Esters of Hydantoic Acids as Prodrugs of Hydantoins

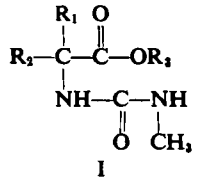
V. STELLA* and T. HIGUCHI▲

Abstract □ Hydantoins used as drugs possess low water solubility, which has led to erratic absorption patterns and dissolution patterns and possible precipitation in the blood after intravenous infusions of solutions of their sodium salts. The aim of this investigation was to elucidate the chemistry, physical properties, and pharmacological activity of some more water-soluble derivatives of hydantoins to determine the feasibility of their use as prodrugs. Various esters of the hydantoic acids of mephenytoin and 3-methylphenytoin were prepared and found to be quantitatively converted to the respective hydantoin under neutral to basic conditions. The β-*N'*,*N'*-diethylaminoethyl esters, in particular, were soluble in water up to pH 8 (presumably in the protonated form) and were converted to the respective hydantoin at pH 7.4 and 37°, with half-lives of under 20 min. At 25° and pH 6.0, the esters were stable for a few hours. An animal study using mice to test for anticonvulsant activity of the esters after intraperitoneal injection gave encouraging results (ED_{50} = 60 mg./kg., LD_{50} = 600 mg./kg.). The results suggested that β-*N'*,*N'*-diethylaminoethyl esters of hydantoic acids were candidates as water-soluble prodrugs of the respective hydantoin.

Keyphrases □ Hydantoic acid esters—evaluated as potential hydantoin prodrugs □ Hydantoin prodrugs—evaluation of water-soluble hydantoic acid esters □ Prodrugs for hydantoins—evaluation of water-soluble hydantoic acid esters

A number of hydantoins, including phenytoin, mephenytoin, and nitrofurantoin, are widely used as drugs. Their medical and veterinary usages, however, have been hampered by their low water solubility combined with their weakly acidic nature. Phenytoin, for example, has shown erratic absorption patterns and dissolution patterns as well as possible precipitation in the body after intravenous infusions of highly alkaline solutions of its sodium salt (1–4). Gastric upset associated with oral doses of sodium phenytoin similarly may be due to this necessary high alkalinity of aqueous solutions of the salt form.

The aim of this investigation was to determine the feasibility of obtaining transient prodrug (5) forms of these drugs which would confer acceptably high water solubility within the physiological pH range but convert to the desired active structure within the body system. Results obtained based on various esters (I) of the



I

R_1, R_2 = aryl or alkyl substituents
R_3 = alkyl (simple esters) or $-C_2H_4N(C_2H_5)_2$ (amino esters)

respective hydantoic acid of the hydantoin, especially those esters that have an ionizable amino group in the alcohol portion of the ester grouping, are reported at this time. Esters of hydantoic acids are known to cyclize *via* an intramolecular reaction under basic conditions to their respective hydantoin (Scheme I), but no actual kinetic data are available. Kinetic data are available, however, on the cyclization of esters of the related *o*-ureidobenzoic acid under aqueous conditions in the neutral to alkaline pH range (6). Intramolecular cyclizations have been postulated as a means of latentiation by Levine *et al.* (7) in their work on the conversions of ω-haloalkylamines to their quaternary analogs. In the past, many derivatives of drugs formed through an ester linkage have been dependent on enzymatic reactions to release the parent compound (8, 9). Intramolecular reactions are known to be many thousand times faster than their intermolecular equivalent (10), forming a basis for the release of the parent compound at a speed that is not totally dependent on enzymatic mediation.

Table I lists the four esters investigated in the present study along with their compound numbers and formulas. Compounds IV and V cyclize to give mephenytoin, while II and III cyclize to give 3-methylphenytoin. Both mephenytoin and 3-methylphenytoin are anticonvulsant drugs.

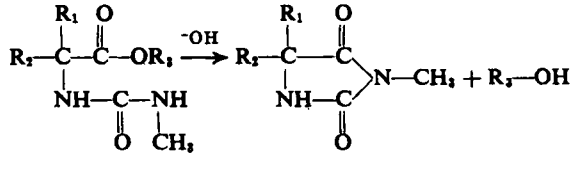
EXPERIMENTAL

Equipment—A recording spectrophotometer[1] with a thermostated cell compartment capable of maintaining temperatures to ±0.1° was used for all spectroscopic measurements. The pH measurements were carried out using a research pH meter[2] standardized at 25° with phosphate buffer (pH 7.40 at 25°) and borate buffer (pH 9.18 at 25°). NMR spectra were measured on an NMR spectrometer[3].

Materials and Reagents—All chemicals used were of analytical or reagent grade. Mephenytoin was supplied commercially[4]. Buffers were made from triply distilled water and analytical grade phosphates and borates, with the ionic strength adjusted to unity with potassium chloride. Buffer concentration was 0.1 *M*.

The four esters were prepared *via* identical pathways. Analyses of the compounds are shown in Table II, and Table III gives some spectral data. An example of the synthetic procedure is listed here, and any differences associated with the synthesis of the other three esters are explained under the particular step.

Synthesis of V—*Step 1*—Ten grams (0.046 mole) of mephenytoin was placed in a plunging autoclave with 200 ml. of 20% NaOH under nitrogen. The mixture was heated at 180–190° for 24 hr., filtered through charcoal, and diluted to 500 ml. The solution was neutralized with glacial acetic acid and concentrated to 150 ml.



Scheme I

[1] Cary 14.
[2] Corning model 12.
[3] Varian T60.
[4] From Sandoz Drug Co.

Table I—Hydantoic Acid Esters Studied with R_i (i = 1, 2, or 3) Referring to the Groups in Scheme I

Chemical Name	Compound Number	R_1	R_2	R_3
Ethyl 5-methyl-2,2-diphenylhydantoate	II	C_6H_5	C_6H_5	C_2H_5
β-N′,N′-Diethylaminoethyl 5-methyl-2,2-diphenylhydantoate	III	C_6H_5	C_6H_5	$C_2H_4N(C_2H_5)_2$
Methyl 2-ethyl-5-methyl-2-phenylhydantoate	IV	C_6H_5	C_2H_5	CH_3
β-N′,N′-Diethylaminoethyl 2-ethyl-5-methyl-2-phenylhydantoate	V	C_6H_5	C_2H_5	$C_2H_4N(C_2H_5)_2$

The crystalline material that precipitated was filtered and washed with 200 ml. of water and 200 ml. of ether. This gave a product of m.p. 288° dec., corresponding to 2,2-ethylphenylglycine (VI) [lit. (11) m.p. 275° dec.]. The yield was 5.5 g.

Step 2—Three grams (0.017 mole) of VI was dissolved in 30 ml. of 1 *N* NaOH. An excess of ethyl chloroformate (5 g., 0.047 mole) was added dropwise to this solution, while the pH of the solution was maintained alkaline by the addition of sodium hydroxide pellets. The solution was allowed to stir until no odor of ethyl chloroformate was detectable. After extraction with 2 × 50-ml. portions of ether (to remove any ether-soluble impurities), the aqueous layer was acidified with hydrochloric acid and again extracted with 4 × 50-ml. portions of ether. After drying over magnesium sulfate, the second ether extraction was evaporated by a rotary evaporator for 1 hr. Fifteen milliliters of benzene was added and warmed to complete solution. *n*-Hexane was added dropwise to cause cloudiness and precipitation of a white solid of m.p. 130–131° and a yield of 1.5 g. This was presumed to be *N*-ethoxycarbonyl-2,2-ethylphenylglycine (VII).

Step 3—Five milliliters of thionyl chloride was refluxed with VII (700 mg., 0.003 mole) for 1 hr. The excess thionyl chloride was removed by a rotary evaporator, leaving a yellow solid. The solid was taken up in benzene and precipitated with *n*-hexane, leaving a white compound of m.p. 99–101° and a yield of 550 mg. This was presumed to be 4,4-ethylphenyloxazolidinedione (2, 5) (VIII).

Step 4—To VIII (500 mg., 0.002 mole), freshly distilled diethylaminoethanol (400 mg., 0.003 mole) was added in a dry flask at 140°. An atmosphere of hydrogen chloride gas was passed over the melt. These conditions were maintained for 20 min. No product was actually isolated in this step.

If the ethyl or methyl esters were being prepared, VIII was refluxed in a solution of the appropriate alcohol which had hydrogen chloride gas passed through it.

Step 5—The syrupy melt of Step 4 was taken up in chloroform and extracted with 0.1 *N* NaOH and water. After drying, the chloroform layer was evaporated to 0.5 ml. and methylisocyanate was added (500 mg., 0.009 mole). The solution was allowed to stand for 20 min. and then evaporated to remove the excess methylisocyanate. The remaining oil was taken up in *n*-hexane and eventually precipitated as a solid of m.p. 83–87°. The solid was taken up in chloroform and extracted with 2 × 50-ml. portions of water. After drying and evaporating, the chloroform layer gave a white solid of m.p. 88–89°, corresponding by elemental analysis and NMR to β-N′,N′-diethylaminoethyl 2-ethyl-5-methyl-2-phenylhydantoate (V).

Table II—Elemental Analyses and Melting Points of the Prepared Esters

Esters	Analysis, % Calc.	Found	Melting Point
II	C 69.25	69.20	220°
	H 6.41	6.69	
	N 8.98	8.84	
IV	C 62.35	62.17	168–169°
	H 7.20	7.42	
	N 11.20	11.37	
III	C 68.95	69.10	144–145°
	H 7.60	7.49	
	N 10.97	10.66	
V	C 64.50	64.77	88–89°
	H 8.66	8.86	
	N 12.52	12.76	

Table III—NMR Data for the Four Esters

Ester	δ Value, Integration, Assignment[a]
II	1.20 δ (triplet), 3, $-OCH_2CH_3$; 2.65 δ (singlet), 3, $-NHCH_3$; 4.25 δ (quartet), 2, $-OCH_2CH_3$; 5.45 δ (broad), 1, $-NHCH_3$; 6.85 δ (broad), 1, $-CONHC(C_6H_5)_2-$; 7.30 δ (multiplet), 10, $-C(C_6H_5)_2$
IV	0.85 δ (triplet), 3, $-C_6H_5C(CH_2CH_3)-$; 2.60 δ (quartet), 2, $-C_6H_5C(CH_2CH_3)-$; 2.65 δ (singlet), 3, $-NHCH_3$; 3.65 δ (singlet), 3, $-OCH_3$; 7.35 δ (multiplet), 5, $-C_6H_5C(C_2H_5)-$
III	0.96 δ (triplet), 6, $-N(CH_2CH_3)_2$; 2.40 δ (quartet), 2.50 δ (singlet), 2.55 δ (triplet) all integrated for 9 protons, $-CH_2N(CH_2CH_3)_2$ and $-NHCH_3$; 4.20 δ (triplet), 2, $-OCH_2CH_3-$; 4.60 δ (broad), 1, $-NHCH_3$; 6.20 δ (broad), 1, $-CO-NH-C(C_6H_5)_2-$; 7.35 δ (multiplet), 10, $-C(C_6H_5)_2-$
V	0.85–1.55 δ (multiple peaks), 9, $-N(CH_2CH_3)_2$ and $-C_6H_5(CH_2CH_3)-$; 2.45–3.00 δ (multiple peaks), 11, $-CH_2N(CH_2CH_3)_2$ and $-NHCH_3$ and $-C_6H_5C(CH_2CH_3)-$; 4.40 δ (triplet), 2, $-OCH_2CH_3$; 5.00 δ (broad), 1, $-NHCH_3$; 6.35 δ (broad), 1, $-CONHC-$; 7.60 δ (multiplet), 5, $-C_6H_5C(C_2H_5)-$

[a] All esters run in $CDCl_3$.

Kinetic Measurements—All kinetic measurements were carried out directly in the thermostated cell compartment of a recording spectrophotometer[1]. At the elevated temperatures, the buffer solution was placed in the cell compartment and allowed to equilibrate before the ester was introduced as a standard dioxane solution. For II and IV, the least water-soluble esters, the final concentration of dioxane was 5%; for III and V, the concentration of dioxane was 2%. The formation of the hydantoin was followed at 240 nm.; the rate constants were determined from plots of log ($A_\infty - A_t$) *versus* time, where A_∞ and A_t are the absorbance readings at infinity and at time t, respectively. Some rate constants were also determined by the Guggenheim (12) method. The closure rates in the presence and absence of horse serum cholinesterase[1], with a concentration of 2 mg./ml. of enzyme in pH 7.4 buffer, were followed directly in the cell compartment of the spectrophotometer.

Product Analysis—All of the esters gave their respective hydantoins on reaction with base. This was checked by spectral comparisons and TLC [solvent system of chloroform using microscopic slide technique (13)]. On heating in water, the four esters gave a near quantitative yield of the parent hydantoin.

Solubility Study—The solubility of the hydantoins and their derivatives in chloroform–carbon tetrachloride (1:3 v/v) at 25° was determined semiquantitatively using a gravimetric technique. The semiquantitative scale was necessary because of the small amounts of materials available. The partitioning of the esters and hydantoins between the chloroform–carbon tetrachloride nonaqueous layer and the pH 6.0 phosphate buffer was also estimated *via* the gravimetric technique by determining the amount of hydantoin or derivative in the nonaqueous layer before and after extraction with the aqueous buffer. The aqueous solubility of V and III in pH 6.0 phosphate buffer was estimated by placing 50-mg. fractions of the esters in 10 ml. of the buffer at room temperature until a saturated solution was obtained. This simple semiquantitative procedure was necessary, because even at 25° and pH 6.0 the esters are converted to their hydantoins with half-lives of under 10 hr.; since the hydantoins (the products of the reaction) are very water insoluble, they would show up as saturated solutions when only a simple observation was being used as the criterion for saturation.

Animal Studies—The animal studies presented here are not meant to represent a full comprehensive study but rather to show that the particular derivatives synthesized and studied in this work are, in fact, pharmacologically active and relatively nontoxic on a short-term scale.

[1] Sigma Chemical Co.

Table IV—Effect of Varying Borate Buffer Concentration on Cyclization[a] of IV at 25°, pH 9.0 (μ = 1.0)

Borate Concentration, M	k_{obs}, min.$^{-1}$
0.017	8.50×10^{-2}
0.029	8.65×10^{-2}
0.050	8.60×10^{-2}
0.100	8.16×10^{-2}
Average	8.58×10^{-2}

[a] k_2 for the cyclization of IV at 25° from the average value of k_{obs} at pH 9.0 is equal to 8.58×10^{3} M^{-1} min.$^{-1}$.

Random bred, albino, female mice, Ha/ICR, age 3–6 weeks, with an average weight of 11 g. (variation 8–15 g.), were obtained[6] and acclimatized for 3 days before use.

Toxicity Studies—Fifty mice were separated into lots of 10. Compound V was dissolved in a 0.1 *M* phosphate buffer of pH 5.9. The concentration of drug was such that when given intraperitoneally, ~0.1 ml. or ~0.2 ml. of the solution gave 100 mg./kg., 200 mg./kg., *etc.*, of drug; *i.e.*, all intraperitoneal injections were of 0.2 ml. or less. The animal weight was used to calculate what volume of solution was necessary to give the desired dose. With the first set of 10 animals, each animal received 100 mg./kg. of V. The second set was given 200 mg./kg. The other three sets were given 400 and 800 mg./kg. and the buffer solution, respectively.

Anticonvulsant Activity Tests—The ED_{50} anticonvulsant activity test is basically an electroshock test[7] where a supramaximal current of 20 ma. is applied across the ears of a mouse for 0.2 sec. to induce convulsion. Forty mice were separated into four sets of 10. Each mouse was submitted to the electroshock and any insensitive mice were discarded. All of the mice required severe resuscitation after the current was disconnected. The first set of mice was then given 50 mg./kg. i.p. of V, the second set 100 mg./kg., and the third set

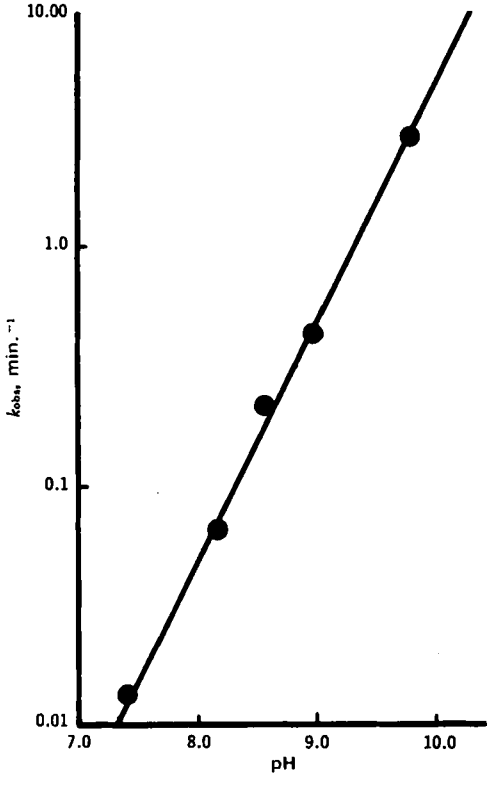


Figure 1—*Log* k_{obs} versus *pH profile for the closure of II at 37°* (μ = *1.0, with potassium chloride*).

[6] From A. R. Schmidt of Wisconsin.
[7] Similar to that used as an undergraduate assignment in the University of Kansas Pharmacology Department.

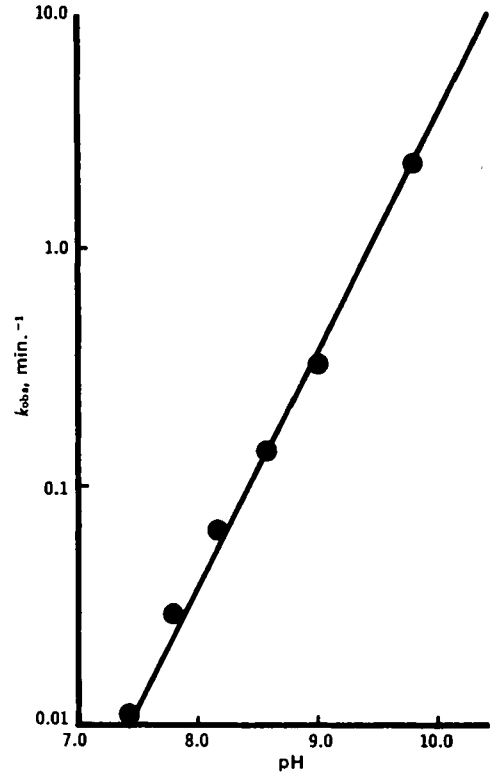
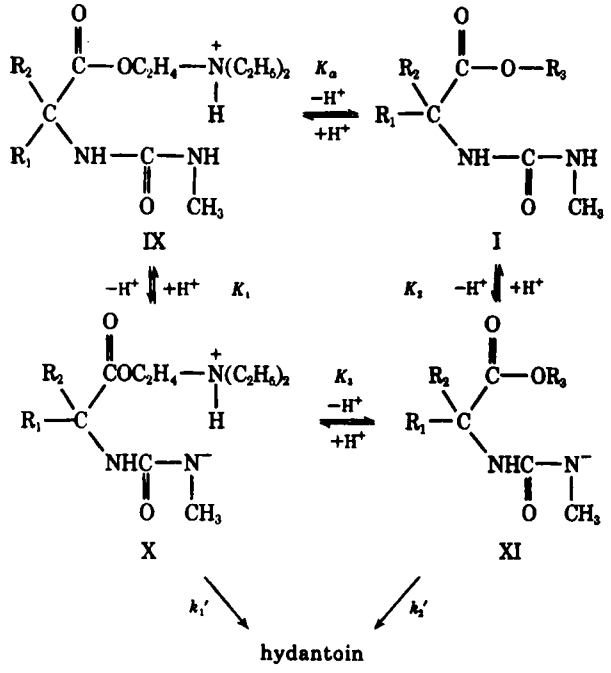


Figure 2—*Log* k_{obs} versus *pH profile for the closure of IV at 37°* (μ = *1.0, with potassium chloride*).

150 mg./kg. The fourth set of mice was given 25 mg./kg. of III. After the injection the mice were returned to their cages for 1 hr. After this 1 hr., each mouse was again submitted to the electroshock test to observe if any protection against electroshock had resulted from the injections.

RESULTS AND DISCUSSION

Chemistry of Cyclization of II, III, IV, and V to Their Respective Hydantoins under Neutral to Basic Aqueous Conditions—In the pH range studied, 7.43–10.80, and at temperatures of 25 and 37°, all



Scheme II

Table V—Effect of Varying Borate Buffer Concentration on Cyclization[a] of II at 25°, pH 9.0 (μ = 1.0)

Borate Concentration, M	k_{obs}, min.$^{-1}$
0.017	1.47×10^{-1}
0.029	1.48×10^{-1}
0.050	1.50×10^{-1}
0.100	1.46×10^{-1}
Average	1.48×10^{-1}

[a] k_2 for the cyclization of II at 25° from the average value of k_{obs} at pH 9.0 is equal to 1.48×10^4 M^{-1} min.$^{-1}$.

four esters cyclized *via* first-order kinetics to their respective hydantoins with the general reaction shown in Scheme II. All reactions were irreversible and complete, as evidenced by spectral comparisons, TLC, and near quantitative recovery of the hydantoins from boiling water solutions of the esters.

Plots of log k_{obs} *versus* pH for the cyclization of the four esters are shown in Figs. 1–4. For the simple esters, II and IV, plots of log k_{obs} *versus* pH (Figs. 1 and 2) gave straight lines whose slopes[b] were approximately one (0.98 and 0.96, respectively), suggesting that the reaction was first-order dependent on hydroxyl ions. For the amino esters, V and III, the plots (Figs. 3 and 4) were predictably nonlinear over part of the pH range studied but did approach linearity at the limits of the plots.

The rates of cyclization of all four esters were found to be buffer concentration independent at constant ionic strength (Tables IV and V), which, along with the log k_{obs} *versus* pH plots, suggest that like the esters of *o*-ureidobenzoic acid (6), the closures to the cyclic hydantoins are specific base catalyzed.

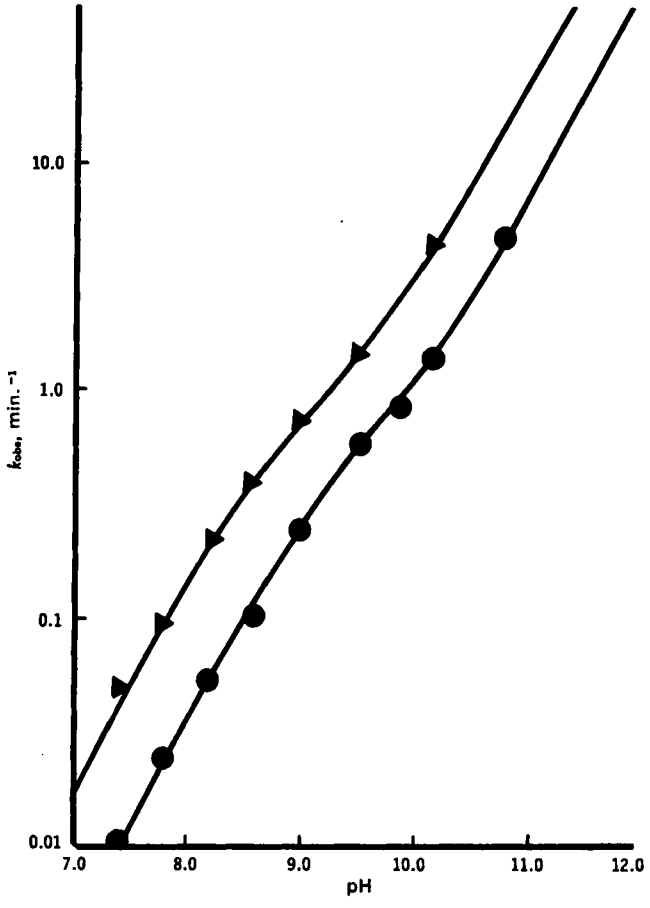


Figure 3—*Log* k_{obs} versus *pH profile for the closure of V at 25°* (●) *and 37°* (▲) (μ = *1.0, with potassium chloride*).

[b] Calculated by standard least-squares techniques, Olivetti Programma.

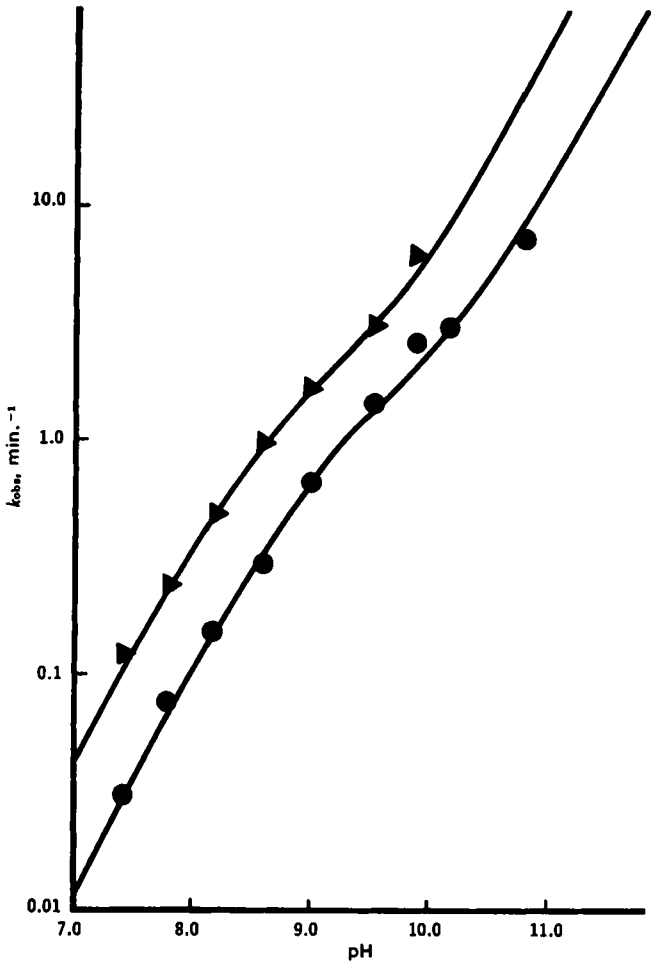


Figure 4—*Log* k_{obs} versus *pH profile for the closure of III at 25°* (●) *and 37°* (▲) (μ = *1.0, with potassium chloride*).

The results presented in Figs. 1–4 and Tables IV and V could be rationalized in terms of the general mechanistic Scheme II, where R_3 = alkyl or $-C_2H_4N(C_2H_5)_2$, and k_1' and k_2' are the spontaneous rate constants for the hydrolytic decomposition of X and XI.

The ionizations described by K_1 and K_2 are due to the microscopic ionization of the ureido group ($-NHCONHCH_3$) to the ureido anion ($-NHCON^-CH_3$). Both K_a and K_3 are due to the dissociation of the protonated amino group which can exist only for the amino esters; *i.e.*, Scheme II is complete for the amino esters but the terms IX, X, K_1, K_3, and K_a are all eliminated from Scheme II for the simple esters. If these terms are eliminated, then the rate of formation of the hydantoin, $d[P]/dt$, from the simple esters may be expressed as:

$$\frac{d[P]}{dt} = k_2'[XI] \quad \text{(Eq. 1a)}$$

$$= k_2'K_2[I]a_{OH^-} \quad \text{(Eq. 1b)}$$

$$= k_2a_{OH^-}[I] \quad \text{(Eq. 1c)}$$

where $k_2 = k_2'K_2$.

The total ester concentration is $[ester]_T = [I] + [XI]$, but since K_2 represents the ionization constant of the ureido group to the ureido anion and this constant is considered to be smaller than 10^{-14} (6), the term [XI] is negligible when compared to [I]. Therefore:

$$\frac{d[P]}{dt} = k_2[ester]_T a_{OH^-} \quad \text{(Eq. 2a)}$$

or:

$$k_{obs} = k_2a_{OH^-} \quad \text{(Eq. 2b)}$$

Since K_2 was not determinable, the results will be expressed in terms of k_2. The a_{OH^-} and a_{H^+} used later refer to the activity of hy-

Table VI—Second-Order Rate Constants and Ionization Constants for the Cyclization of Esters II–V to Their Respective Hydantoins at 25 and 37° in Aqueous Solution (μ = 1.0)

Ester	Temperature	k_1, × 10^{-4} (M^{-1} min.$^{-1}$)	k_2, × 10^{-4} (M^{-1} min.$^{-1}$)	pKa
II	25°	—	1.48	—
	37°	—	2.00	—
IV	25°	—	0.86	—
	37°	—	1.52	—
III	25°	10.5	1.10	9.20
	37°	17.3	1.93	8.65
V	25°	3.82	0.64	9.25
	37°	6.70	1.01	8.75

droxyl ion and hydrogen ion, respectively, which were calculated from pH measurements. Equation 2*b* predicts that a plot of log k_{obs} *versus* pH will be linear with a positive slope of unity, which is consistent with the present findings.

For the amino esters (III and V), the rate equation was more complex:

$$\frac{d[P]}{dt} = k_1'[X] + k_2'[XI] = k_1'[IX]K_1a_{OH^-} + k_2'[I]K_2a_{OH^-} \quad \text{(Eq. 3)}$$

The total ester concentration is:

$$[\text{ester}]_T = [IX] + [I] + [X] + [XI] \quad \text{(Eq. 4)}$$

Since K_1 and K_2 represent the ionization constants of the ureido group (substituted urea) and are considered to be smaller than 10^{-14} (6), the terms [X] and [XI] are negligible compared to [IX] and [I]. The fraction of ester present as [IX], expressed as β, therefore, can be defined as:

$$\beta = \frac{[IX]}{[I] + [IX]} = \frac{a_{H^+}}{a_{H^+} + K_a} \quad \text{(Eq. 5a)}$$

$$\frac{[dP]}{dt} = [\text{ester}]_T \{k_1a_{OH^-}\beta + k_2a_{OH^-}(1 - \beta)\} \quad \text{(Eq. 5b)}$$

$$k_{obs} = k_1a_{OH^-}\beta + k_2a_{OH^-}(1 - \beta) \quad \text{(Eq. 5c)}$$

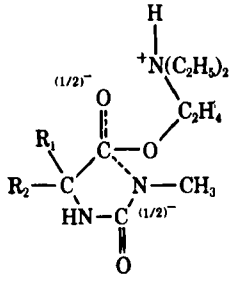
where $k_1 = k_1'K_1$, and $k_2 = k_2'K_2$.

Since K_1 and K_2 could not be determined, the results will be expressed in terms of k_1 and k_2. Equation 5*c* predicts that plots of log k_{obs} *versus* pH for the amino esters will be complex and nonlinear in the pH range where β does not approximately equal zero or one. The plot resulting from such an equation has been adequately described (14) and will not be discussed here.

The rate and equilibrium constants consistent with the experimental results are shown in Table VI. Variation of pKw with temperature was taken into account in the calculation of the rate and equilibrium constants. The solid lines drawn in Figs. 1–4 have been calculated by substituting the rate and equilibrium constants shown in Table VI into Eqs. 2*b* and 5*c*.

The rate constant k_1 has been defined by Scheme II and Eq. 5*c* as the second-order base-catalyzed rate constant for the attack of the ureido group on the protonated amino ester. However, its kinetic equivalent, the spontaneous attack of the neutral ureido group on the free base form of the ester, cannot be fully discounted.

In summary, the cyclization of esters of hydantoic acid in neutral to alkaline solution appears to be a specific base-catalyzed intramolecular closure, with the rate-determining transition state involving the attack of the ureido anion at the ester carbonyl group.



XII

Table VII—Half-Lives for Closure of Esters of Hydantoic Acids to Their Hydantoins

Ester	$t_{1/2}$, min.[a]	$t_{1/2}$, min.[b,c]
II	57.3	4680
IV	73.0	8170
III	6.8	600
V	16.9	1710

[a] pH 7.4 and 37°. [b] pH 6.0 and 25°. [c] Estimated from Figs. 3 and 4 and Tables IV and V.

It appears that the diethylamino moiety helped increase the ester's rate of closure at pH's below the pKa of the amino group. For example, k_{III}/k_{II} at pH 7.40 and 37° is 8.5. This increased rate of closure over the simple esters and the free base form of the amino esters may be due to electrostatic stabilization of the negatively charged reaction center (see XII), although the possibility of intramolecular general acid catalysis cannot be discounted. The transition states for the esters where $-C_2H_4N^+(-H)(C_2H_5)_2$ is replaced by a simple alkyl group or $-C_2H_4N(C_2H_5)_2$ would be the same as XII, except that no electrostatic stabilization of the negative reaction center would be possible.

Hegarty and Bruice (6) recently demonstrated the intramolecular nucleophilicity of the ureido group. They showed a rate acceleration of 1.5 × 10^5-fold for the attack of the ureido group in the intramolecular cyclization of the methyl ester of *o*-ureidobenzoic acid to that of its intermolecular equivalent. The results and conclusion presented here are in perfect accordance with their findings.

Table VII shows the half-lives for the conversion of the four esters to their hydantoin at pH 7.4 and 37°, suggesting that at least under *in vitro* conditions the esters are quickly converted to their hydantoin, while at pH 6.0 and 25° the conversion is quite slow. This means that the esters could be used in a reconstitutable injection if they are water soluble. The solubility of the esters will be discussed later.

Because the derivatives appeared promising, a test was run to observe the effect of an esterase enzyme on the product and rate of cyclization of one of the esters, III. Horse serum cholinesterase[5] at a concentration of 2 mg./ml. in buffer solution did not affect the hydrolytic intramolecular closure of the ester. The $t_{1/2}$ values at pH 7.43 and 23.6° with and without enzyme were 31.1 and 31.9 min., respectively. The products of the two reactions, *i.e.*, with and without enzyme, gave the same final UV spectrum corresponding to 3-methyl-5,5-diphenylhydantoin. Of particular importance was the enzyme's inability to cleave the ester linkage before the intramolecular reaction was able to take place. The product of such a

Table VIII—Results of LD_{50} Study of V[a]

Dose, mg./kg.	Mortality, %	Observations
100	0	All mice acted quite normal with no observable toxic effects over 4 hr.
200	0	Slightly sedated and ataxic over 4 hr. but no critical effects.
400	0	After 1 hr., the mice were very ataxic and it did appear that a few of them may die. Their breathing appeared normal, but there was kicking of the hindlegs. After 4 hr., all the mice had recovered and appeared normal.
800	100	All mice died within 10–15 min. They became very ataxic, with increased breathing rate and kicking of the hindlegs, indicative of convulsions.
Buffer solution (no drug)	0	All mice appeared quite normal.

[a] The mice given III in the ED_{50} experiment at a dose of 25 mg./kg. appeared quite normal over a 3-hr. range with no obvious toxic effects.

Table IX—Effect of V and III on Protection of Mice against Electroshock (an ED_{50} Study)

Number	V, 50 mg./kg.	V, 100 mg./kg.	V, 150 mg./kg.	III, 25 mg./kg.
1	−+[a]	+	+	−
2	−[a]	+	+	−−+
3	−++	+	+	−+
4	−	+	+	+
5	+[a]	+	+	−+
6	−	+	+	−
7	+	+	+	−
8	−	+	+	−+
9	+	−	+	−
10	−+	+	+	−

[a] Key: −, no protection; +, full protection; and −+, borderline; *i.e.*, mice actually extended their hindlegs, but there was a very definite lag time before this actually occurred.

reaction would have been the hydantoic acid, and the hydantoic acid under *in vitro* physiological conditions is closed very slowly to the hydantoin (15).

Solubility Studies—The order of solubility of the esters in chloroform–carbon tetrachloride (1:3 v/v) on a molar basis compared to the hydantoins was found to be amino ester (in the free base form) > parent hydantoin > simple ester. A study of the partitioning between chloroform–carbon tetrachloride (1:3 v/v) and 0.1 *M* phosphate buffer (pH 6.0) showed that very little of the hydantoin and the simple ester passed into the aqueous layer from the organic layer in which they were dissolved, whereas virtually all the amino ester did pass into the aqueous layer presumably in the protonated form. The aqueous solubility of V and III in 0.1 *M* phosphate buffer (pH 6.0) was estimated at 2 and 1% w/v, respectively. An accurate estimate could not be made because of the fast cyclization of the esters to the hydantoins. It appears, therefore, that the diethylamino moiety not only helped increase the esters' rate of closure under *in vitro* physiological conditions but also helped increase the aqueous solubility of the esters below their pKa.

Toxicity and Activity Studies—A problem that may arise when attempting chemical modification of a drug is that the derivative itself may possess increased toxicity or have a pharmacological effect of its own. The β-*N'*,*N'*-diethylaminoethyl moiety is found in a diverse group of compounds: local anesthetics, cholinergic drugs, antiasthmatics, and tranquilizers.

Table VIII shows the results of LD_{50} determinations on mice *via* intraperitoneal injections of V dissolved in 0.1 *M* phosphate buffer (pH 5.9). Table IX shows the results of an anticonvulsant activity ED_{50} determination on mice *via* intraperitoneal injections of V dissolved in the same buffer used for the LD_{50} determination. The results of one run using III at a concentration of 25 mg./kg. is also given.

The results displayed in Tables VIII and IX suggest that the ED_{50} of V against electroshock in mice after intraperitoneal injection is approximately 60 mg./kg. and its LD_{50} is between 400 and 800 mg./kg. For III an ED_{50} and LD_{50} could not be estimated; but at 25 mg./kg., 16% protection was observed. In the ED_{50} experiments with doses that gave only slight or no protection, the animals after convulsion did not require any resuscitation, whereas the mice not given the drug had to be revived for breathing to begin. This suggests that even at low doses the drugs were having an effect. Efforts to compare solutions of V and III with the parent hydantoins were not successful, because the hydantoins were not soluble in the buffer of pH 5.9. Suspensions of the hydantoin given intraperitoneally through a wide bore needle and tested for anticonvulsant activity after 1 hr. offered no protection, *i.e.*, the lack of protection against electroshock was probably due to the method of administration.

SUMMARY

The results of the present study showed that esters of hydantoic acids may be of use as prodrugs for the respective hydantoin. The β-*N'*,*N'*-diethylaminoethyl esters in particular showed superior physical properties to those of the parent hydantoin. Their cyclization rates, half-lives of under 20 min., under physiological conditions were unaffected by an esterase enzyme, and a preliminary animal study showed interesting and encouraging results. The amino esters may be of use as components of a reconstitutable injection, because their rate of cyclization and aqueous solubility at physiological pH would not lead to the precipitation in the bloodstream that was seen with the sodium hydantoin. Similarly, because solutions can be maintained at around neutral pH, the solution would be more compatible in intravenous infusions with other possible components of the infusion.

Normally, prodrugs formed through the formation of an ester linkage require enzyme mediation to regenerate the parent drug. Intramolecular nucleophilic ureido participation at an ester linkage has been shown to give a rate acceleration of 10^5-fold over the intermolecular equivalent. If the product of the intramolecular reaction happens to be the parent drug, then the acylic ester is a chemical modification whose closure rate is independent of the need of enzymatic mediation. In the present study, an ionizable group has been built into the alcohol portion of a hydantoic acid ester, giving it increased aqueous solubility and an increased rate of closure under physiological conditions. These desirable properties make V and III possible candidates as prodrugs of their respective hydantoin.

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