2H_5—	26.10	6
CH_3—CH_2—CH_2—CH_2—	23.80	6
CH_3 \ CH—CH_2— / CH_2	21.08	6
	3.33	8
	2.58	8
CH_3—CH_2—CH(—CH_3)—	1.46	9
CH_3—CH_2—CH_2—CH(—CH_3)—	1.16	9
CH_3—CH_2—CH=C(—CH_3)—	4.07	8
	39.13	7

Effect of Substituents on Rate of Hydrolysis—Several barbituric acid derivatives were hydrolyzed in 0.4 N NaOH at 80° to determine the effect of various substituents on the rates of hydrolysis. Tables V and VI show the quantitative results obtained.

Separation and Identification of Degradation Products of Barbital—Barbital as free acid (1.6 g.), pKa 7.86 (29), was refluxed with 100 ml. of phosphate buffer (pH 5.8) composed of 2.76 g. of sodium phosphate monobasic $NaH_2PO_4 \cdot H_2O$ and 0.47 g. of sodium phosphate dibasic (anhydrous) for 4 days. The final pH of the reaction mixture was 6.15. The solution was cooled, and the crystals were filtered and dried, yielding 0.6 g. of white needles, m.p. 209–210°. This corresponded to the literature value of 207–208° for diethylacetylurea (7), one of the reported hydrolysis products of barbital. No melting-point depression was observed on mixing with an authentic sample of diethylacetylurea. After prolonged cooling in the refrigerator, another portion (0.1 g.) of diethylacetylurea was obtained. No attempt was made to recover unhydrolyzed barbital.

TLC—TLC was used to separate and identify some of the degradation products of barbituric acid and thiamylal. After degrading a 0.1% solution of barbituric acid in phosphate buffer (pH 8.2) at 80° for 97 hr., only unhydrolyzed starting material (R_f 0.37) and urea (R_f 0.65) could be identified.

Five different compounds were separated by TLC after the hydrolysis of a 0.1 M solution of thiamylal in 0.4 N NaOH for 3 hr. at 80°. Two of these compounds were identified as unhydrolyzed thiamylal (R_f 0.76) and thiourea (R_f 0.56). Two others (R_f 0.24 and 0.67) showed an acidic character by giving yellow spots with bromcresol green. Attempts to isolate these compounds on a larger scale were unsuccessful.

Hydrolysis of Diethylmalonuric Acid—Diethylmalonuric acid has been shown to be one of the products of barbital hydrolysis (7). The hydrolysis of diethylmalonuric acid in phosphate and carbonate buffers was monitored by UV spectroscopy, and an unexpected increase in absorbance was observed. The new peak formed showed the same spectral characteristics as barbital. The absorbance decreased after reaching a maximum ($\lambda_{max.}$ = 238 nm.) by an apparent first-order process. These spectral changes were followed over the pH region 7.45–10.12 at 80.0°. Typical examples are shown in Figs. 9 and 10. The data of Fig. 10 were quantitated by substracting the apparent A_∞ values from each absorbance reading and mathematically evaluating the resulting data by the "feathering" technique to obtain the two apparent

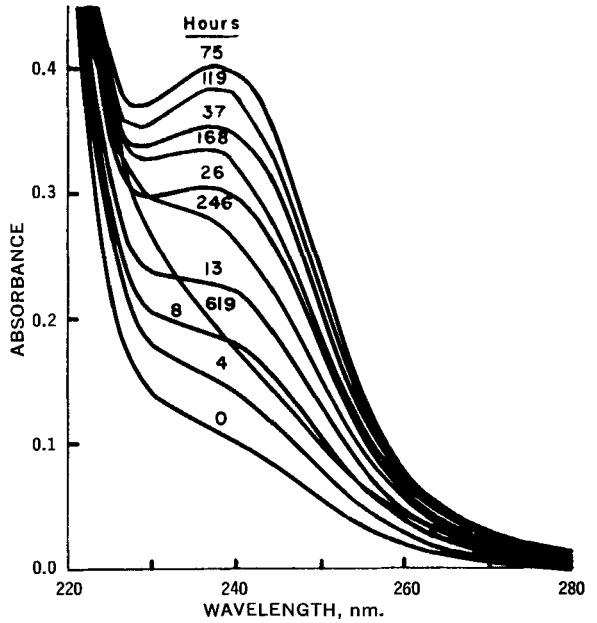
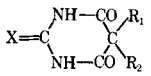


Figure 9—*Typical spectral changes for hydrolysis of 5 × 10^{-4} M diethylmalonuric acid in pH 6.98 phosphate buffer at 80.0°; μ = 0.15. The samples were adjusted to an alkaline pH of 11.5 by diluting 1:1 with an appropriate NaOH solution immediately prior to spectrophotometric measurement. The curves are labeled as to hours after the start of the reaction.*

first-order rate constants governing the increase and subsequent decrease of absorbance at 238 nm. with time. The larger rate constant was designated as k_1, while the smaller rate constant was called k_2. The values of k_1 and k_2 for each pH studied are given in Table VII. The reaction products were identified by dissolving 0.023 g. of diethylmalonuric acid in 100 ml. of phosphate buffer (pH 6.49), heating at 80° for 75 hr., cooling, and extracting the solution with two 25-ml. portions of ether. The ether was evaporated and the residue dissolved in 1.5 ml. of acetone. This solution (30 μl.) was spotted on a TLC plate along with barbital and diethylacetylurea standards (50 μl. of about 10^{-2} M solutions in acetone). The hydrolysis reaction products had R_f values identical to the barbital (0.65) and diethylacetylurea (0.82) standards. This suggests that diethylmalonuric acid simultaneously degrades to diethylacetylurea and cyclizes to form barbital.

Ionic Strength Effects—Variations in the apparent first-order rate constants with ionic strength changes were observed for the hydrolysis of amobarbital and phenobarbital at pH 11.20 where the barbiturate anion would exist (Fig. 11). The use of the Bronsted–

Table VI—Effect of Substituents on Rate of Hydrolysis



X	R_1	R_2	$k \times 10^5$ [sec.$^{-1}$] 0.4 N NaOH, 80°
O	CH_3—CH_2—CH(—CH_3)—	CH_3—CH_2—	1.46
		CH_2=CH—CH_2—	1.59
O	CH_3—CH_2—CH_2—CH(—CH_3)—	CH_3—CH_2—	1.16
		CH_2=CH—CH_2	1.52
S	CH_3—CH_2—CH_2—CH(—CH_3)—	CH_3—CH_2—	15.5
		CH_2=CH—CH_2—	40.02

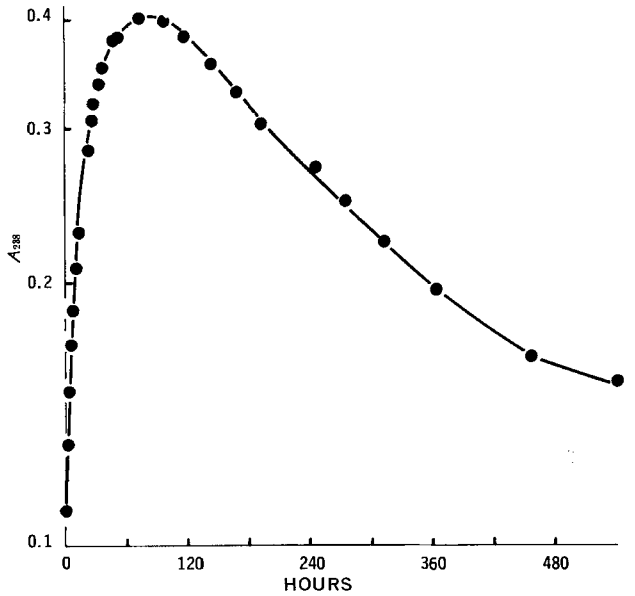


Figure 10—*Logarithm of absorbance changes at 238 nm.* versus *time for hydrolysis of* 5×10^{-4} M *diethylmalonuric acid in pH 6.98 phosphate buffer at 80.0°;* $\mu = 0.15$. *The samples were adjusted to an alkaline pH of 11.5 by diluting 1:1 with an appropriate NaOH solution immediately prior to spectrophotometric measurement.*

Bjerrum equation, together with the extended Debye–Huckel equation, results in the relationship (30):

$$\log k = \alpha + 2QZ_AZ_B \frac{\sqrt{\mu}}{1 + \sqrt{\mu}} \qquad \text{(Eq. 11)}$$

A plot of the logarithm of the apparent first-order rate constant *versus* $\sqrt{\mu}/(1 + \sqrt{\mu})$ was linear for both barbiturates (Fig. 11). The plots had significant positive slopes of 1.1507 for phenobarbital and 1.0666 for amobarbital. The value of $2Q$ at 80° is 1.145 (30).

DISCUSSION

General Structure–Hydrolytic Reactivity Correlations—Nonkinetic information has been used as a basis for qualitative appreciation of substituent effects on the hydrolysis of barbituric acid derivatives (6, 7, 12, 32, 33). Lamb (31) claimed a correlation between the rates of base-catalyzed decomposition of some 5,5-disubstituted barbiturates and the Taft E_s steric substituent constants. He stated that the variation in rate of decomposition in the barbiturate series appears to be only a function of the steric effects of the substituents and argued that the steric effects on the mechanism of phenobarbital decomposition were similar to the related steric effects for ester hydrolysis proposed by Pavelich and Taft (36). Eriksson (16, 17) also pointed out the importance of steric effects on substituent-modified hydrolyses of 1-methyl-5,5-disubstituted barbituric acids. He stated his belief that Taft-type parameters cannot be used to evaluate substituent effects for barbiturate-type compounds; instead, he utilized isokinetic relationships to group equivalent degrees of steric hindrance in the transition state. In contrast, Carstensen *et al.* (37) showed the validity of prediction of stability for a series of 5-substituted allylbarbituric acids on the basis of the Hammet $\rho\sigma$ linear free energy relation, in which the pKa values of the unsubstituted and substituted compounds were used to determine the reaction constant.

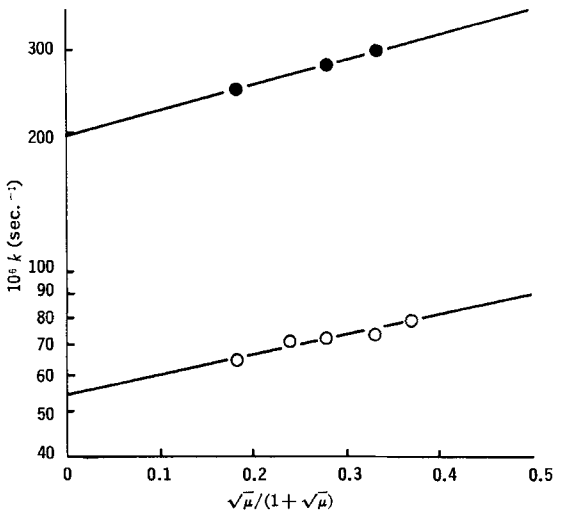


Figure 11—*Effect of varying ionic strength on apparent first-order rate constant at pH 11.20 for hydrolysis of some barbiturates at 80.0°. Key:* ●, *phenobarbital; and* ○, *amobarbital.*

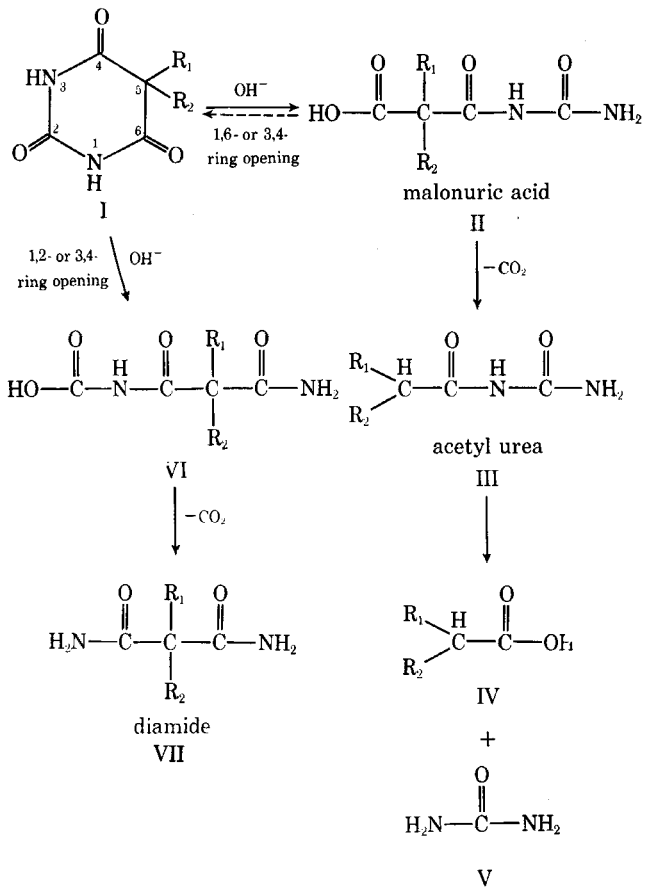
Table VII—Apparent First-Order Rate Constants[a] for Hydrolysis of Diethylmalonuric Acid at Various pH Values at 80.0°

pH	10^6k_1[b]	10^6k_2[c]
10.12	37.6	8.94
9.89	17.6	6.85
9.58	10.9	6.83
9.08	12.0	4.13
7.91	13.0	1.47
7.45	11.4	1.81

[a] Apparent first-order rate constants, k_1 and k_2, were determined by the "feathering" technique based on the assumption of an $A \rightarrow B \rightarrow C$ model. The faster rate constant was designated as k_1 and the smaller rate constant as k_2, and they are purely arbitrary estimates as described in the text. The true model may be $A \rightleftharpoons B \rightarrow C$, and k_1 (or k_2) may be more complex functions of the contant for the reversible equilibrium between A and B. [b] k_1 is the rate constant assigned to the cyclization equilibrium between I and II (Scheme I). [c] k_2 is the rate constant assigned to the decarboxylation of the malonuric acid derivative II (Scheme I).

Studies using the rate constants for the hydrolysis of many commonly used barbiturates under constant conditions (Tables V and VI) also show the effects of substituents in the 5-position. Substitution of sulfur for oxygen in the 2-position and replacement of one of the imide protons with a methyl group increase the susceptibility to hydrolysis. Increased alkyl group length decreases the rate of hydrolysis slightly, but branching at the first carbon of the alkyl chain or 5-substitution of a bulky cyclic group markedly decreases hydrolytic rate.

Newman Rule of Six and Hydrolytic Reactivity—The Newman rule of six (38), which essentially states that those atoms most effective in sterically hindering addition are separated from the oxygen of the attacked carbonyl in the transition state by a chain of four atoms, provides further evidence of the importance of steric effects in the hydrolysis of barbiturates. The order of reactivity (Table V) follows the "six number," *i.e.*, the number of atoms in the 6-position where the carbonyl oxygen is considered number "one." A more systematic approach would be to relate the bimolecular rate constants to the Newman "six numbers." Our data could not be subjected to this type of analysis due to the limited number of compounds for which the necessary complete rate–pH profiles were studied to determine exactly the bimolecular rate constant for hydroxide-ion attack on the monoanion. However, the excellent data of Eriksson (16, 17) were utilized to show this relationship since he studied derivatives that do not undergo a second dissociation; therefore, the bimolecular rate constants could be calculated from one first-order rate constant at a given alkali concentration. The use of the "six number" to classify relative reactivities provides interesting relations when Eriksson's detailed data of second-order rate constants for the alkaline hydrolysis of 1-methyl-5,5-disubstituted barbituric acids at 25° (16, 17) are considered. When the logarithms of the second-order rate constants are plotted against the total "six number," *i.e.*, for both 5-substituents, three distinct linear relations can be observed (Fig. 12). Curve A represents those compounds in which only one of the 5-substituents is exerting steric influence since the other group is a methyl which, in the barbiturate system, has a "six number" of zero. Curve B represents those compounds in which both substituents are interacting, and Curve C shows those compounds in which one of the substituents is a phenyl. Curves B and C, both of which are for compounds containing two interacting groups at the 5-position, have essentially the same slopes but different intercepts. Since the slopes of Curves B and C differ from that of Curve A,



Scheme I—Simplified scheme for hydrolysis of barbituric acid derivatives

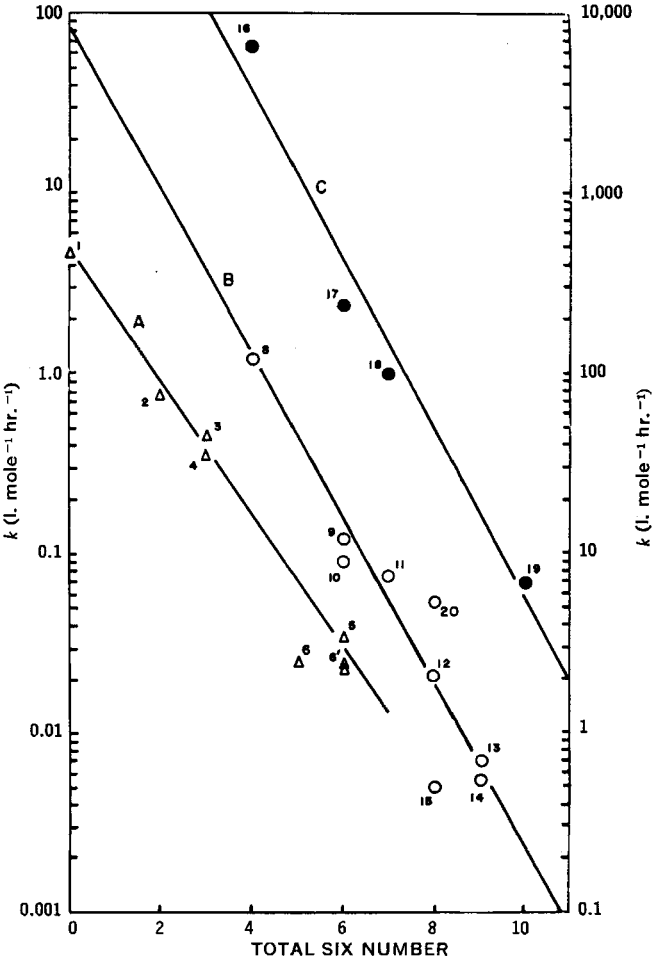


Figure 12—*Logarithm of bimolecular rate constant for hydrolysis of various substituted barbituric acid derivatives* versus *the total "six number" for the compound. The rate constants are taken from* References 16 *and* 17. *The numbers next to the various points represent 1-methyl barbituric acids substituted at the 5-position in the following manner: (1) methyl, methyl; (2) methyl, allyl; (3) methyl, ethyl; (4) methyl, propyl; (5) methyl, isopropyl; (6) methyl, 1-cyclohexenyl; (6′) methyl, cyclohexyl; (7) methyl,* sec*-butyl; (8) allyl, allyl; (9) ethyl, ethyl; (10) ethyl, butyl and propyl, propyl; (11) allyl, 1-methyl-2-pentynyl; (12) allyl, isopropyl; (13) propyl, isopropyl and ethyl, cyclohexyl; (14) propyl, 1-methylpentyl; (15) isopropyl, 2-bromoallyl; (16) phenyl, methyl; (17) phenyl, allyl; (18) phenyl, ethyl; (19) phenyl, isopropyl; and (20) ethyl, 1-cyclohexenyl. Curve A should be read using the ordinate on the right-hand side of the plot; for Curves B and C, use the ordinate on the left.*

it may indicate that the rate of hydrolysis of barbiturates is not a function of steric effects alone but that electronic effects also play some role. In line with this, the only two compounds that fall markedly off their respective lines are the cyclohexenyl and bromoallyl derivatives. More derivatives with electronegative groups in the 5-position should be studied to confirm this finding.

Mechanism of Barbiturate Hydrolysis—Considerable work has been done on the isolation and identification of the products of barbiturate hydrolysis (7–10, 33–35). The most comprehensive studies were published by Fretwurst (7) and Aspelund and coworkers (33–35). Barbiturate hydrolytic pathways are complex due to the possible alternative routes of sequential hydrolyses of the intermediates (39). The simplified scheme shown in Scheme I includes the major postulated routes.

Studies with the hydrolysis of barbital and its degradation product, diethylmalonuric acid, have demonstrated a previously unmentioned reversible system between the barbiturate and the ring-opened malonuric acid derivative (Figs. 9 and 10)[6]. The malonuric acid hydrolysis product can simultaneously degrade to the acetylurea derivative and cyclize to form the barbiturate (Scheme I).

Several investigators argued or stated that the hydrolysis of barbiturates proceeds through two different pathways (13, 15, 16). The unionized form of the barbiturate has been said to hydrolyze *via* ring opening at the 1,2- or equivalent 2,3-position to yield a substituted diamide, VII, and the ionized form to hydrolyze *via* opening at the 1,6- or equivalent 3,4-position to produce a substituted acetylurea derivative, III (Scheme I). There are rate–pH profiles in uridine solvolysis (2), similar to those of the barbiturates, in which different products do indeed result from hydroxyl-ion attack on the undissociated and dissociated species. The mechanism proposed by Hasegawa *et al.* (14) did not allow for production of the diamide and was criticized by the proponents of the dual pathways (13).

The argument (13) for different products of hydrolysis from the ionic and nonionic forms was based on two factors: (*a*) analysis of equilibria phenomena and series first-order reaction kinetics showed that the fraction decomposing by the ureide path (1,6-ring opening) was dependent on pH changes which could be related to the concentration of the ionic form of 1, and (*b*) the diamide, VII (1,2-ring opening) was isolated as a hydrolysis product by two investigators (7, 9). However, the diamide, supposedly a product of nonionized barbiturate solvolysis, was isolated by Fretwurst (7) from alkaline solutions of the ionized barbiturate. No mention of diamide isolation was made by Kapadia *et al.* (9).

The hydrolysis of barbital in phosphate buffer at a pH value (6.15) well below the first pKa′ (7.86) of the compound, where the barbiturate was largely unionized, yielded diethylacetylurea as 84% of the hydrolyzed barbital. The extent of hydrolysis at 100° to correct for unreacted barbital present was calculated for the time of reaction on the basis of the determined heat of activation (Table III) and the catalytic constants in Table IV. This diethylacetylurea

[6] Subsequent to submission of this manuscript for pu lication, the authors learned that Aspelund recently described the cyclization of a malonuric acid derived from a tetrasubstituted barbituric acid. [H. Aspelund, *Acta Acad. Aboensis Math. Phys., Ser. B*, **29** (8), (1969).]

cannot be formed if ring opening occurs at the 2-position of the barbiturate ring. This strongly identifies an acetylurea as the major hydrolysis product of *both* the ionized and unionized form of the barbiturates. A close look at the Fretwurst data (7) indicates that the diamide is most easily formed from those barbiturates with a 1-methyl substituent or with bulky groups substituted at the 5-position. This argues that 1,6-ring opening would be favored in all barbiturates and that 1,2-splitting is of minor importance except when 1,6-opening is sterically hindered by a bulky 5-substituent or the combination of a 5-substituent and a 1-methyl group.

These observations are difficult to reconcile with the proposed sole dependence of phenylethylacetylurea formation on the concentration of the ionized parent barbiturate, phenobarbital (13). The basis of this proposal (13) was the postulated instability of the presumed irreversibly formed malonuric acid intermediate, II (Scheme I), so that the analyses of the acetylurea, III, with time could be taken as measures of the rate of hydroxyl-ion attack on the ionized form. The determination of the total rate of loss of barbiturate, I, permitted the assumption that the difference between this rate and the rate of acetylurea, III, formation could be attributed to the rate of formation of intermediate VI and the diamide VII. The resultant observed pH dependence (13) of the fraction of acetylurea, III, formed was assigned to the attack of hydroxyl ion on the ionized barbiturate where the diamide was considered to result from the attack of hydroxyl ion on the uncharged barbiturate.

The data in Table VII indicate that the cyclization of II to I occurs predominantly with the anion of the malonuric acid derivative since the rate of formation and amount of chromophore assigned to the cyclized product, *i.e.*, barbital, decrease with decreasing pH. In addition, a study of the cyclization of II to I at pH 1.1 indicated that no chromophore at 238 nm. was formed with time, while a similar study at pH 4.55 showed the slow formation of this chromophore assigned to barbital. Since decarboxylation of the anion of the malonuric acid derivative II is hydroxide-ion catalyzed (40) and the cyclization of II to I, if assignable to a function of the magnitude of k_1, appears to be pH independent up to a pH of 9.5 (Table VII), it is possible that the rate of formation of the acetylurea derivative, III, decreases with decreasing pH. The net effect would be a buildup of II and an increase in the rate of cyclization to the parent barbiturate. The result would be an apparent decrease in acetylurea III formation and an apparent increase in hydrolysis *via* 1,2-ring opening which is not related to any relative increase in hydroxyl-ion attack on one position of the barbiturate ring over another for the ionized or nonionized state.

Thus, it is possible that the data showing that the fraction decomposing by the ureide path (1,6-ring opening) is a function of pH in accordance with the observed experimental data (13) is not a direct consequence of a unique degradation pathway for the ionic form of the barbiturate, as was implied. The prime factor responsible for a pH-dependent path for barbiturate hydrolysis would then be the balance struck between hydroxyl-ion-catalyzed decarboxylation of the malonuric acid anion and the pH-independent recyclization below pH 9.5 (Scheme I).

Steric effects of the substituents may further affect the yield through each path of hydrolysis. Thus, barbital, with very little steric hindrance to 1,6-ring opening, produces primarily the acetylurea derivative even from the unionized form (84% of reacted barbital). The data of Fretwurst (7) were confirmatory. 1-Methyl barbiturates with bulky 5-substituents give the highest yields of diamide, even from alkaline solutions of ionized compounds (7).

These observations indicate the need for further studies to determine the rates of formation and relative amounts of barbiturate hydrolysis products as a function of pH. Various chromatographic techniques could be employed to separate and quantitate the reaction products. In addition, studies should be attempted to isolate the malonuric acids of various barbiturates and to investigate the rates of cyclization as functions of the 5-substituents and pH.

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ACKNOWLEDGMENTS AND ADDRESSES

Received February 8, 1971, from the *College of Pharmacy, University of Florida, Gainesville, FL 32601*

Accepted for publication April 21, 1971.

Presented in part to the Basic Pharmaceutics Section, APhA Academy of Pharmaceutical Sciences, Montreal meeting, May 1969.

This work was supported in part by Grants GM-09864-05 and CA10738-06 from the National Institutes of Health, U. S. Public Health Service, Bethesda, Md.

The authors are grateful to the Abbott, Eli Lilly, McNeil, Merck Sharp and Dohme, Parke-Davis, and Sterling Winthrop pharmaceutical companies for supplying some of the compounds used in this study.

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