Alkaline Hydrolysis of 1,3-Dimethylphenobarbital

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Abstract □ The reaction of 1,3-dimethylphenobarbital (I) with 0.02–0.32 *M* KOH in aqueous methanol was studied. The barbiturate ring cleaved reversibly at the 1,6-position, forming a malonuric acid which was stable to further hydrolysis but which could readily recyclize to I. *N,N*-Dimethylethylphenylmalondiamide arose from decarboxylation of the carbamic acid formed by a 1,2-ring opening; this irreversible decarboxylation determined the diamide as the only final reaction product. The malonuric acid, which could be isolated in solid form, appeared as *N*-methyl-2-phenylbutyramide (GLC) following thermal decarboxylation and degradation of the acid. The I disappearance rate was biphasic, and the kinetics were consistent with the described reaction. The individual rate constants and the equilibrium constant for the reaction between I, the malonuric acid, and hydroxide were determined.

Keyphrases □ Dimethylphenobarbital—alkaline hydrolysis, kinetics, reaction products □ Hydrolysis, alkaline—dimethylphenobarbital, kinetics, reaction products □ Kinetics—dimethylphenobarbital alkaline hydrolysis

The 5,5-disubstituted barbituric acid alkaline hydrolysis has recently received attention (1–5). The pyrimidine ring may cleave at the 1,6-position, yielding a malonuric acid, or at the 1,2-position, forming a carbamic acid. Further hydrolysis leads to the acetic acid derivative and urea. The predominant pathway depends on the barbituric acid ionization state (2) and on the C-5 substituent size (1).

1,3-Dimethyl-5,5-disubstituted barbituric acid hydrolysis yielded only the *N,N'*-dimethylmalondiamides (6). Neither a malonuric acid nor a carbamic acid was isolated from the hydrolysis of phenobarbital, its *N*-methyl derivative (7), and 1,3-dimethylphenobarbital (6). A diamide was the main product in 1,3-dimethylbarbital alkaline hydrolysis, which also produced an acid intermediate. Acid intermediates that can recyclize to the parent barbituric acids have been reported (2, 3).

As a result of a related study (8), interest arose in the base-catalyzed degradation of 1,3-dimethylphenobarbital (I) and the alkaline hydrolysis of I in aqueous methanol was examined. Initial results were sufficiently different from those found with other barbituric acids to warrant detailed study. This paper presents a kinetic and product analysis investigation of the I reaction with potassium hydroxide.

EXPERIMENTAL

Materials—Compound I and *N*-methyl-2-phenylbutyramide (VI) were obtained as described previously (8). Compound IV was prepared by I hydrolysis in 0.7 *M* KOH in methanol–water for 24 hr. After acidification, the reaction mixture was extracted with chloroform and IV was purified by treatment with charcoal[1] in ethanol. The product, mp 106–110° [lit. (6) mp 108–109°], was characterized by mass spectrometry and yielded a single peak by GLC. Ethylphenylmalonate ethyl ester[2] was purified by distillation under reduced pressure.

The malonuric acid (II) was prepared by treating I with 0.1 *M* KOH in methanol–water for a few minutes, acidifying, and extracting with chloroform. The organic layer was shaken with pH 7 phosphate buffer, and the chloroform phase was discarded. Compound II was then obtained by chloroform extraction of the acidified aqueous phase.

All salts and solvents were reagent grade.

GLC—GLC[3] was performed on a 1.82-m × 2-mm i.d. silanized glass column packed with OV-17 on Chromosorb WHP[4]. The carrier gas was nitrogen (30 ml/min). The injection port temperature was maintained at 280°, as was the flame-ionization detector. For detailed reaction product examination, the oven temperature was programmed from 160 to 260° at 10°/min. In the kinetic studies, it was programmed from 220 to 260° at 10°/min.

TLC—TLC[5] was performed using silica gel sheets with a fluorescent indicator[6]. Petroleum ether (bp 60–110°)–ether (65:35) was used to separate reaction products. The R_f values were: I, 0.65; VI, 0.15; and IV, 0.06. In some experiments, ethanol–chloroform was used to isolate II (R_f 0.75). In this latter system, I, IV, and VI moved with the solvent front. The compounds were visualized under UV light, and the spots were scraped off the sheet and eluted with chloroform or methanol.

Kinetic Studies—Kinetic measurements were made at 25° in various potassium hydroxide concentrations with potassium chloride added to

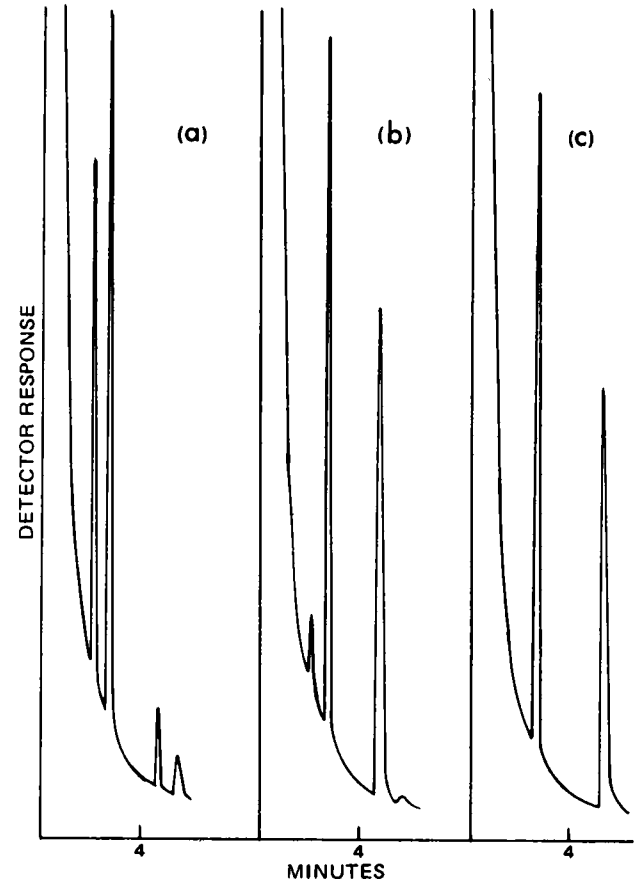

Figure 1—*Gas chromatograms of the reaction mixture of 1,3-dimethylphenobarbital with potassium hydroxide. Key:* a, *0.319* M *KOH after 1 min;* b, *0.0199* M *KOH after 2 min; and* c, *0.319* M *KOH after 50 min. Peaks in order of increasing retention time are* N*-methyl-2-phenylbutyramide, internal standard, 1,3-dimethylphenobarbital, and* N,N'*-dimethylethylphenylmalondiamide.*

[1] Norit-A, Fisher Scientific Co.
[2] Eastman.
[3] Hewlett-Packard 7620A.
[4] Applied Science Laboratories.
[5] Eastman chromatogram chamber set.
[6] Eastman 6060.

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Table I—Rate and Equilibrium Constants [a] for the Reaction of 1,3-Dimethylphenobarbital with Potassium Hydroxide in 50% Aqueous Methanol at 25°

[KOH], M	λ_1	λ_2	k_1	k_2	k_3	k_3[b]	K[c]	K[d]
0.319	0.101	—	16.9[e]	3.14[f]	0.658[g]	0.660	16.9	20.6
0.239	0.0867	—	9.47[e]	2.35[f]	0.449[g]	0.474	16.9	18.6
0.160	0.0713	—	4.25[e]	1.56[f]	0.275[g]	0.277	17.0	20.4
0.0798	0.0486	1.85	1.04	0.738	0.122	0.119	17.6	18.4
0.0606	0.0373	1.33	0.678	0.607	0.0817	0.0894	18.4	18.8
0.0399	0.0223	0.600	0.253	0.329	0.0407	0.0430	19.3	16.2
0.0303	0.0155	0.451	0.164	0.277	0.0252	0.0267	19.5	17.6
0.0199	0.00761	0.229	0.0685	0.157	0.0111	0.0114	21.9	17.1

[a] Rate constants are in minutes^{-1}. [b] Determined from $(\Delta IV/\Delta t)/[I]$. [c] Calculated from $(k_1/k_2)/[KOH]$. [d] Calculated from $([II]/[I])_{max}/[KOH]$. [e] Extrapolated from plot of k_1 and $[KOH]^2$. [f] Extrapolated from plot of k_2 and [KOH]. [g] Calculated using k_1, k_2, and λ_1.

maintain a 0.32 ionic strength. Stock I solutions were prepared by dissolving 10 mg of I in 5 ml of methanol and diluting to 10 ml with water. After thermal equilibration, 0.200 ml of the hydroxide solution in methanol–water was added rapidly to 0.200 ml of the stock I solution. The solution was mixed[7] rapidly and allowed to stand in a constant-temperature bath for the required time.

The solution was then acidified rapidly with hydrochloric acid, 0.400 ml of the internal standard solution (ethylphenylmalonate ethyl ester, 44 mg/100 ml of chloroform) was added, and the mixture was agitated vigorously for 1 min. This volume of solvent and duration of mixing were sufficient to extract the reaction products completely from the aqueous phase. Immediately after the layers had settled, 4 μl of the chloroform solution was injected into the gas chromatograph.

The I and IV peak height ratios to the internal standard were assumed to be proportional to the concentration of these compounds studied (0–0.5 μg/μl). The relative response of I to IV was determined by comparing the peak heights of I aliquots before and after completion of the reaction with potassium hydroxide. Since VI was a decomposition product in GLC and tailed on the packing used, peak height ratios were only an approximate measure of the II concentration.

IR, NMR, and Mass Spectra—IR[8] spectra were obtained in potassium bromide pellets. The NMR[9] spectrum was determined in deuterated chloroform[10] containing 1% tetramethylsilane. Mass spectra were obtained as described previously (8).

RESULTS

Compound I reacted slowly with potassium hydroxide in methanol but readily in the presence of water. The hydrolysis was studied in 50% (v/v) methanol–water.

Hydrolysis Products—When the chloroform extracts of the acidified reaction mixtures were injected into the gas chromatograph, three peaks in addition to that of the internal standard generally were observed (Fig. 1). On the basis of retention times and mass spectrometry (8), the peaks were identified as the butyramide VI, I, and the diamide IV, in order of increasing retention times. The relative peak heights depended on the reaction time and hydroxide concentration. Details are presented under *Kinetic Studies*.

TLC of the extracts using the petroleum ether–ether system showed three spots. Upon elution and GLC, two spots corresponded to I and IV, as did the R_f values. The third spot, at the origin, did not have the VI R_f value, although it yielded the butyramide by GLC. Similar behavior was observed with TLC using ethanol–acetone except that the reference I, IV, and VI moved with the solvent front, as did all of the reaction mixture components except the one that yielded VI by GLC. Since VI initially increased and then decreased to zero as hydrolysis proceeded, it must be derived from an intermediate.

Compound VI did not appear on the gas chromatograms when the reaction chloroform extract was shaken with pH 7 phosphate buffer prior to injection. This finding suggested that the VI precursor was acidic. This extraction procedure was used as the basis for VI isolation.

The IR spectrum of the intermediate was consistent with II, a malonuric acid formed by 1,6-cleavage of the pyrimidine ring (broad regions centered around 3000 cm^{-1}, 1730 cm^{-1}, —COOH; 3280, 1680, 1635, and 1530 cm^{-1}, amide functions). Solubility difficulties precluded obtaining an NMR spectrum. The intermediate melted at ~115° with effervescence. After melting, the product did not correspond on TLC to any of the previously mentioned compounds, although it yielded VI upon GLC. Its R_f value was 0.4 in petroleum ether–ether. An IR spectrum was consistent with the acetylureide V; the carboxyl group bands were no longer present, but bands at 3300, 1700, 1655, and 1530 cm^{-1} showed that the amide functions were intact. The V structure was confirmed by NMR of material purified by TLC: δ 7.29 (s, 5H, C_6H_5), 6.69 (broad peak, 1H, NH), 3.77 (t, 1H, CH), 3.22 (s, 3H, NCH_3), 2.87 [d, 3H, N-(H)CH_3], 1.87 (broad, diffuse, probably —CH_2—), and 0.88 (t, 3H, CH_3).

The possibility that the intermediate was the carbamic acid derivative was ruled out since it would have formed the diamide IV upon decarboxylation.

Samples of II gradually produced increasing I quantities on standing in methanol or chloroform or on a TLC sheet, indicating the capability of the malonuric acid to recyclize back to I. This recyclization was shown to be base catalyzed by treating the isolated intermediate with hydroxide for a short time and acidifying. Substantial I quantities were demon-

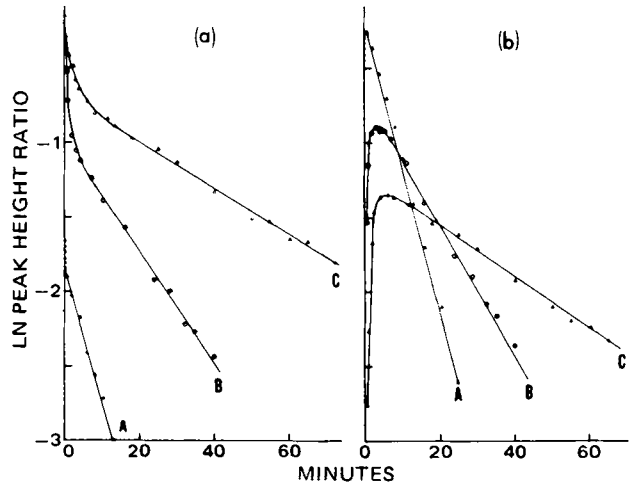


Figure 2—*Rate of change of 1,3-dimethyphenobarbital* (a) *and the intermediate (as N-methyl-2-phenylbutyramide)* (b). *Key: A* (▲), *0.239* M *KOH; B* (○), *0.0606* M *KOH; and C* (△), *0.0303* M *KOH.*

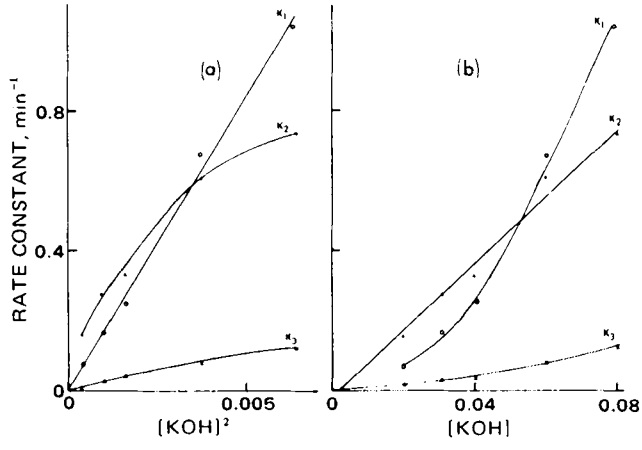
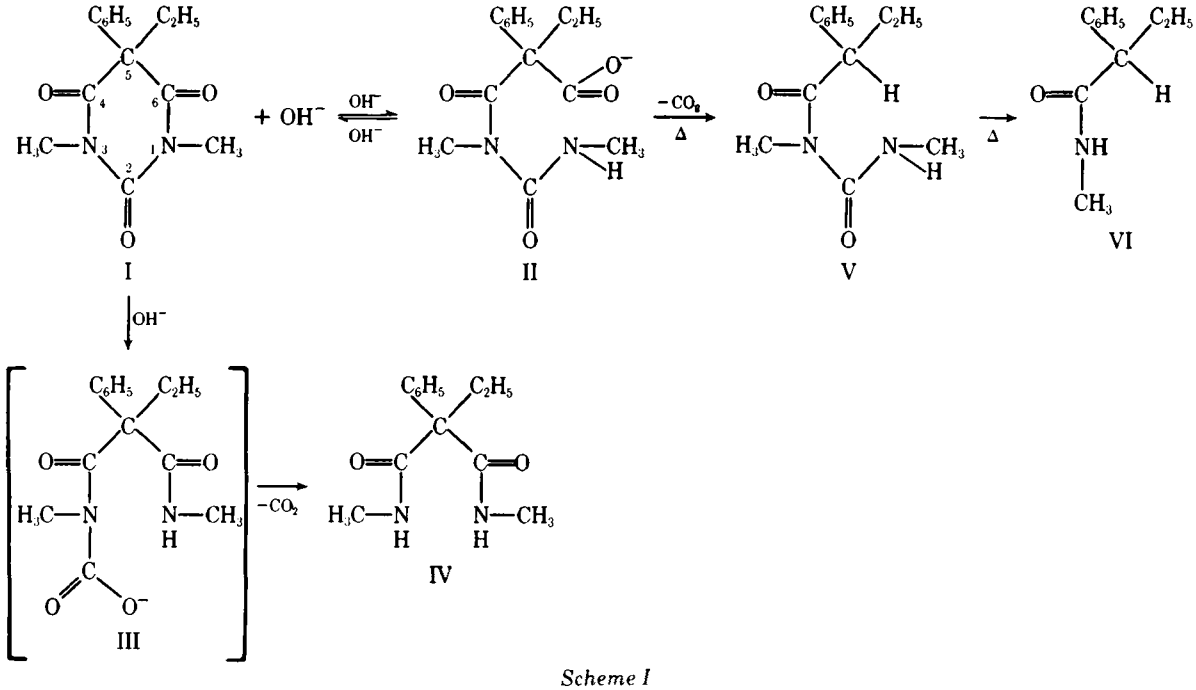


Figure 3—*Plots of* k_1, k_2, *and* k_3 *against* $[KOH]^2$ (a) *and* [KOH] (b).

[7] Vari-Whirl, VWR Scientific Inc.
[8] Perkin-Elmer 257 grating spectrophotometer.
[9] Varian T60.
[10] Norell Chemical Co.



Scheme I

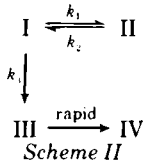
strated by GLC and TLC. Chloroform extraction of an alkaline (no acidification before extraction) II solution resulted in all of it being converted to I (except for a small amount converted to IV). This finding showed that malonurate anion recyclization occurs rapidly and that as I is formed it is removed continuously into the organic layer. When II was allowed to react to completion with potassium hydroxide, only IV could be isolated. Scheme I summarizes the previous reactions.

Kinetic Studies—At all hydroxide concentrations used, the I disappearance rate and the II change rate were biphasic; *i.e.*, a rapid change was followed by a more gradual one, the final phase being linear on a semilogarithmic plot (Fig. 2). The extent of the initial change and the terminal phase slope were strongly dependent on the hydroxide concentration. The initial phase was so fast in hydroxide concentrations greater than 0.1 *M* that it could not be followed kinetically.

The biphasic I disappearance could be fitted to:

$$[I] = Ae^{-\lambda_1 t} + Be^{-\lambda_2 t} \qquad \text{(Eq. 1)}$$

using standard curve-fitting techniques (9); A and B are constants, and λ_1 and λ_2 are rate constants (arbitrarily $\lambda_2 > \lambda_1$). This equation is consistent with the reaction sequence shown in Scheme II.



Scheme II

The constant A is the terminal line intercept at $t = 0$. From the observed A, λ_1, and λ_2 values, the rate constants k_1, k_2, and k_3 were calculated (Table I) using:

$$k_2 = \lambda_1 + A(\lambda_2 - \lambda_1)/[I]_0 \qquad \text{(Eq. 2)}$$

$$k_3 = \lambda_1\lambda_2/k_2 \qquad \text{(Eq. 3)}$$

$$k_1 = \lambda_1 + \lambda_2 - k_2 - k_3 \qquad \text{(Eq. 4)}$$

The rate constants k_1, k_2, and k_3 were plotted against various hydroxide concentration functions. The best correlations (Fig. 3) for k_1 and k_2 were:

$$k_1 = 166[OH^-]^2 + 0.0118 \qquad r = 0.996 \qquad \text{(Eq. 5)}$$

$$k_2 = 9.97[OH^-] - 0.0382 \qquad r = 0.993 \qquad \text{(Eq. 6)}$$

No hydroxide function was found that yielded a satisfactory correlation with k_3: the plots of k_3 *versus* [KOH] and [KOH]^2 (Fig. 3) show a definite curvature.

In principle, the rate constants could also be obtained from the II rate of change with time. However, at all potassium hydroxide concentrations, the terminal slope, *i.e.*, λ_1 for the II disappearance rate, measured by GLC as VI, was slightly higher than the rate for I. This finding was attributed to the nonlinearity of the peak height ratios with II concentration because of column tailing. The error is amplified in obtaining λ_2. Since II is an unstable compound, no standard response on the GLC can be obtained and the II concentration at the intercept that is needed for the rate constant calculation can only be approximated. The response factor was approximated by calculating the II concentration from the difference between the amount of I added and the amount of I unreacted and IV formed.

With the following appropriate relationships:

$$k_1 = (\text{intercept of terminal line}) \times (\lambda_2 - \lambda_1)/[I]_0 \qquad \text{(Eq. 7)}$$

$$k_2 + k_3 = \lambda_1 + \lambda_2 - k_1 \qquad \text{(Eq. 8)}$$

$$k_2k_3 = \lambda_1\lambda_2 \qquad \text{(Eq. 9)}$$

k_1, k_2, and k_3 can be estimated from the corrected peak height ratios. For example, for the reaction in 0.0606 *M* KOH, λ_1, λ_2, k_1, k_2, and k_3 were estimated to be 0.0421, 1.05, 0.558, 0.436, and 0.102 min^{-1}, respectively, compared to 0.0373, 1.33, 0.678, 0.607, and 0.0817 min^{-1}, respectively, obtained for the I rate of change. The agreement is reasonable considering the approximations made; the data are consistent with the reactions in Scheme II.

Since the III to IV conversion is rapid, the IV formation rate is proportional to the I concentration. Therefore, approximate values for k_3 can be derived using:

$$(\Delta IV/\Delta t)/[I] \simeq k_3 \qquad \text{(Eq. 10)}$$

By using increments of IV and time from [IV] *versus* t plots, approximate k_3 values were calculated (Table I). The agreement with the k_3 values obtained by the previous method is remarkable but to some extent fortuitous.

Since $d[II]/dt = k_1[I] - k_2[II] = 0$ at the maximum of the plot of [II] *versus* time, and since k_1 is proportional to [KOH]^2 and k_2 is proportional to [KOH], Eq. 11 can be derived:

$$([II]/[I])_{max}/[KOH] = (k_1/k_2)/[KOH] = K \qquad \text{(Eq. 11)}$$

where K is the equilibrium constant for the reaction between I and II and $([II]/[I])_{max}$ is the ratio of the I and II concentrations at the maximum in II. Values of K obtained in this way using the maximum obtained graphically are listed in Table I, as are values of $(k_1/k_2)/[KOH]$. Although there is a 12-fold change in hydroxide concentration, the values of K are reasonably constant.

Although λ_2 could not be obtained directly from the experimental data for the higher hydroxide concentrations, the rate and equilibrium constants were estimated as indicated in the footnotes of Table I.

DISCUSSION

Unlike 1-methylphenobarbital and phenobarbital itself (7), I alkaline hydrolysis yielded only the substituted diamide IV. Although this compound could be the result of decarboxylation of a carbamic acid intermediate formed by a 1,2-ring opening, the acid intermediate actually isolated was identified as a malonuric acid formed by a 1,6-ring opening. The formation of the diamide as the sole final reaction product is in agreement with a previous report (6), although no acid intermediates were isolated in that work, probably due to the relatively low hydroxide concentrations used and to the reaction mixture manipulation.

The malonurate (II) did not decarboxylate but recyclized to the parent barbituric acid. The overall irreversibility of IV hydrolysis was due to the decarboxylation of a carbamic acid, which was too unstable to isolate. Phenobarbital and its *N*-methyl derivative yielded acetylureides and diamides, but no malonuric acids (7), upon hydrolysis. The simplicity of I hydrolysis is due to dimethylated diamide and malonuric acid stability. A diamide is the only product of 1,3-dimethylbarbital hydrolysis (3). An acid intermediate proposed to be a carbamic acid could be a malonuric acid in rapid equilibrium with the barbituric acid.

A malonurate anion would not be expected to recyclize readily, but similar compounds form cyclic products. Diethylacetylurea cyclizes to barbital (2), 2-ureidobenzoate forms 2,4-dihydroxyquinazoline at a rate proportional to the hydroxide-ion activity (10), and 2-iodo-3-ureidopropionic acid yields 2-amino-2-oxazoline-3-carboxylic acid (11).

The observed reaction kinetics of I with hydroxide are consistent with the reaction sequence in Scheme I and agree with the qualitative observations that ring opening occurs predominantly at the 1,6-position. This is the first report of a biphasic decrease in barbiturate concentration upon alkaline hydrolysis, and its observation is due in part to the reaction mixtures being analyzed by a technique that permits separation of products. The simplicity of the reactions enabled the rate constants and equilibrium constant to be determined. The terminal disappearance of the barbituric acid is not a simple function but depends on all of the rate constants in Scheme II.

The rate of II formation is dependent on the second power of the hydroxide concentration. This result can be explained by tetrahedral complex formation between I and hydroxide ion, followed by a base-catalyzed ring opening to the malonurate. Such a sequence was proposed (12) for barbituric acid hydrolysis. The reverse reaction cyclization would have to be first order in hydroxide concentration, as was observed in this study.

The lack of a simple k_3 dependence on hydroxide concentration probably reflects the fact that the irreversible step is complex, involving ring opening and decarboxylation. Although a carbamic acid was not isolated in this work, it may be present in the reaction mixture and undergo decarboxylation during the workup. In view of carbamic acid instability, the quantity would be insignificant.

Although the reaction rate of I is biphasic, this behavior becomes modified at the lower and higher hydroxide concentrations and approaches first-order kinetics. Therefore, the complex behavior observed in the hydrolysis of barbituric acids possibly could be explained by closer examination of the experimental data.

The pyrimidine ring in 1,3-dimethylphenobarbital is preferably cleaved at the 1,6-position, as established by detailed kinetic studies, whereas final product analysis could lead to the conclusion that ring opening occurs at the 1,2-position. This system may serve as a useful model for a study of the effects of substituents at the 5 carbon without the complications of ionization or degradation to a large number of products.

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