Mechanism of Phenobarbital Degradation

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Previous studies on the degradation of barbiturates have indicated that the products of hydrolysis of the ionic and nonionic forms might be different. During the course of a study on the analysis of phenobarbital it was discovered that one of the postulated products (phenylethylacetylurea) could be determined in the presence of phenobarbital. Experiments were therefore undertaken to follow the breakdown of phenobarbital and the ureide at different pH values. The pseudo first-order rate constants for phenobarbital and the ureide were then determined. Analysis of equilibria phenomena and series first-order reaction kinetics show that the fraction decomposing by the ureide path is dependent on the concentration of the ionic form as previously postulated.

PREVIOUS WORK on the degradation of barbiturates (1) led to the postulate that the mechanism of hydrolysis of the ionized and nonionized forms was different, leading to distinguishable intermediates[1] (Fig. 1). This postulate could be tested by determining the concentration of these intermediates as a function of varying concentrations of the ionic and nonionic forms. Unfortunately, the determination of these intermediates is difficult for most of the barbiturate series. During a study on the analysis of phenobarbital (2) it was found that both phenobarbital and the ureide (G) can be determined spectrophotometrically in the presence of the decomposition products of phenobarbital. This, therefore, makes it possible to test the postulated mechanism.

EXPERIMENTAL

Reagents and Apparatus.—Phenobarbital, U.S.P. grade was recrystallized from dilute ethanol, m.p. 176–178°. Phenylethylacetylurea was prepared by thermal degradation of a 5% aqueous solution of phenobarbital and isolation of the precipitate. The ureide was recrystallized from dilute ethanol, m.p. 147–149° (3–5).

Ammonia buffer solutions (0.2, 0.4, 0.6, and 0.8 total molarity NH_3 and NH_4^+) at pH values of 8.0, 8.5, 9.0, and 0.8 *M* at a pH of 8.2, 8.7, and 9.5 were used. The total ionic strength was adjusted to 1.0 with potassium chloride in each case. A Beckman model DU spectrophotometer and a Beckman zeromatic pH meter were used.

Assays.—The concentration of phenobarbital was followed by determining the change in absorbance at 241 mμ in the following manner: a 10-ml. sample was diluted to 100 ml. with ammonia buffer (pH 10.0) and two 10-ml. aliquots of this were taken. One of these was diluted to 100 ml. with the same ammonia buffer while the second sample was diluted with 10 ml. of 1.0 *N* hydrochloric acid and sufficient buffer to make 100 ml. (Final pH = 1.5.) This absorbance difference is now used to obtain the phenobarbital concentration free of interference by the breakdown products (2).

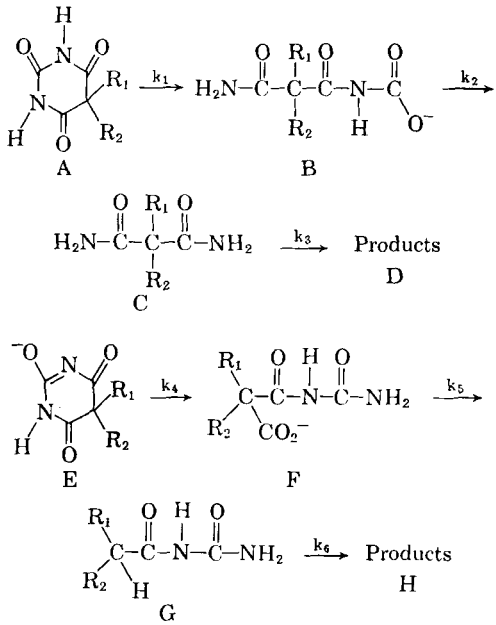

Fig. 1.—Degradation pathways of the nonionic and ionic species.

The concentration of phenylethylacetylurea during the degradation of phenobarbital was followed by measuring the absorbance of a 5-ml. sample at 245 and 260 mμ after the addition of 10 ml. of 1.0 *N* hydrochloric acid and dilution to 100 ml. with the pH 10.0 ammonia buffer. The use of simultaneous equations (after correction for the absorbance of phenobarbital) then made possible the determination of the ureide in the presence of diamide, or other interfering degradation products, all of which have approximately the same molar absorptivities at 245 and 260 mμ.

Concentration of Phenobarbital and Phenylethylacetylurea as a Function of Time.—Samples of phenobarbital (0.1% w/v final concentration) and

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[1] A different mechanism has been proposed by Hasegawa, *et al.* (7). Their mechanism, however, does not allow for the production of the diamide which has been isolated as a hydrolysis product (3, 8).

phenylethylacetylurea (0.01% w/v final concentration) were made up at different pH values using various concentrations of ammonium chloride for each pH value. (See Table I.) The samples were sealed in ampuls and kept in a constant temperature water bath at 60 ± 0.1°.

The reactions followed a pseudo first-order course since the log of the absorbance plotted *vs.* time was a straight line (Figs. 2 and 3). Table I gives the pseudo first-order rate constants for the different samples as determined by the method of least squares.

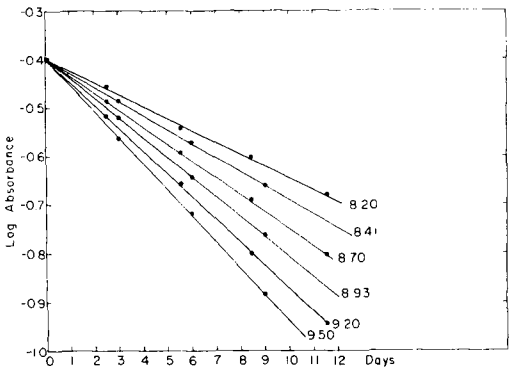

Fig. 2.—Degradation of phenobarbital at 60°. All at 0.8 *M*, numbers on individual lines are pH values.

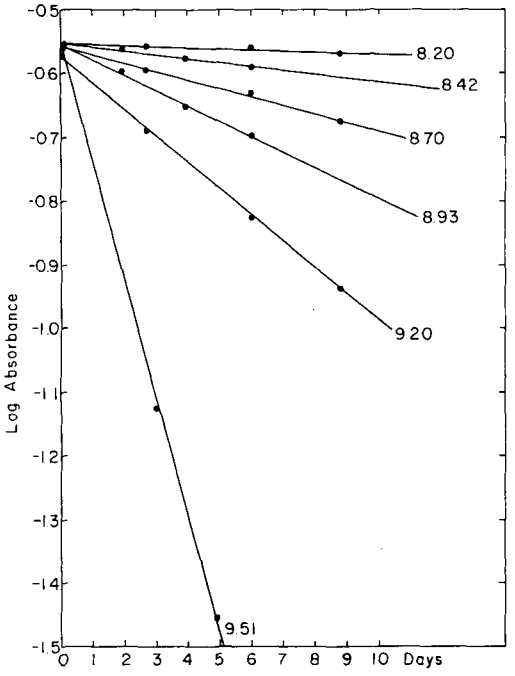

Fig. 3.—Degradation of phenylethylacetylurea at 60°. All at 0.8 *M*, numbers on individual lines are pH values.

DISCUSSION

In order to verify the similarity between the hydrolytic breakdown of phenobarbital and other barbiturates previously studied, a plot of the pseudo first-order rate constants *vs.* ammonium ion concentration was made (Fig. 4). These curves show a marked similarity to the curves obtained in previous work (1).

TABLE I.—FIRST-ORDER RATE CONSTANTS FOR THE DEGRADATION OF PHENOBARBITAL AND PHENYLETHYLACETYLUREA AT 60°

	Phenobarbital		
NH_4^+ *M*	pH at 25°	K, days^{-1}	Phenylethylacetylurea K, days^{-1}
0.1896	8.20	4.13×10^{-2}	...
0.3792	8.20	4.52×10^{-2}	...
0.5687	8.20	5.10×10^{-2}	...
0.7583	8.20	5.66×10^{-2}	4.03×10^{-3}
0.7030	8.42	6.70×10^{-2}	1.63×10^{-2}
0.1704	8.70	5.38×10^{-2}	...
0.3408	8.70	6.13×10^{-2}	...
0.5112	8.70	7.01×10^{-2}	...
0.6815	8.70	7.99×10^{-2}	3.10×10^{-2}
0.5569	8.93	9.29×10^{-2}	5.55×10^{-2}
0.1291	9.20	6.98×10^{-2}	...
0.2581	9.20	8.27×10^{-2}	...
0.3872	9.20	9.59×10^{-2}	...
0.5163	9.20	1.11×10^{-1}	9.55×10^{-2}
0.2922	9.51	1.23×10^{-1}	4.24×10^{-1}

The concentration of the ureide as a function of time may be predicted in the following manner (from Fig. 1)

$$E \xrightarrow{k_4} F \xrightarrow{k_5} G \xrightarrow{k_6} H$$

$$\frac{dE}{dt} = -k_4 [E], \quad \frac{dF}{dt} = -k_5 [F] + k_4 [E]$$

$$\frac{dG}{dt} = -k_6 [G] + k_5 [F]$$

Since species (F), the malonuric acid, is a very unstable compound (3, 5), it was assumed that $k_5 \gg k_4$ and the steady state approximation

$$-k_5 [F] + k_4 [E] = 0$$

or

$$[F] = \frac{k_4}{k_5} [E]$$

was made.

$$\therefore \frac{dG}{dt} = -k_6 [G] + k_4 [E]$$

This equation is solved in the usual manner (6) to obtain

$$[G]_T = \frac{E^0 k_4}{k_6 - k_4} [e^{-k_4 t} - e^{-k_6 t}] \quad \text{(Eq. 1)}$$

where E^0 is the original concentration of E.

If it is assumed that all of the barbiturate decomposes by the ureide path, E^0 becomes the original phenobarbital concentration, k_4 the pseudo first-order rate constant for the degradation of phenobarbital, and k_6 the pseudo first-order rate constant for the degradation of the ureide. The fraction of the total degradation going by the ureide path can then be defined as

$$f = \frac{[G]_M}{[G]_T} \quad \text{(Eq. 2)}$$

where $[G]_M$ is the measured concentration of ureide

TABLE II.—ANALYSIS OF FRACTION BY UREIDE PATH

pH	8.20		8.42		8.70		8.93		9.20		9.50	
Days	2.50	5.56	2.96	5.98	2.50	5.56	2.96	5.98	2.50	5.56	2.96	5.98
G_T, mg.	0.646	1.320	0.859	1.529	0.835	1.605	1.089	1.811	1.105	1.780	0.832	0.810
G_M	0.025	0.049	0.046	0.092	0.093	0.193	0.173	0.281	0.315	0.500	0.400	0.382
f	0.039	0.037	0.054	0.060	0.111	0.120	0.159	0.155	0.285	0.281	0.481	0.472
Av. f	0.038		0.057		0.116		0.157		0.283		0.477	

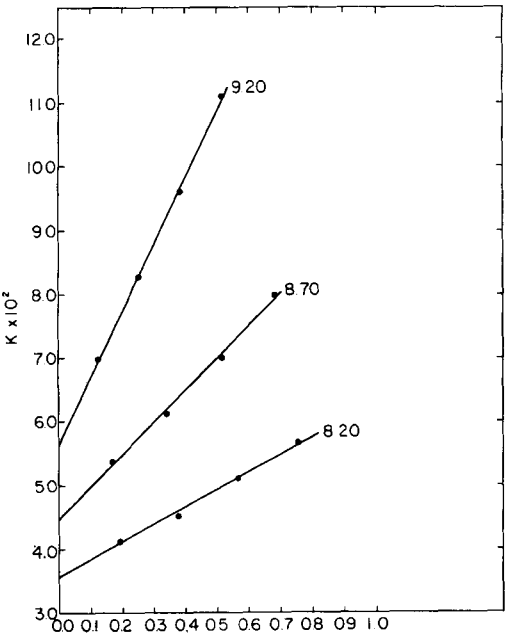

Fig. 4.—Pseudo first-order rate constants plotted *vs.* ammonium ion concentration at 60°. Numbers on individual lines are pH values.

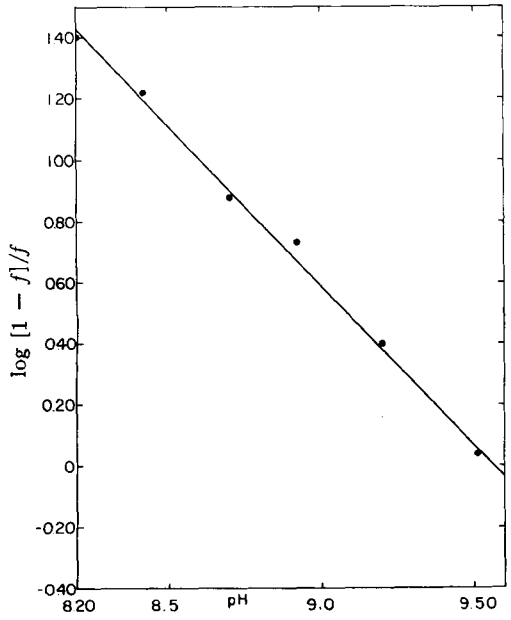

Fig. 5.—The effect of pH on fraction (f) degrading by ureide pathway.

at some given time and $[G]_T$ is the "theoretical" concentration computed by Eq. 1.

The assumption that the ureide pathway is taken by the ionized form of phenobarbital may now be formulated as follows: equilibrium requires

$$K_A = \frac{[E]\,[H^+]}{[A]} \qquad \text{(Eq. 3)}$$

where $[H^+]$ is the hydronium ion concentration of the solution

$$R_1 = k_1\,[A] \qquad \text{(Eq. 4)}$$

$$R_2 = k_4\,[E] \qquad \text{(Eq. 5)}$$

where R_1 and R_2 are the rates of degradation of the two forms, then

$$f = \frac{R_2}{R_1 + R_2} = \frac{k_4\,[E]}{k_1\,[A] + k_4\,[E]} = \frac{k_4\,[K_A]}{k_1[H^+] + k_4[K_A]} \qquad \text{(Eq. 6)}$$

and

$$1/f = 1 + \frac{k_1}{k_4\,K_A}\,[H^+] = 1 + \alpha\,[H^+] \qquad \text{(Eq. 7)}$$

or

$$\log\left[\frac{1-f}{f}\right] = \log\alpha - \text{pH} \qquad \text{(Eq. 8)}$$

Equation 8 therefore predicts that if the proposed mechanism is correct, a plot of log $[1 - f]/f$ *vs.* pH should be a straight line with a slope of -1. The experimental results are summarized in Table II and Fig. 5. The least squares slope of the line in Fig. 5 is -1.035. Thus, it appears that the proposed mechanism fits the experimental facts.

SUMMARY

1. The degradation of phenobarbital and phenylethylacetylurea have been followed concurrently in solution.

2. The results of these studies have been used to corroborate previous hypotheses about the degradation mechanism of barbiturates.

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