Phenytoin Prodrugs IV: Hydrolysis of Various 3-(Hydroxymethyl)phenytoin Esters

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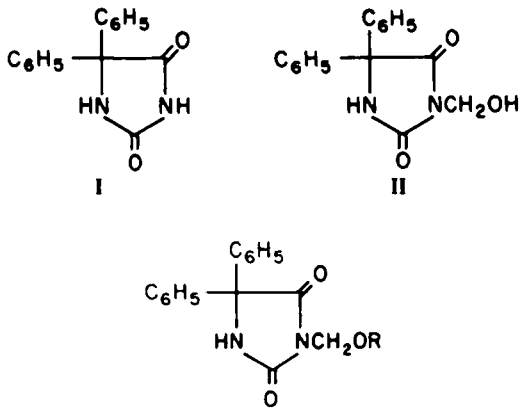
Abstract □ The aqueous chemical stability of various bioreversible derivatives or prodrugs of phenytoin, a poorly water-soluble and erratically absorbed drug after both oral and intramuscular parenteral dosing, were evaluated. This study, together with assessments of other physicochemical properties including cleavage in the presence of various animal tissues and anticonvulsant activity in mice, helped identify a number of promising candidate prodrugs. Various amino groups containing acyl esters of 3-(hydroxymethyl)phenytoin [3-(hydroxymethyl)-5,5-diphenylhydantoin] were identified as potential orally and perhaps parenterally useful prodrugs, while the disodium phosphate ester of 3-(hydroxymethyl)phenytoin appears to be ideally suited as a parenteral form of phenytoin.

Keyphrases □ Prodrugs—water-soluble, phenytoin stability, pH profiles □ Phenytoin—water-soluble prodrugs, stability, pH profiles □ Anticonvulsants—phenytoin, water-soluble prodrugs, stability, pH profiles

The aqueous chemical stabilities of various selected prodrugs and model compounds of phenytoin (I), *i.e.*, various esters (III–VII) of 3-(hydroxymethyl)phenytoin [II, 3-(hydroxymethyl)-5,5-diphenylhydantoin], are presented in this paper. The synthesis and a preliminary evaluation (including aqueous solubility determination, cleavage rates in the presence of various animal tissues, the chemical degradation of II to I under simulated physiological conditions, and, in some cases, the anticonvulsant activity in mice) of II–VII and other potential prodrugs of phenytoin were presented in the previous paper (1).



I

II

III: R = $COCH_3$
IV: R = $COCH_2\overset{+}{N}H(CH_3)_2CH_3SO_3^-$
V: R = $CO(CH_2)_2\overset{+}{N}H(C_2H_5)_2$
VI: R = $CO_2(CH_2)_2\overset{+}{N}H(CH_3)_2CH_3SO_3^-$
VII: R = $PO_3^{2-}Na_2^+$

The overall goal of this series of studies was to identify orally bioavailable phenytoin prodrugs with delivery characteristics superior to phenytoin itself, and parenterally useful prodrugs of phenytoin with good aqueous solubility and stability at a physiologically acceptable pH, a goal that was only partially achieved in a previous study (2).

Since any prodrug, to be useful as either an oral or parenteral form of phenytoin, must display adequate chemical stability, a study of the aqueous stability pH profiles for III–VII was undertaken. Because phenytoin has poor aqueous solubility (1, 3–6), the stability-limiting factor for a potential parenteral prodrug form of phenytoin was not considered to be maintenance of some percentage of the labeled amount of prodrug, but rather, the time that it takes for the poorly water-soluble phenytoin to begin precipitating from an aqueous solution of the prodrug on chemical degradation (2). This question will also be addressed briefly in this paper.

EXPERIMENTAL SECTION

Materials—Unless otherwise stated, all reagents used were analytical grade. Phenytoin was obtained from a commercial source[1]; the prodrugs were synthesized previously and were analytically pure (1). The water used in the stability studies was deionized (≤1-mho conductance) and charcoal-filtered prior to use or freshly distilled from an all-glass still. All buffers were prepared in carbonate-free (freshly boiled) deoxygenated (nitrogen-purge) water.

Kinetic Studies—Stock solutions of III in dry acetonitrile, IV–VII in water, and V in methanol–water were prepared immediately before use. An appropriate aliquot of the stock solution was mixed with the aqueous buffers to initiate kinetic runs. The final concentration of methanol in the case of V was <0.2% v/v and that of acetonitrile for III was <0.01% v/v.

All kinetic studies were carried out at a constant temperature maintained by a circulating-water bath[2]. The kinetics of conversion of IV–VI in the pH range of 3–5 and VII at all pH values were studied in aqueous buffers by the

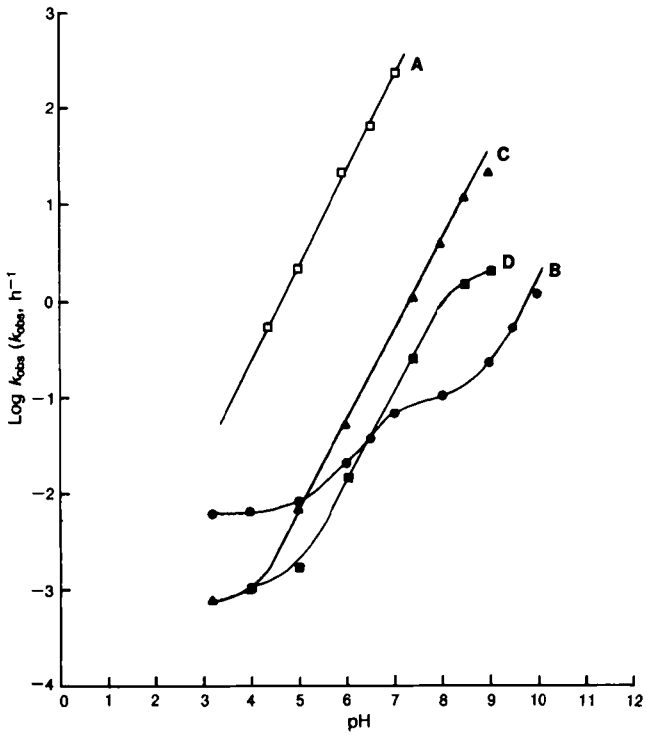


Figure 1—*pH-rate profiles for the hydrolysis of II (A), IV (B), V (C), and VI (D) to phenytoin at 25°C, μ = 0.5 M.*

[1] Sigma Chemical Co., St. Louis, Mo.
[2] Brinkman Model, Landa RC 20.

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Table I—Buffers Used in the Hydrolysis of the Esters III–VII at Various pH Values

pH Range	Buffer
3.0–5.0	Acetate
6.0–8.1	Phosphate
8.5–9.5	Borate
10.0	Carbonate
11.0–12.2	Sodium hydroxide

Table II—Observed First-Order Rate Constants for the Hydrolysis of III at Various pH Values [a]

pH[b]	Observed First-Order Rate Constant (k_{obs}), h^{-1}
12.19	39.7
11.92	17.2
11.8	15.3
11.5	11.08
11.1	3.24
11.01	3.34
10.8	2.01

[a] μ = 0.5, 25°C. [b] pH adjusted with sodium hydroxide.

initial rate method (7) since the half-lives of the esters under these conditions were long. The prodrugs in their stock solutions were mixed with the temperature-equilibrated buffer solution, and phenytoin production was followed until ~5% of the reaction was complete. All other kinetic studies were followed to completion by monitoring the appearance of phenytoin.

The chemical stability of III–VI was assessed in aqueous buffer solutions at 25 ± 0.1°C, and VII was studied at 70 ± 0.1°C (and at 50°C and 90°C at pH 7.4). A list of the buffers used at the various pH values is shown in Table I. The ionic strength was adjusted to 0.5 by the addition of potassium chloride. The stability studies were carried out at different buffer concentrations to allow for the determination of the rate constant at zero buffer concentration. Unless otherwise specified, all rate constants used in the pH–rate profiles were obtained by extrapolation to zero buffer concentration. The hydrolysis of III was monitored by UV spectroscopy[3], and the stability of VI–VII was studied by following the formation of phenytoin as a function of time using GC[4] as described previously by Stella (8).

Specifically, the rate of hydrolysis of III was studied at 25°C by following the decrease in absorbance at 256 nm. The initial concentration of the ester was 2×10^{-5} M. The reaction followed pseudo-first-order kinetics, and apparent first-order rate constants were determined from plots of log $(A_t - A_\infty)$ *versus* time, where A_t and A_∞ are the absorbance readings at time t and at the completion of the reaction, respectively. In all cases, the final spectrum corresponded to that of phenytoin. Conversion to phenytoin was also confirmed by TLC and GC.

The stability of IV–VII was studied by observing the appearance of phenytoin as a function of time using GC analysis (1, 8). In the case of esters possessing an amino group in the ester moiety (IV–VI), 100-μL samples were removed from the temperature-equilibrated reaction vessels at regular intervals and added to 100 μL of 10% metaphosphoric acid solution. The metaphosphoric acid helped quench the reaction due to the increased stability of these compounds under acidic conditions and also protonated the amino group of the ester, which prevented its extraction into toluene, the next step required for the work-up for the GC analysis (8).

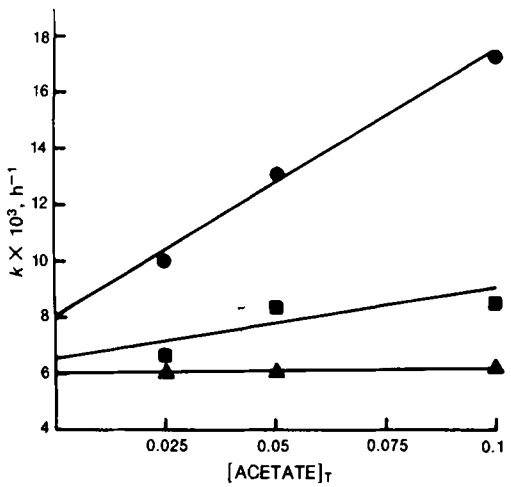


Figure 2—*Effect of acetate buffer concentration on the hydrolysis of IV at pH 3.2 (▲), 4.0 (■), and 5.0 (●); 25°C, μ = 0.5 M.*

[3] Cary Model 118, Varian Instruments.
[4] Varian Model 2100 or 3700 gas chromatograph.

Table III—Effect of Buffer Concentration on the Rates of Hydrolysis of IV at Various pH Values [a]

Buffer	Buffer Conc., M	Apparent First-Order Rate Constant (k), × 10^4 h^{-1}	k_{obs} at Zero Buffer Conc., × 10^4 h^{-1}
Acetate			
pH 3.2	0.025–0.1	60.4[b]	60.4
pH 4.0	0.025	66.7	
	0.05	83.9	
	0.1	85.7	65.8
pH 5.0	0.025	103.0	
	0.05	131.3	
	0.1	173.9	81.7
Phosphate			
pH 6.0	0.01	243	
	0.02	267	
	0.03	315	204.0
pH 6.5	0.01	422.5	
	0.02	468.2	
	0.03	515.1	376.0
pH 7.0	0.01	788	
	0.02	856	
	0.03	963	695.0
pH 8.0	0.01	1103.0	
	0.02	1194.0	
	0.03	1280.0	1015
Borate			
pH 9.0	0.02–0.04	2310[a]	2310
pH 9.5	0.01–0.04	5500[a]	5500
Carbonate			
pH 10.0	0.01	12882	12499.7
	0.03	13500	
	0.04	13980	

[a] μ = 0.5, 25°C. [b] No buffer catalysis.

For conversion of VII to phenytoin at elevated temperatures in aqueous buffer solutions, aliquots (100 μL) were removed from the reaction vessel at various intervals and transferred to tubes immersed in dry ice in order to quench the reaction. The samples were then assayed by the same technique used for following the hydrolysis of VII in tissue samples (1).

The conversion of the esters to phenytoin followed first-order kinetics. Apparent first-order rate constants for the production of phenytoin from the various prodrugs were determined from plots of log $(D_\infty - D_t)$ *versus* time where D_t and D_∞ are the concentrations of phenytoin at time t and infinity, respectively. The apparent first-order rate constants for the decompositions of IV–VI in the pH range of 3–5 and VII were determined by the initial rate method, in which <5% of the reaction was followed (7).

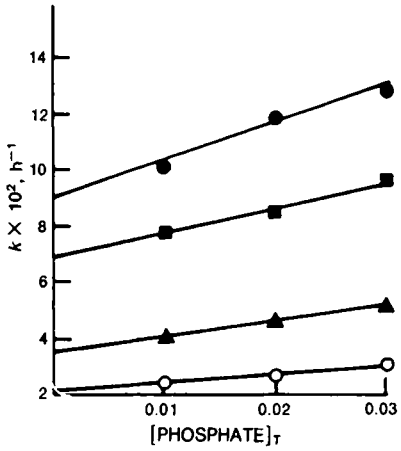


Figure 3—*Effect of phosphate buffer concentration on the hydrolysis of IV at pH 6.0 (○), 6.5 (▲), 7.0 (■), and 8.0 (●); 25°C, μ = 0.5 M.*

Table IV—Effect of Buffer Concentration on the Rates of Hydrolysis of V at Various pH Values [a]

Buffer	Buffer Conc., M	Apparent First-Order Rate Constant (k), × 10^4 h^{-1}	k_{obs} at Zero Buffer Conc., × 10^4 h^{-1}
Acetate			
pH 3.2	0.02	7.9	
	0.05	8.5	
	0.2	10.9	7.6
pH 4.0	0.025	10.7	
	0.1	12.6	
	0.2	15.2	10.0
pH 5.0	0.02	69.2	
	0.1	74.2	
	0.2	80.1	68.0
Phosphate			
pH 6.0	0.02-0.04	507.0[b]	507
pH 7.4	0.01-0.04	10,014.5[b]	10,014.5
pH 8.0	0.02-0.04	35,568.0[b]	35,568.0
Borate			
pH 8.5	0.02-0.04	112,200[b]	112,200
pH 9.0	0.02-0.04	221,405[b]	221,405

[a] μ = 0.5, 25°C. [b] No buffer catalysis.

Precipitation Time Study—This study was carried out to determine the time for precipitation of phenytoin from aqueous solutions of IV and VII. Various aqueous solutions of IV, *i.e.*, 1, 10, and 100 mg/mL, were prepared and filtered, and the pH values of the solutions were measured. Visual observations and spectral determinations of the precipitation times were recorded by the following technique. Three-milliliter aliquots of the solutions were placed in cylindrical 1-cm quartz spectrophotometer cells. These cells were then placed in a constant-temperature shaking water bath[5] and maintained at 25 ± 0.1°C. The absorbance of these solutions as a function of time was followed at 500 nm, a wavelength at which there was no absorbance due to the compounds, and the light absorption due to precipitate formation could be easily assessed. Plots of absorbance *versus* time were made and the precipitation time determined when a significant break occurred in the plot.

To determine the time for precipitation of phenytoin from a 61-mg/mL aqueous filtered solution of VII, visual observations were made. The solution was stored in sealed vials, and these were kept at ambient temperature (23-26°C). The vials were not subjected to any agitation during storage. The vials were inspected at regular intervals for the presence of any turbidity, in order to get an estimate of the time for phenytoin to precipitate from solution.

RESULTS AND DISCUSSION

Esters III-VII would be expected to revert to phenytoin in aqueous solution according to Scheme I, with the cleavage of the ester being the rate-determining step. The pH-rate profile for the cleavage of II to I was presented in the previous paper in this series (1), as well as by Bundgaard and Johansen (5). The log k_{obs} *versus* pH profile is reproduced in Fig. 1 (pH profiles at 25°C for II and IV-VI) to show that for all the esters at all the pH values studied, k_2 was indeed fast relative to k_1.

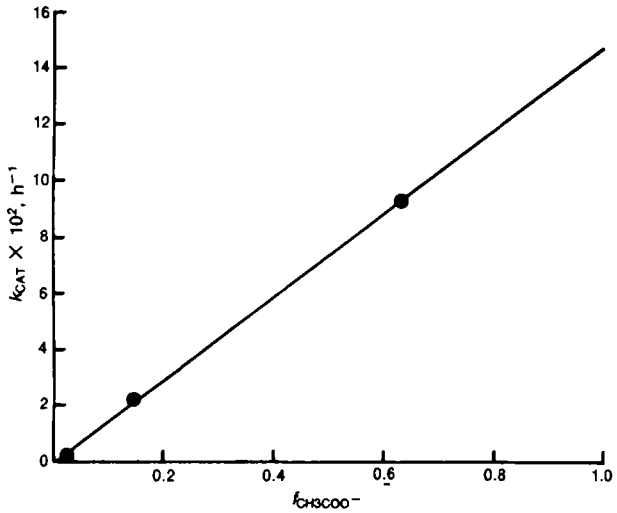


Figure 4—*Dependence of the catalytic constant,* k_{cat}, *on the fraction of acetate ion,* $f_{CH_3COO^-}$, *for the hydrolysis of IV.*

[5] AO Model No. 2156; American Optical Corp., Buffalo, N.Y.

Table V—Effect of Buffer Concentration on the Rates of Hydrolysis of VI at Various pH Values

Buffer	Buffer Conc., M	Apparent First-Order Rate Constant (k), × 10^4 h^{-1}	k_{obs} at Zero Buffer Conc., × 10^4 h^{-1}
Acetate			
pH 4.01	0.05	10.4	
	0.15	11.2	
	0.2	12.2	9.8
pH 4.98	0.1	18.2	
	0.15	19.4	
	0.2	20.7	15.7
Phosphate			
pH 6.0	0.01-0.03	143.6[b]	143.6
pH 7.4	0.01-0.03	2480[b]	2480
Borate			
pH 8.5	0.02-0.04	15180[b]	15180
pH 9.0	0.03-0.04	20550[b]	20550

[a] μ = 0.5, 25°C. [b] No buffer catalysis.

The observed pseudo-first-order rate constants (k_{obs}) for the hydrolysis of III to phenytoin at alkaline pH (μ = 0.5, 25°C) is given in Table II, and a plot of log k_{obs} *versus* pH was linear with a slope of 0.92, indicating a hydroxide ion-catalyzed reaction. The observed pseudo-first-order constant follows the form of:

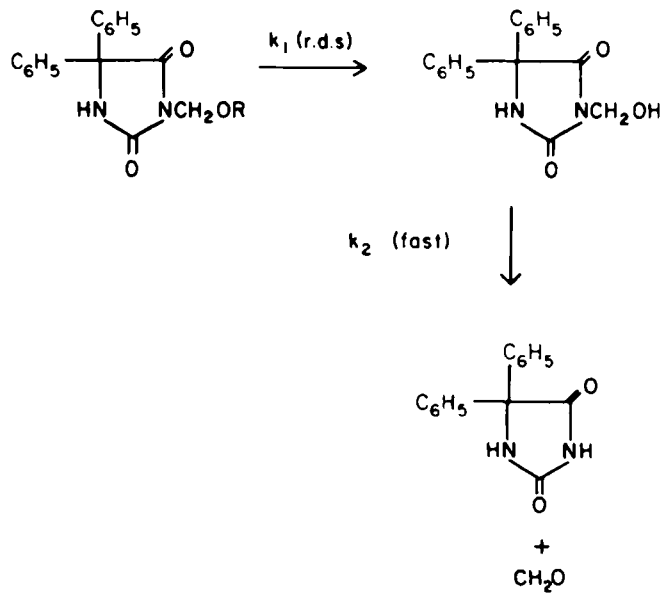
$$k_{obs} = k_{OH}a_{OH} \qquad \text{(Eq. 1)}$$

where a_{OH} is the hydroxide ion activity (calculated from k_w/a_H) and k_{OH} is the second-order hydroxide ion-catalyzed rate constant for the hydrolysis of III. A value of 50.8 M^{-1} min^{-1} was calculated for k_{OH} at 25°C, which was consistent with similar findings by Bundgaard and Johansen (5) for the hydrolysis of III at 37°C.

The purpose of studying the hydrolysis of III was twofold: (*a*) to observe the hydrolysis rate of III as a standard for the accelerative effect of amino groups in the acyl function, *e.g.*, how much faster does IV cleave relative to III, and (*b*) the determination of k_{OH} for III allows an estimate of the pK_a' of the hydroxyl group of II. For example, Bruice *et al.* (9) have shown that a linear relationship exists between the logarithm of the second-order rate constants for the alkaline hydrolysis of various acetyl esters and the pK_a' for the corresponding leaving-group alcohol. This relationship is given by:

$$\log k_{OH} = -0.253 \cdot pK_a' + 4.9 \qquad \text{(Eq. 2)}$$

Substituting the k_{OH} value for III into this equation gives a pK_a' = 12.6, a



Scheme I

Table VI—Rate and Dissociation Constants for the Hydrolysis of IV–VI in Buffered Aqueous Solutions

	IV	V	VI
k_0, $\times 10^4$ h^{-1}	60.0	7.5	9.8
k_{OH}, $\times 10^{-6}$ $M^{-1}h^{-1}$	2.4	3.6	1.44
k'_{OH}, $\times 10^{-4}$ $M^{-1}h^{-1}$	1.2	ND[b]	ND
K_a, $\times 10^9$	253	<1	6.3
pK_a	6.60	>9.0	8.20

[a] μ = 0.5, 25°C. [b] ND = Not determinable.

Table VII—Effect of Buffer Concentration on the Rate of Hydrolysis of VII at Various pH Values[a]

Buffer	Buffer Conc., M	Apparent First-Order Rate Constant (k), $\times 10^4$ h^{-1}	k_{obs} at Zero Buffer Conc., $\times 10^4$ h^{-1}
Acetate			
pH 3.9	0.025	50.1	
	0.05	50.7	
	0.1	62.1	44.4
Phosphate			
pH 6.5	0.02	13.4	
	0.03	15.2	
	0.04	18.3	8.3
pH 7.4	0.01–0.04	2.9[b]	2.9
pH 8.1	0.02–0.04	1.1[b]	1.1

[a] μ = 0.5, 70°C. [b] No buffer catalysis.

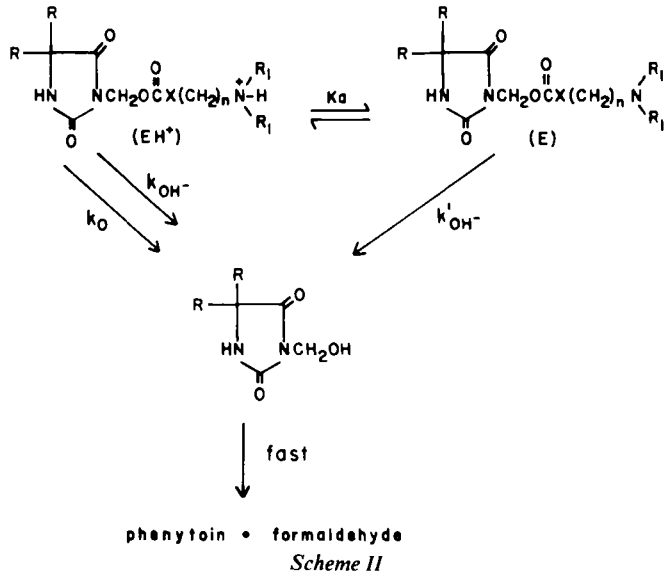
value comparable to the apparent pK_a' of 13.1 at 37°C previously reported for *N*-(hydroxymethyl)benzamide (4).

In aqueous solution, IV–VI hydrolyzed to yield phenytoin quantitatively. The effect of pH and buffer concentration on the apparent first-order rate constants for the hydrolyses of IV–VI are shown in Tables III–V, respectively. The buffer-free apparent first-order rate constants (k_{obs}) at various pH values were obtained from intercepts of linear plots of the apparent first-order rate constants against the total buffer concentration (see Fig. 2). The slopes of these plots are the catalytic constants for buffer catalysis, k_{cat}. Figure 1 is a plot of the logarithm of k_{obs} *versus* pH for the hydrolysis of II and IV–VI to phenytoin at 25°C, μ = 0.5.

The pH–rate profiles for IV–VI are consistent with Scheme II, where R_1 = —CH_3 or —C_2H_5, n = 0–2, x = —CH_2— or —O— and can be adequately described by[6]:

$$k_{obs} = \frac{k_0 a_H}{K_a + a_H} + \frac{k_{OH} a_H}{K_a + a_H}[OH^-] + \frac{k'_{OH} K_a}{K_a + a_H}[OH^-] \qquad \text{(Eq. 3)}$$

where k_0 is the pseudo-first-order rate constant for the pH-independent hydrolysis of the protonated form of the ester (pH < 4), and k_{OH} and k'_{OH} are the second-order rate constants for the apparent attack of the hydroxide ion on the protonated and unprotonated form of the esters, respectively. K_a is the dissociation constant of the protonated amino group. Equation 3 predicts a break in the pH profile at around the pK_a if k_{OH} is significantly different from k'_{OH} (as expected). Breaks can be seen at pH values close to the expected pK_a' values of the amino group for IV and VI. Ester V did not show a break up to pH 9.0, suggesting its pK_a may be >9.0. The lines drawn for the pH–rate profiles for IV–VI in Fig. 1 were constructed from Eq. 3 and the appropriate rate constants given in Table VI. These pH profiles were similar to the pH–rate profile of phenyl-3-dimethylaminopropionate reported by Kirby and Lloyd (10), as well as other amino side-chain esters (11–15).



Scheme II

[6] It is recognized in this and subsequent cases that kinetically equivalent terms may be equally correct and indistinguishable.

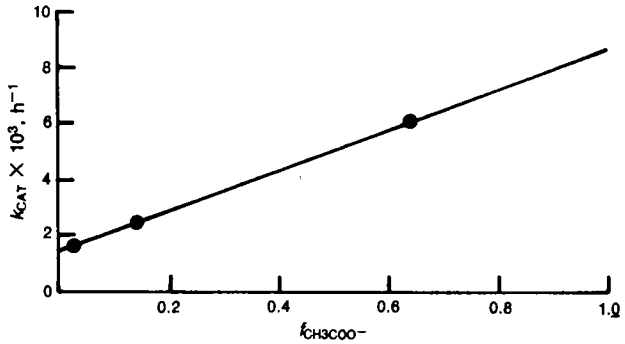


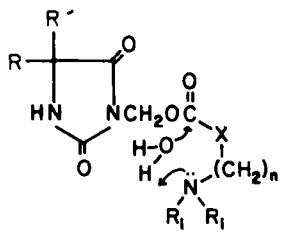
Figure 5—*Dependence of the catalytic constant, k_{cat}, on the fraction of acetate ion, $f_{CH_3COO^-}$, for the hydrolysis of V.*

Based on the pH profiles alone, it is difficult to assign mechanisms for these reactions. For example, the reaction term defined by k_{OH} in Scheme II and Eq. 3 is kinetically equivalent (mathematically equivalent) to the water-catalyzed hydrolysis of the unprotonated ester, perhaps facilitated intramolecularly through a general-base mechanism by the unprotonated tertiary amino group; alternatively, the possibility of an intramolecular nucleophilic attack by the tertiary amine on the acyl function displacing the alcohol has to be considered. A clue as to the possible mechanisms can be seen from an analysis of the buffer catalysis. The hydrolysis of IV exhibited buffer catalysis in the presence of acetate and phosphate buffers (see Figs. 2 and 3). Plots of k_{cat} *versus* the fraction of acetate ion $f_{CH_3COO^-}$ for each pH (Fig. 4) shows that the only catalytic species is the acetate ion. Similarly, reference to Fig. 3 shows that HPO_4^{2-} is a better catalyst than $H_2PO_4^-$. All these results are consistent with a general-base catalysis mechanism for these buffers, where the buffers are facilitating the attack by a water molecule on the acyl function or stabilizing a transition state.

In the case of V and VI, little or no buffer catalysis was observed, except in the case of acetate buffer for V. A plot of k_{cat} *versus* $f_{CH_3COO^-}$ (Fig. 5) for the cleavage of V again showed that general-base catalysis by the acetate anion was the primary catalyst; however, $k_{CH_3COO^-}$ for IV is 1.5×10^{-1} $M^{-1}h^{-1}$, while $k_{CH_3COO^-}$ for V is 8.5×10^{-3} $M^{-1}h^{-1}$, *i.e.*, acetate anion is a 17.6-fold better catalyst of IV hydrolysis compared with V. No significant buffer catalysis was seen for the hydrolysis of VI. It would appear from these results that the following mechanisms for the hydrolysis of IV–VI are operative:

1. At pH values <4, the primary reaction, k_0, involves water attack on the protonated ester, facilitated by general bases such as acetate and phosphate.

2. At pH values >5 but <pK_a, the primary reaction (defined mathematically in Eq. 3 as k_{OH}) is probably hydroxide ion attack on the protonated amine or attack of water on the unprotonated amine. The reason it is difficult to distinguish between these two mechanisms is that if the first mechanism is operative, no catalysis by buffers should be seen provided the rate-determining step is the actual attack on the acyl function by the hydroxide ion. This is usually seen with esters that have good leaving groups, *e.g.*, esters of activated phenols. Compound II should show behavior intermediate between a phenol and an aliphatic alcohol (a conclusion based on a pK_a of 12.6). Water



Scheme III

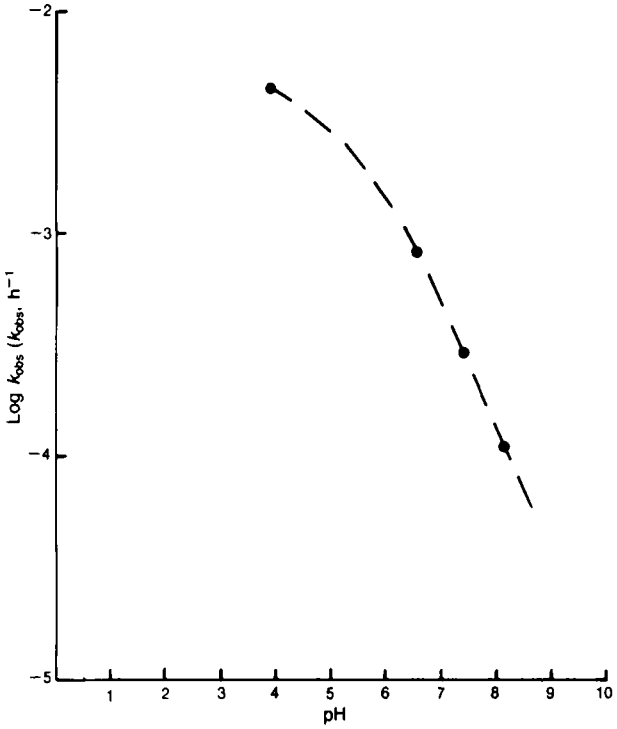


Figure 6—*pH-rate profile for the hydrolysis of VII at 70°C, μ = 0.5.*

attack on the unprotonated ester should be subject to general-base catalysis, but as seen in Scheme III intramolecular general-base catalysis by the dialkylamino group may be operative.

Such a mechanism is unfavorable for IV because a *five*-membered ring including a hydrogen bond would be required. Hydrogen-bonded systems favor a linear hydrogen bond (16) which, therefore, is not favored in a five-membered ring. Compounds V and VI form six- and seven-membered rings, respectively. Such cyclic structures are favorable for hydrogen-bond formation (16, 17). This type of intramolecular general-base catalysis has been shown by Kirby and Lloyd (10) to occur in the hydrolysis of other 3-dimethylaminopropionates. To fully differentiate between the two mechanisms, techniques such as proton inventory solvent isotope effects will have to be employed. Alternatively, ionic strength and solvent polarity changes could be used. However, interpretation of results obtained from such studies are complicated by changes in pK_a values, *etc.*

3. More than likely at pH > pK_a (*e.g.*, pH > 9 for IV), the major reaction is probably hydroxide attack on the unprotonated amine. Consistent with this is the lack of buffer catalysis by carbonate or borate[7] in the hydrolysis of IV.

Phosphate esters such as VII are of interest pharmaceutically because their polar nature provides a means of making water-soluble derivatives of sparingly soluble compounds such as those containing a hydroxyl group. In addition phosphate esters have been found to be chemically stable at neutral to slightly alkaline pH (18–22). The hydrolysis of such esters follow pseudo-first-order kinetics; it is assumed that VII would behave similarly.

Table VII contains the data on the rates of hydrolysis of VII to phenytoin at 70°C and various pH values and buffer concentrations, while Fig. 6 shows the pH–rate profile for the hydrolysis of VII at 70°C.

To determine the apparent energy of activation and the half-life for the hydrolysis of VII at 25°C and pH 7.4, rate constants were determined at various temperatures. Figure 7 shows the Arrhenius plot for the hydrolysis of VII, obtained by plotting the apparent first-order rate constants, determined at pH 7.4 and in 0.04 M phosphate buffer, *versus* the reciprocal of the absolute temperatures. From the slope of the Arrhenius plot, the apparent energy of activation and the half-life for the hydrolysis of VII at 25°C were calculated.

The data on the hydrolysis of VII suggests that it is catalyzed by acetate and phosphate buffers at pH 3.9 and 6.5. The pH–rate profile for VII is similar to the pH–rate profiles reported for the hydrolysis of various other phosphate monoesters (18–22). The rate of hydrolysis increases with decreasing pH suggesting that the monoanionic form of VII is the reactive species. The concentration of the monoanion would be at a maximum at pH ~4.0, as the

[7] This was also not unexpected, as borate is a poor general-base catalyst.

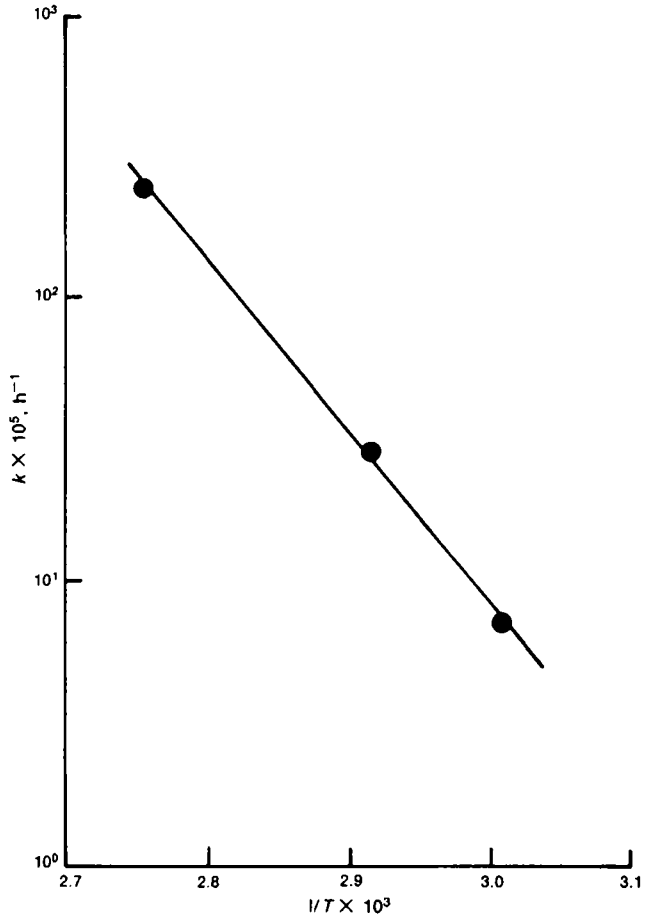
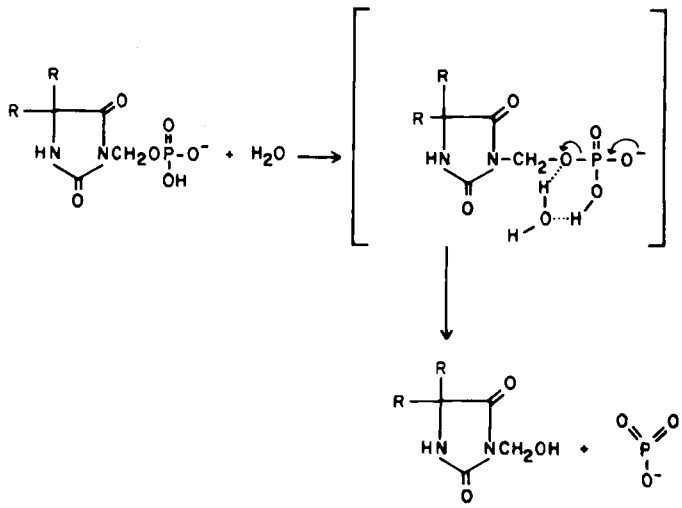
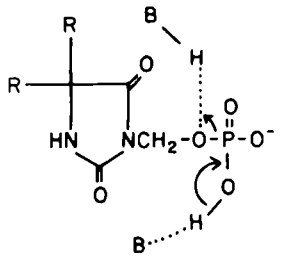


Figure 7—*Arrhenius plot for the hydrolysis of VII to phenytoin in 0.04 M phosphate buffer, pH 7.4, μ = 0.5.*

second pK_a of most phosphate esters is 6.0–6.5 (18–21) at this ionic strength. The pK_{a_2} of VII in water at 25°C was determined to be 6.2 by titration with hydrochloric acid. Thus, the pH–rate profile for VII began to plateau at pH 4.0; however, the rate of degradation of VII below pH 4.0 was not studied. The hydrolysis of other phosphate esters at pH <4.0 has been shown to increase (20) or decrease (23, 24) depending on the reactivity of the neutral species. The neutral species may undergo degradation, with carbon–oxygen bond cleavage. The mechanism for the hydrolysis of VII in the pH range 3.9–8.1 is probably similar to that proposed for the hydrolysis of other phosphate monoesters (20–22). The probable reaction mechanism is shown in Scheme IV. The mechanism involves the negative charge on the phosphate oxygen acting as the driving force for the reaction. Proton transfer to the alkoxide group occurs by formation of a six-membered ring through hydrogen



Scheme IV



Scheme V

bonding with a single water molecule. This would be consistent with the dianion being less susceptible to hydrolysis, as observed in the pH-rate profile.

Another concerted mechanism can be proposed to explain the buffer catalysis observed at pH 3.9 and 6.5 (Scheme V). The mechanism involves the buffer acting as both a proton donor and a proton acceptor.

The apparent energy of activation of 20.5 kcal·mol^{-1} obtained for the hydrolysis of VII was consistent with the activation energies reported for other phosphate esters (18). Ester VII had a long half-life of ~6800 d at 25°C and pH 7.4, which is characteristic of most phosphate esters.

Based on the pH profiles (Figs. 1 and 7) and the data presented in the rest of this study, esters IV–VII hydrolyze to phenytoin *via* pseudo-first-order kinetics at fixed pH values with no accumulation of the decomposition intermediate, II. The amino acyl esters exhibit maximum stability in the pH range 3–4 (Table VIII), which is within the physiologically acceptable range for parenteral dosage forms. The superior stability of VII over IV–VI makes it an attractive prodrug candidate for parenteral administration provided it is nontoxic and rapidly and quantitatively reverts to phenytoin *in vivo*.

Table VIII points out the $t_{90\%}$ (time for 90% of the ester remaining at the indicated pH values), perhaps suggesting that this may be the determining factor controlling the shelf life of a reconstituted dosage form of these prodrugs if they were to be formulated as reconstituted parenteral dosage forms (sterile powder or freeze-dried powder). However, as pointed out earlier, the $t_{90\%}$ will probably not be the stability-limiting factor for these products, since hydrolysis of the esters produces the sparingly soluble parent drug, phenytoin. For example, if a 50-mg/mL solution (phenytoin equivalents) of IV is prepared, then theoretically, when 0.02 mg/mL of phenytoin is produced, phenytoin should begin precipitating from solution; *i.e.*, the reaction would only have to proceed to the extent of 0.04% before problems could occur. For this reason, and because IV and VII (25, 26) were considered to be the two most likely candidates as oral/parenteral and parenteral prodrugs, respectively, a limited study[8] on the theoretical and observed precipitation times for phenytoin was undertaken

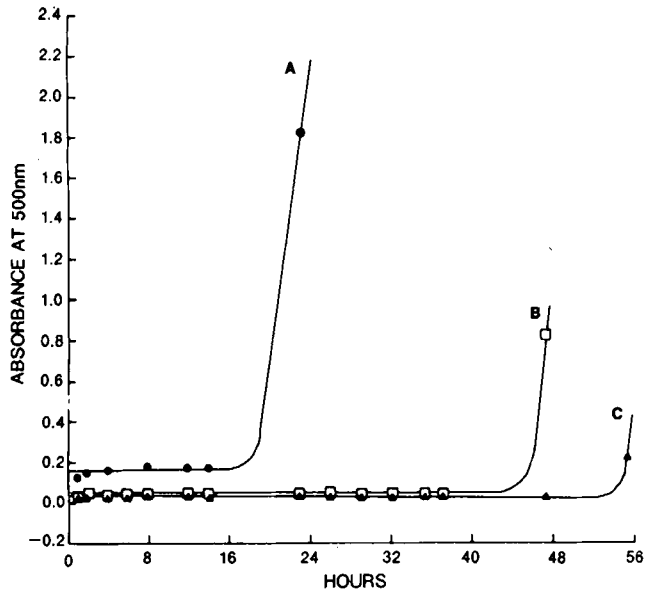


Figure 8—*Plot of change in the absorbance at 500 nm of aqueous solutions of IV due to precipitation of phenytoin. Solutions are 100 (A), 10 (B), and 1 mg/mL (C) of IV.*

[8] A more comprehensive study of this problem of precipitation is currently underway in our laboratory. Results will be formally presented in a later paper.

Table VIII—Time for 10% Hydrolysis ($t_{90\%}$) of IV–VI at pH Values of Maximum Stability and of VII at pH 7.4 [a]

Compound	pH	$t_{90\%}$, h
IV	3.2	17.4
V	3.2	138.1
VI	4.0	107.1
VII	7.4	24,769.0 (2.8 years)

[a] μ = 0.5, 25°C.

Table IX—Theoretical ($t_{ppt,calc}$) and Experimental ($t_{ppt,obs}$) Precipitation Times for the Formation of Phenytoin from Aqueous Solutions of IV and VII, Determined at 25°C

Compound	Initial Conc. of Ester (Phenytoin equivalent), mg/mL	pH of Solution	$t_{ppt,calc}$	$t_{ppt,obs}$
IV	1 (0.54)	3.86	6 h[a]	~55 h
IV	10 (5.44)	2.85	0.6 h[a]	~43 h
IV	100 (54.43)	1.94	0.06 h[a]	~19 h
VII	61.3 (38.1)	8.5	13.7 d[b]	>114 d[c]

[a] The half-life for the degradation of IV to phenytoin was taken as 115.0 h at pH 3.2 and 25°C. [b] The apparent first-order rate constant for the degradation of VII to phenytoin was taken as 68.9 × 10^{-8} h^{-1} at pH 8.5 and 25°C. [c] No precipitation was observed at the end of the 114-d period.

using aqueous solutions of 1–100 mg/mL of IV at 25 ± 0.1°C and a 61.3-mg/mL aqueous solution of VII at pH ~8.5 and ambient room temperature.

In the case of IV, 1-, 10-, and 100-mg/mL solutions of IV were prepared and the presence of a precipitate was detected by following absorbance changes at 500 nm (Fig. 8). The break in the absorbance *versus* time plots was due to the light absorption by the solid phenytoin when it began to precipitate from solution. In the case of VII, only periodic visual inspection over a 114-d period was attempted. Table IX summarizes the calculated and observed times for phenytoin to precipitate from aqueous solutions of esters IV and VII. The calculated times were based on the assumption that the rate constant for hydrolysis was independent of initial concentration and that precipitation would occur when a 7.9 × 10^{-5} M (20-μg/mL) solution of phenytoin was produced at 25°C. It was also assumed that the prodrug solutions contained no phenytoin as an initial impurity.

The results shown in Table IX show no correlation between the calculated (theoretical) and observed precipitation times. Possible explanations for this behavior have appeared in the literature (2). In an extension of the results presented in Table IX, we have tentatively shown[8] that the following processes contribute to this lack of correlation.

1. In the case of IV, associated solutions (probably micellar) occur at ~3–5 mg/mL of IV, resulting in a slowing of the rate of phenytoin production at concentrations >3–5 mg/mL than would be predicted by the dilute solution kinetics. This contributes approximately a factor of two to the discrepancy between $t_{ppt,calc}$ and $t_{ppt,obs}$.

2. Phenytoin is significantly solubilized by IV at concentrations >3–5 mg/mL, *e.g.*, a 50-mg/mL solution of IV has been shown to dissolve ~1.6 mg/mL of phenytoin[8], an increase in aqueous solubility of phenytoin of ~80-fold over water alone. This increased solubility by the prodrug, along with the processes mentioned above, can account for all but about a factor of three in the discrepancy between the theoretical and observed precipitation times. A similar study with VII showed that in the presence of 74 mg/mL of VII, the solubility of phenytoin was approximately double that in water[8].

3. Therefore, it appears from our preliminary results that there may also be a contribution from supersaturation (a factor of two to five).

Based on the data presented in this paper and the solubility data on IV–VII presented in the previous paper (1), it seems that all the compounds should be sufficiently stable for oral usage, especially in solid dosage forms; *i.e.*, the compounds do not appear to be excessively unstable in aqueous solution. It also seems likely that any injectable form of IV, VI[9], and VII would have to be of the reconstitutable type, although there may be a chance that VII maintained at pH ~9–9.5 would be sufficiently stable as a constituted injectable[10]. Based on stability considerations alone, the best candidate for an injectable form of phenytoin appears to be the phosphate ester, VII.

[9] Compound VI will be rejected later due to toxicity concerns. Compound V had already been rejected as a parenteral form due to its low aqueous solubility.

[10] Such studies on VII are ongoing.

REFERENCES

(1) S. A. Varia, S. Schuller, K. B. Sloan, and V. J. Stella, *J. Pharm. Sci.*, **73**, 1068 (1984).

(2) V. Stella, T. Higuchi, A. Hussain, and J. Truelove, in "Prodrugs as Novel Drug Delivery Systems," T. Higuchi and V. Stella, Eds., American Chemical Society Symposium Series #14, 1975, pp. 154–183.

(3) K. Arnold, N. Gerber, and G. Levy, *Can. J. Pharm. Sci.*, **5**, 89 (1970).

(4) M. Johansen and H. Bundgaard, *Arch. Pharm. Chem., Sci. Ed.*, **7**, 175 (1979).

(5) H. Bundgaard and M. Johansen, *Int. J. Pharm.*, **5**, 67 (1980).

(6) D. W. Newton and R. B. Kluza, *Am. J. Hosp. Pharm.*, **37**, 1647 (1980).

(7) A. A. Frost and R. G. Pearson, in "Kinetics and Mechanism," 2nd ed., Wiley, New York, N.Y., 1960, p. 28.

(8) V. J. Stella, *J. Pharm. Sci.*, **66**, 1510 (1977).

(9) T. C. Bruice, T. H. Fife, J. J. Bruno, and N. E. Brandon, *Biochemistry*, **1**, 7 (1962).

(10) A. J. Kirby and G. J. Lloyd, *J. Chem. Soc., Perkin II*, **1976**, 1748.

(11) T. Higuchi, A. Havinga, and L. W. Busse, *J. Am. Pharm. Assoc., Sci. Ed.*, **39**, 405 (1950).

(12) M. Johansen and H. Bundgaard, *Arch. Pharm. Chem., Sci. Ed.*, **9**, 43 (1981).

(13) P. Y. Bruice and H. G. Mautner, *J. Am. Chem. Soc.*, **95**, 1582 (1973).

(14) T. C. Bruice and S. J. Benkovic, *J. Am. Chem. Soc.*, **85**, 1 (1963).

(15) J. P. Patel and A. J. Repta, *Int. J. Pharm.*, **9**, 29 (1981).

(16) S. S. Minor and R. L. Schowen, *J. Am. Chem. Soc.*, **95**, 2279 (1973).

(17) M. D. Joesten and L. J. Schaad, "Hydrogen Bonding," Dekker, New York, N.Y., 1974, chap. 5, pp. 253–279.

(18) G. L. Flynn and D. J. Lamb, *J. Pharm. Sci.*, **59**, 1433 (1970).

(19) T. O. Oesterling and E. L. Rowe, *J. Pharm. Sci.*, **59**, 175 (1970).

(20) J. Kumamoto and F. H. Westheimer, *J. Am. Chem. Soc.*, **77**, 2515 (1955).

(21) C. A. Bunton, D. R. Llewellyn, K. G. Oldham, and C. A. Vernon, *J. Chem. Soc.*, **1958**, 3574.

(22) W. W. Butcher and F. H. Westheimer, *J. Am. Chem. Soc.*, **77**, 2420 (1955).

(23) M. C. Bailley, *Bull. Soc. Chim. France*, **9**, 340 (1942).

(24) A. Desjobert, *Bull. Soc. Chim. France*, **14**, 809 (1947).

(25) S. A. Varia and V. J. Stella, *J. Pharm. Sci.*, **73**, 1080 (1984).

(26) S. A. Varia and V. J. Stella, *J. Pharm. Sci.*, **73**, 1087 (1984).

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Phenytoin Prodrugs V: *In Vivo* Evaluation of Some Water-Soluble Phenytoin Prodrugs in Dogs

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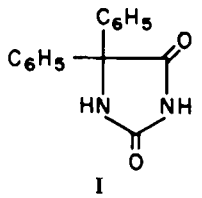
Abstract □ Phenytoin bioavailability was evaluated in beagle dogs after oral and intravenous administrations of sodium phenytoin and two amino acyl esters and a disodium phosphate ester of 3-(hydroxymethyl)phenytoin (three prodrugs of phenytoin). Phenytoin displayed nonlinear pharmacokinetics in the dogs, complicating the determination of the absolute bioavailability of phenytoin from sodium phenytoin and the prodrugs. All three prodrugs essentially released phenytoin after intravenous administration in a quantitative manner, and all gave plasma levels of phenytoin after oral administration greater than those found after administration of sodium phenytoin. Based on the behavior in dogs and the earlier determination of the physicochemical properties of the prodrugs, it was concluded that one of the amino acyl esters, 3-(hydroxymethyl)-5,5-diphenylhydantoin *N,N*-dimethylglycine ester methanesulfonate, would be the most useful prodrug for oral administration, while 3-(hydroxymethyl)-5,5-diphenylhydantoin disodium phosphate ester would be the most useful for parenteral administration.

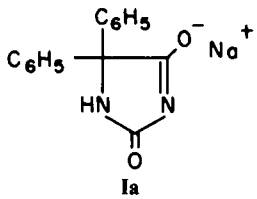
Keyphrases □ Prodrugs—phenytoin, bioavailability, dogs □ Phenytoin—hydroxymethyl esters, prodrugs, bioavailability, dogs □ Bioavailability—phenytoin prodrugs, dogs

Phenytoin (I), because of its weakly acid nature (1–4) and poor aqueous solubility (3–5), shows erratic absorption patterns after oral administration of either the sodium salt (Ia) or the free acid in both humans (6–19) and dogs (20, 21). Sodium phenytoin (Ia) in the parenteral dosage form is hazardous if rapidly injected intravenously (22, 23), and the free acid appears to precipitate at intramuscular injection sites (24–29), leading to prolonged and marginal phenytoin release.

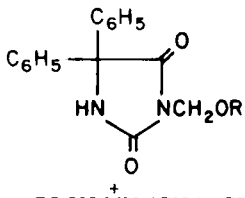
In the previous papers in this series (5, 30), a number of water-soluble prodrugs of phenytoin were evaluated with respect to their physicochemical properties (*e.g.*, solubility and stability), their cleavage to phenytoin in animal tissues, and their anticonvulsant activity in mice. Based on those studies, three of the prodrugs, 3-(hydroxymethyl)-5,5-diphenylhydantoin *N,N*-dimethylglycine ester methanesulfonate (II)[1], 3-(hydroxymethyl)-5,5-diphenylhydantoin *N,N*-dimethylaminoethyl carbonate methanesulfonate (III)[1], and 3-(hydroxymethyl)-5,5-diphenylhydantoin disodium phosphate



I



Ia



II: R = $-COCH_2\overset{+}{N}H(CH_3)_2\ CH_3SO_3^-$

III: R = $-CO_2(CH_2)_2\overset{+}{N}H(CH_3)_2\ CH_3SO_3^-$

IV: R = $-PO_3^{2-}\ Na_2^+$

[1] Compounds II, III, and IV are equivalent to IV, VI, and VII, respectively, in papers III and IV in this series.

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