Phenytoin Prodrugs III: Water-Soluble Prodrugs for Oral and/or Parenteral Use

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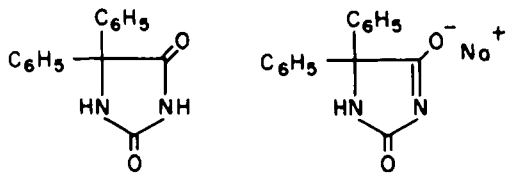
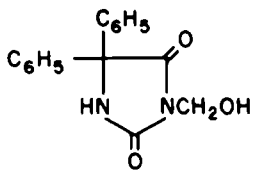
Abstract □ Various bioreversible derivatives of phenytoin, a poorly water soluble and erratically absorbed drug after both oral and parenteral dosing, were synthesized. Initial evaluation of these expected prodrugs, *i.e.*, their aqueous solubility, cleavage in the presence of various animal tissues, and anticonvulsant activity in mice, confirmed that a number of the derivatives did indeed behave as prodrugs. The more promising prodrugs were the disodium phosphate ester and various amino groups containing acyl esters of 3-(hydroxymethyl)-5,5-diphenylhydantoin.

Keyphrases □ Prodrugs—phenytoin, water-soluble, hydroxymethylation, anticonvulsants □ Anticonvulsants—phenytoin prodrugs, water-soluble, hydroxymethylation □ Phenytoin—water-soluble prodrug, hydroxymethylation

Phenytoin (I) is a high melting (293°C), weakly acidic (1, 2) drug exhibiting poor solubility (3–5) in water. These properties lead to erratic absorption after oral dosing with both the free acid and the sodium salt (Ia) (3, 6–19). For parenteral use, sodium phenytoin is formulated in an aqueous alkaline medium of pH ~12 containing 40% propylene glycol and 10% ethanol. The parenteral dosage form is hazardous if the intravenous injection is rapid (20, 21), and the free acid appears to precipitate at intramuscular injection sites (22–25). The parenteral dosage form has also presented handling problems, especially if admixing or dilution was desired (26, 27).

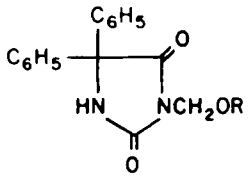


I Ia



II

One solution to these problems was to develop prodrugs of phenytoin with more desirable physicochemical properties[1]. The overall goal of this study, therefore, was to synthesize various water-soluble phenytoin prodrugs and to evaluate their potential oral and parenteral use. Recently, interest has arisen in *N*-hydroxymethyl derivatives of hydantoins, amides, and imides (4, 5, 28–31). In the present study 3-(hydroxymethyl)-5,5-diphenylhydantoin or 3-(hydroxymethyl)phenytoin (II) was synthesized with the expressed purpose of providing a "synthetic handle" in the molecule to which various other potential bioreversible water-solubilizing functional groups could be added (esters III–X).



III: R = $COCH_3$
IV: R = $COCH_2\overset{+}{N}H(CH_3)_2\ CH_3SO_3^-$
V: R = $CO(CH_2)_2\overset{+}{N}H(C_2H_5)_2$ (2-naphthalenesulfonate)
VI: R = $CO_2(CH_2)_2\overset{+}{N}H(CH_3)_2\ CH_3SO_3^-$
VII: R = $PO_3^{2-}Na_2^+$
VIII: R = $SO_3^-Na^+$
IX: R = $CO(CH_2)_2CO_2^-Na^+$
X: R = $CO(CH_2)_3CO_2^-Na^+$

All the esters except III have an ionizable group in the ester moiety, and the salt forms of esters would be expected to have an increased aqueous solubility over that of phenytoin. Compounds IV–VI, IX, and X would be expected to be cleaved *in vivo* by esterases to II, which will be shown in this study to break down further to phenytoin and formaldehyde with a half-life of ~2 s at 37°C and pH 7.4, an observation consistent with an earlier study (5). Similarly, VII and VIII were expected to be catalytically hydrolyzed by phosphatase and sulfatase enzymes, respectively.

The evaluation of VIII as a potential prodrug of phenytoin has been presented elsewhere (32); VIII was found not to behave as a prodrug of phenytoin. The syntheses and preliminary evaluations of compounds IV–VII, IX, and X are presented herein.

EXPERIMENTAL SECTION

All compounds were synthesized by standard techniques. The specific details for the synthesis of each compound are presented below.

3-(Hydroxymethyl)phenytoin [II, 3-(Hydroxymethyl)-5,5-diphenylhydantoin]—A suspension of 20 g (0.08 mol) of phenytoin, 80 mL of formalin (1.07 mol, 37% formaldehyde in water), 1 g (0.007 mol) of potassium carbonate, and 720 mL of water were stirred at room temperature for 24 h. The mixture was filtered and the resulting white solid was washed with 3% aqueous formaldehyde, air dried for 24 h to give 21.5 g of II (92% yield), mp 187–189°C. ^{1}H-NMR (Me_2SO-d_6): δ 4.85 (d, CH_2), 6.4 (t, OH), 7.4 (s, 10), and 9.6 ppm (s, NH).

Anal.—Calc. for $C_{16}H_{14}N_2O_3$: C, 68.08; H, 5.00; N, 9.92. Found: C, 68.15; H, 4.93; N, 9.97.

3-(Hydroxymethyl)-5,5-diphenylhydantoin Acetate (Ester III)—A solution of 1.5 g (0.0058 mol) of II, 1 mL of acetic anhydride, and 10–20 μL of sulfuric acid was allowed to stand for 10 min. The solid precipitate was suspended in 10 mL of ether, filtered, washed with 30 mL of ether, and then recrystallized from ethanol to give 1.69 g of ester III (90% yield), mp 154–157°C. ^{1}H-NMR ($CDCl_3$): δ 2.0 (s, CH_3), 5.55 (s, CH_2), 7.4 (s, 10), and 7.7 ppm (s, NH).

Anal.—Calc. for $C_{18}H_{16}N_2O_4$: C, 66.65; H, 4.97; N, 8.64. Found: C, 66.66; H, 5.01; N, 8.61.

[1] Paper I in this series was published in "Prodrugs as Novel Drug Delivery Systems", ACS Symposium Series, No. 14, 1975, Chap. 3, paper II in *J. Pharm. Sci.*, **72**, 400 (1983).

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3-(Hydroxymethyl)-5,5-diphenylhydantoin Ester with *N,N*-Dimethylglycine, Monomethanesulfonate (IV)—A suspension of 3.65 g (0.035 mol) of *N,N*-dimethylglycine, 7.3 g (0.035 mol) of dicyclohexylcarbodiimide, and 10 g (0.035 mol) of II in 50 mL of dry pyridine was stirred at room temperature for 24 h. After evaporation under reduced pressure at 50°C, the syrupy contents were triturated with 100 mL of dichloromethane and the dicyclohexylurea was removed by filtration. The dichloromethane was removed under reduced pressure, and the trituration was repeated twice to produce a syrup, to which was added 50 mL of ether. After standing, the resulting precipitate was removed by filtration and washed with 25 mL of ether and dried *in vacuo* to give 11.3 g of IVa (88% yield), mp 133–137°C. ^{1}H-NMR and elemental analysis confirm the structure of the product as 3-(hydroxymethyl)-5,5-diphenylhydantoin ester with *N,N*-dimethylglycine (IVa). A solution of 1.92 g (0.005 mol) of IVa, 0.5 g (0.005 mol) of methanesulfonic acid, and 25 mL of dichloromethane was stirred, and the resulting precipitate was removed by filtration and recrystallized from ethanol–acetone–ether to give 2.15 g of IV (93% yield), mp 173–175°C. ^{1}H-NMR (D_2O): δ 3.05 (s, CH_3), 3.2 (s, 2, CH_3), 4.4 (s, CH_2), 5.85 (s, CH_2), and 7.5 ppm (s, 10).

Anal.—Calc. for $C_{21}H_{25}N_3O_7S$: C, 54.42; H, 5.44; N, 9.07. Found: C, 54.22; H, 5.40; N, 8.92.

3-(Hydroxymethyl)-5,5-diphenylhydantoin Ester with *N,N*-Diethyl-β-alanine, Mono-2-naphthalenesulfonate (V)—A suspension of 3.54 g (0.0195 mol) of 3-*N,N*-diethylaminopropionic acid hydrochloride, 4.02 g (0.0195 mol) of dicyclohexylcarbodiimide, and 5 g (0.0177 mol) of II in 20 mL of dry pyridine was stirred at room temperature for 20 h and filtered. The glassy residue was triturated with 100 mL of dichloromethane, and the suspended dicyclohexyl urea was removed by filtration. The dichloromethane was removed under reduced pressure and the trituration was repeated twice. The syrup was dissolved in 100 mL of water, the pH was lowered to 3 with dilute HCl, and the mixture was extracted twice with 100 mL of ether. The pH of the aqueous layer was raised to 9 with solid potassium carbonate, and the mixture was extracted with 100 mL of ether. The organic phase was dried (magnesium sulfate), and the solvent was removed under reduced pressure to yield 3-(hydroxymethyl)-5,5-diphenylhydantoin ester with *N,N*-diethyl-β-alanine (Va). A solution of 4.35 g (0.0106 mol) of Va and 2.6 g (0.0106 mol) of 2-naphthalenesulfonic acid, dissolved in a minimum amount of methanol, in 80 mL of dry ether was stirred until the precipitation of a white solid. The precipitate was removed by filtration, washed with 50 mL of ether, and dried in a vacuum desiccator to give 4.0 g of V (44.5% yield), mp 174–176°C. ^{1}H-NMR (Me_2SO-d_6): δ 1.15 (t, $2CH_3$), 3.1 (m, 8), 5.55 (s, CH_2), 7.4 (m, 17), and 9.9 ppm (s, NH).

Anal.—Calc. for $C_{33}H_{35}N_3O_7S$: C, 64.17; H, 5.17; N, 6.80. Found: C, 63.90; H, 5.72; N, 6.62.

3-(Hydroxymethyl)-5,5-diphenylhydantoin Ester with 2-(Dimethylamino)-ethyl Carbonate, Monomethanesulfonate (VI) *Via* 3-(Hydroxymethyl)-5,5-diphenylhydantoin, *p*-Nitrophenyl Carbonate (XI)—A solution of 6.78 g (0.033 mol) of *p*-nitrophenylchloroformate and 9.5 g (0.033 mol) of II in 30 mL of dry pyridine was stirred at room temperature for 5 h, poured with vigorous stirring into ice water, and acidified with 0.38 mol of sulfuric acid. The pale yellow solid was removed by filtration and dried in a vacuum oven at 40°C overnight to give 12.9 g of XI (88% yield), mp 191–193°C.

A solution of 4 mL of 2-(*N,N*-dimethylamino)ethanol, previously dried by distillation, and 4.5 g (0.01 mol) of XI in 25 mL of acetonitrile was stirred at room temperature for 5 h, poured into ice water, and extracted with 100 mL of dichloromethane. The organic layer was extracted twice with 25 mL of 5% aqueous potassium carbonate solution, dried over anhydrous magnesium sulfate, filtered, and evaporated under reduced pressure to give 1.42 g of a white glassy solid, (35% yield), mp 88–90°C (VIa).

The methanesulfonate salt of VIa (VI) was prepared in the same manner as IV from IVa, except that on filtration from dichloromethane, the salt was refluxed in acetone to remove phenytoin contamination (46% yield), mp 186–189°C. ^{1}H-NMR (D_2O): δ 3.05 (s, CH_3), 3.2 (s, $2CH_3$), 3.7 (t, CH_2), 4.75 (t, CH_2), 5.8 (s, CH_2), and 7.55 ppm (s, 10).

Anal.—Calc. for $C_{22}H_{27}N_3O_8S$: C, 53.57; H, 5.47; N, 8.51. Found: C, 53.2; H, 5.5; N, 8.3.

3-(Hydroxymethyl)-5,5-diphenylhydantoin Disodium Phosphate Ester (VII)—Sodium dibenzylphosphate and silver dibenzylphosphate were synthesized according to the method of Clark and Todd (33) from dibenzylphosphite[2].

3-(Chloromethyl)-5,5-diphenylhydantoin (XII)—A solution of 20 g (0.071 mol) of II and 6.2 mL of phosphorous trichloride in 100 mL of dichloromethane was stirred at room temperature for 24 h, diluted with a further 50 mL of dichloromethane, and extracted once with 100 mL of water and twice with 250 mL of 5% w/v aqueous sodium carbonate solution. The separated dichloromethane was dried over anhydrous magnesium sulfate, filtered, and evaporated under reduced pressure to give 13.1 g of a white solid (61% yield), mp 155.0–156.5°C.

[2] Aldrich Chemical Co., Milwaukee, Wis.

Table I—Aqueous Solubilities and pH Values of the Saturated Solutions of Phenytoin, II (in 1 M Aqueous Formaldehyde), and IV–VII at 25°C

Compound	pH	Solubility (Phenytoin Equivalent), mg/mL	Solubility Relative to Phenytoin
I	Neutral	0.02[a] (0.02)	1
II	Neutral	0.19[b] (0.17)	8.5
IV	~2	140 (76.2)	3810
V	~4	1.9 (0.78)	39
VI	~2	185 (94.6)	4730
VII	~9	142 (88.17)	4408.5

[a] Literature values vary from 0.01–0.04 mg/mL (1, 26). A value of 0.02 mg/mL was found in our studies. [b] The solubility was determined in aqueous formaldehyde (1 M).

3-(Hydroxymethyl)-5,5-diphenylhydantoin Dibenzyl Phosphate Ester (XIII)—A suspension of 13.13 g (0.034 mol) of silver dibenzylphosphate and 10 g (0.033 mol) of XII in 300 mL of benzene was refluxed for 2 h, hot-filtered under reduced pressure, extracted with 250 mL of 5% aqueous potassium carbonate, dried over magnesium sulfate, filtered, and evaporated under reduced pressure. The remaining syrup was triturated with 60 mL of ether and allowed to stand overnight; the white precipitate (XIII) which formed was removed by filtration, washed with 60 mL of ether, and dried in a vacuum oven to give 12 g of XIII (67% yield), mp 116–118°C.

Phosphate Ester of II (VIIa) and Its Disodium Salt (VII)—A suspension of 1 g of palladium (5%) on activated carbon[2] and 2 g (0.0037 mol) of XIII in 100 mL of ethyl acetate was subjected to hydrogenation[3] at 60 psi for 25 min (34). The suspension was filtered and the filtrate was evaporated under reduced pressure to give 0.7 g of a white solid (VIIa) which readily recrystallized from acetone (54.5% yield), mp 173–176.5°C.

Anal.—Calc. for $C_{16}H_{15}N_2O_6P$: C, 53.05; H, 4.17; N, 7.73. Found: C, 52.88; H, 4.02; N, 7.60.

A solution of 1.8 g (0.005 mol) of VIIa in 100 mL of methanol was titrated with 1 M NaOH to the second end point as determined by pH monitoring. The solution was filtered, evaporated under reduced pressure, and the white solid recrystallized from water–ethanol–acetone to give 1.1 g of VII (56% yield), mp 220°C (softens). ^{1}H-NMR (D_2O): δ 5.3 (d, CH_2) and 7.45 ppm (s, 10).

Anal.—Calc. for $C_{16}H_{13}N_2O_6PNa_2{\cdot}2H_2O$: C, 43.4; H, 3.87; N, 6.33. Found: C, 43.25; H, 3.82; N, 6.31.

3-(Hydroxymethyl)-5,5-diphenylhydantoin Sodium Succinate (Ester IX)—A solution of 10 g (0.035 mol) of II in 100 mL of dry pyridine was stirred at room temperature for 5 d with the gradual addition of 4.85 g (0.043 mol) of succinic anhydride. The solution was poured into 400 mL of 5% w/v aqueous sodium bicarbonate and immediately extracted with 250 mL of chloroform. The aqueous layer was isolated and the pH was lowered to 2 with hydrochloric acid; the aqueous layer was then rapidly extracted with 400 mL of chloroform. The chloroform layer was dried over anhydrous magnesium sulfate, filtered, and evaporated under reduced pressure; the resulting white solid (IXa) was triturated with ether and removed by filtration, mp 144.5–147.5°C. ^{1}H-NMR ($CDCl_3$): δ 2.6 (s, $2CH_2$), 5.55 (s, CH_2), 6.2 (s, 1), 7.4 (s, 10), and 7.6 ppm (s, 1).

Anal.—Calc. for $C_{20}H_{18}N_2O_6$: C, 62.83; H, 4.75; N, 7.33. Found: C, 62.74; H, 4.71; N, 7.20.

The sodium salt of IXa was prepared by titrating IXa with an equimolar solution of sodium methoxide in anhydrous methanol to give 8.8 g (62% yield), mp 141.5–146°C.

Anal.—Calc. for $C_{20}H_{17}N_2O_6Na{\cdot}0.5\ H_2O$: C, 58.13; H, 4.36; N, 6.78. Found: C, 57.87; H, 4.18; N, 6.57.

3-(Hydroxymethyl)-5,5-diphenylhydantoin Sodium Gluturate (Ester X)—Compound X was synthesized by allowing glutaric acid anhydride to react with II in dry pyridine and isolated by a method identical to that described for the synthesis of IX (30% yield), mp 137.5–144°C. ^{1}H-NMR of Xa ($CDCl_3$): 2.8 (m, 3 CH_3), 5.55 (s, CH_2), 6.6 (s, 1), 7.4 (s, 10), and 7.7 ppm (s, 1).

Anal.—Calc. for $C_{21}H_{20}N_2O_6$ (Xa): C, 63.6; H, 5.09; N, 7.07. Found: C, 62.87; H, 5.07; N, 6.91.

Anal.—Calc. for $C_{21}H_{19}N_2O_6Na{\cdot}H_2O$ (X): C, 57.81; H, 4.81; N, 6.41. Found: C, 57.71; H, 4.77; N, 5.47.

Aqueous Solubility of II and IV–VII—The aqueous solubility of II was determined by adding an excess of II to a solution of formaldehyde (1 M) in vials maintained at 25 ± 0.1°C in a constant-temperature water bath. The solubility was determined in 1 M formaldehyde solution in order to prevent

[3] Parr Instrument Co., Moline, Ill.

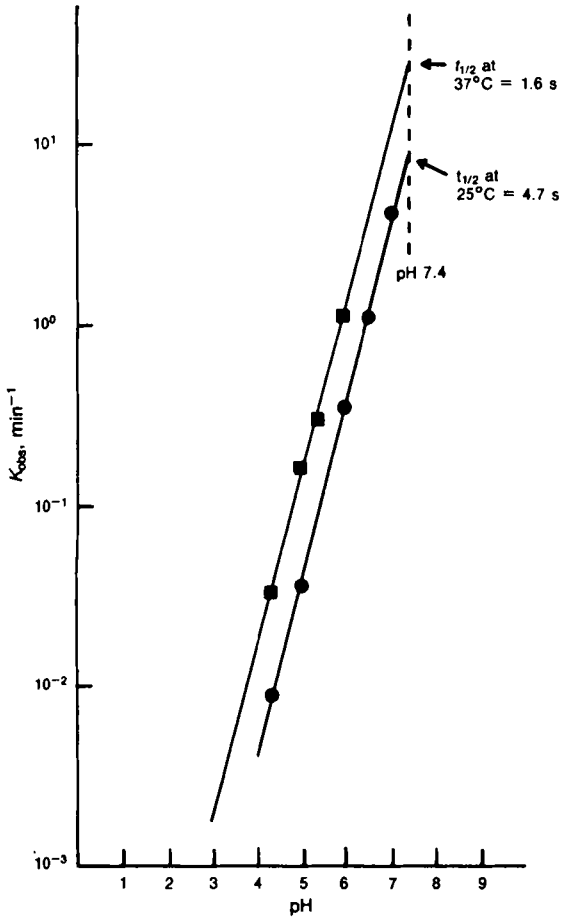


Figure 1—*pH-rate profile for the conversion of II to phenytoin at 25°C (●) and 37°C (■), μ = 0.5.*

the decomposition of II to phenytoin. The vials were shaken for 24 h so that equilibrium solubility was attained. The contents of the vials were then filtered. Appropriate dilutions of the filtrate with 1 M formaldehyde were made, and the absorbances of the diluted solutions at 260 nm were measured. The concentration of II was calculated from the measured absorbances by reference to standard curves. The aqueous solubilities of IV–VII were estimated at 25 ± 0.1°C by shaking an excess of the compound with a known volume of water, over a limited time period of 3–6 h to minimize the extent of degradation of the esters to phenytoin. The saturated solutions were filtered, diluted, and the concentrations of the esters were calculated from measured UV absorbances of the diluted solutions by reference to standard curves. The pH values of the saturated solutions were also measured.

Cleavage of IV–VII to Phenytoin in Biological Tissues—Conversion of IV–VII to phenytoin was carried out at 25°C and 37°C in plasma or whole blood obtained from rats[4], beagle dogs[5], and human subjects[6]. Blood was treated with EDTA to prevent coagulation. Plasma was obtained by centrifuging[7] blood for 15–20 min at 1000×*g*.

The conversion of VII to phenytoin was also studied in rat and dog tissue homogenates at 37°C. Sörensen's isotonic phosphate buffer (pH 7.4) was used to prepare the tissue homogenates and also to dilute plasma in cases where the reversion of the esters to phenytoin was too fast to follow in undiluted plasma. Reaction was initiated by injecting an appropriate amount of a stock solution of the ester in an appropriate solvent into temperature-equilibrated plasma, whole blood, or tissue homogenates. Samples obtained for the hydrolysis of IV–VI were quenched with *m*-phosphoric acid and assayed for phenytoin by the GC[8] method of Stella (35). In the case of the hydrolysis of VII, 100 μL of blood, plasma, or tissue homogenate samples were extracted directly into toluene containing the gas chromatography internal standard, 5-(*p*-methylphenyl)-5-phenylhydantoin, 0.7 μg/mL. The rest of the procedure

[4] Harlan Sprague-Dawley, Madison, Wis.
[5] Kindly supplied by INTERx Research Corp.
[6] Community Blood Bank, Kansas City, Mo.
[7] Dynac II; Beckton, Dickinson and Co.
[8] Varian model 2100 or 3700 gas chromatograph fitted with a flame-ionization detector.

Table II—Half-Lives for the Conversion of IV–VII to Phenytoin in Rat, Dog, and Human Plasma at 25°C and 37°C

Compound	Temperature, °C	Half-Life Rat	Dog	Human
IV	25	1.3 min	19.5 min	1.1 min
	37	16 s	10.7 min	23 s
V	25	27 s[a]	20.3 min	24.0 min
	37	—[c]	8.3 min	6.8 min
VI	25	1.1 min[b]	28.6 min	9.3 min
	37	33 s[b]	14.1 min	1.7 min

[a] In 10% rat plasma diluted with isotonic Sörenson's phosphate buffer, pH 7.4. [b] In 0.5% rat plasma diluted with isotonic Sörenson's phosphate buffer, pH 7.4. [c] Not determined.

was as described by Stella (35). Apparent first-order kinetics over 2–4 half-lives for the appearance of phenytoin were observed in all instances. Quantitative recovery (or conversion) of all the prodrugs were observed.

The tissue homogenates studied were rat and dog liver and rat intestinal homogenates prepared in pH 7.4, isotonic Sörensen's phosphate buffer (one part tissue to four parts buffer). The tissue was homogenized for 10 min using a 100-mL glass homogenizer with a polytef pestle having radial serrations[9]. The homogenizer was mechanically driven using a 3/8-hp variable-speed drill. The homogenates were centrifuged[10] for 90 min at 100,000×*g* at 5°C. The temperature was maintained between 5–10°C during the preparation of the tissue homogenate to prevent inactivation of the enzymes.

Hydrolysis of II to Phenytoin—The rates of decomposition of II in buffer solutions at 25°C and 37°C were studied spectrophotometrically[15] by recording the decrease[11] in absorbance at 255 nm. A 10-cm thermostated quartz cuvette was filled (30 mL) with pre-equilibrated buffer solutions [0.1 M acetate buffer (pH 3.0–5.0), 0.1 M phosphate buffer (pH 5.0–7.05), ionic strength 0.5], and the reaction was initiated by injecting a 25-μL aliquot of a stock solution of II in acetonitrile to yield a final concentration of II of 8×10^{-5} M. All reactions followed apparent first-order kinetics as 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 completion of the reaction, respectively. In all cases the final spectrum corresponded to that of phenytoin. In a few studies, the effects of varying buffer concentrations at constant pH were evaluated. Since no buffer catalysis was noted at any pH value, no further studies on the effect of buffers were attempted.

Anticonvulsant Activity and Toxicological Evaluation of IV–VII—Compounds IV–VII were evaluated using the anticonvulsant drug screening program (36) conducted by the National Institute of Neurological and Communicative Disorders and Stroke at the National Institutes of Health, Bethesda, Md. All four compounds underwent the primary evaluation (phase I); in a minimal number of animals, the anticonvulsant activity of each compound was evaluated by the maximal electroshock test (MES) and the subcutaneous pentylenetetrazol test (ScMET); the CNS toxicity of each compound was evaluated by the rotorod test using mice[12] (36). After this initial evaluation, any potentially useful derivatives may be subjected to further comprehensive screening for activity and toxicity. Specifically, this consists of the phase II and phase IV studies. The phase II study requires administering the drug intraperitoneally to mice and determining the time of peak effect (activity and toxicity) by the MES and the rotorod test. From this, the effective dose 50% (ED_{50}) and toxic dose 50% (TD_{50}) for the prodrugs could be determined. The phase IV study is similar to the phase II study except that the drug is administered orally.

For specific details of the procedures used in the screening, the reader is directed to Ref. 36. With the current compounds, IV–VII underwent the initial screening (Phase I), VI underwent phase II, while IV and VII underwent both phase II and phase IV evaluations.

RESULTS AND DISCUSSION

Compounds IV–X were synthesized by standard procedures as indicated in the *Experimental Section*. All compounds were found to conform to the proposed structures and showed only one spot on TLC. Compounds IX and X showed less than theoretical values for carbon and nitrogen analyses. Recrystallization attempts did not improve the results. The precursors IXa and Xa gave reasonable C, H, N analyses, so it appears that during the formation

[9] Series 3431-E04; A. H. Thomas Co., Philadelphia, Pa.
[10] Beckman Model L-5-50 Ultracentrifuge, T-35 rotor.
[11] Cary Model 118; Varian Instruments.
[12] Carworth Farm # 1 mice.

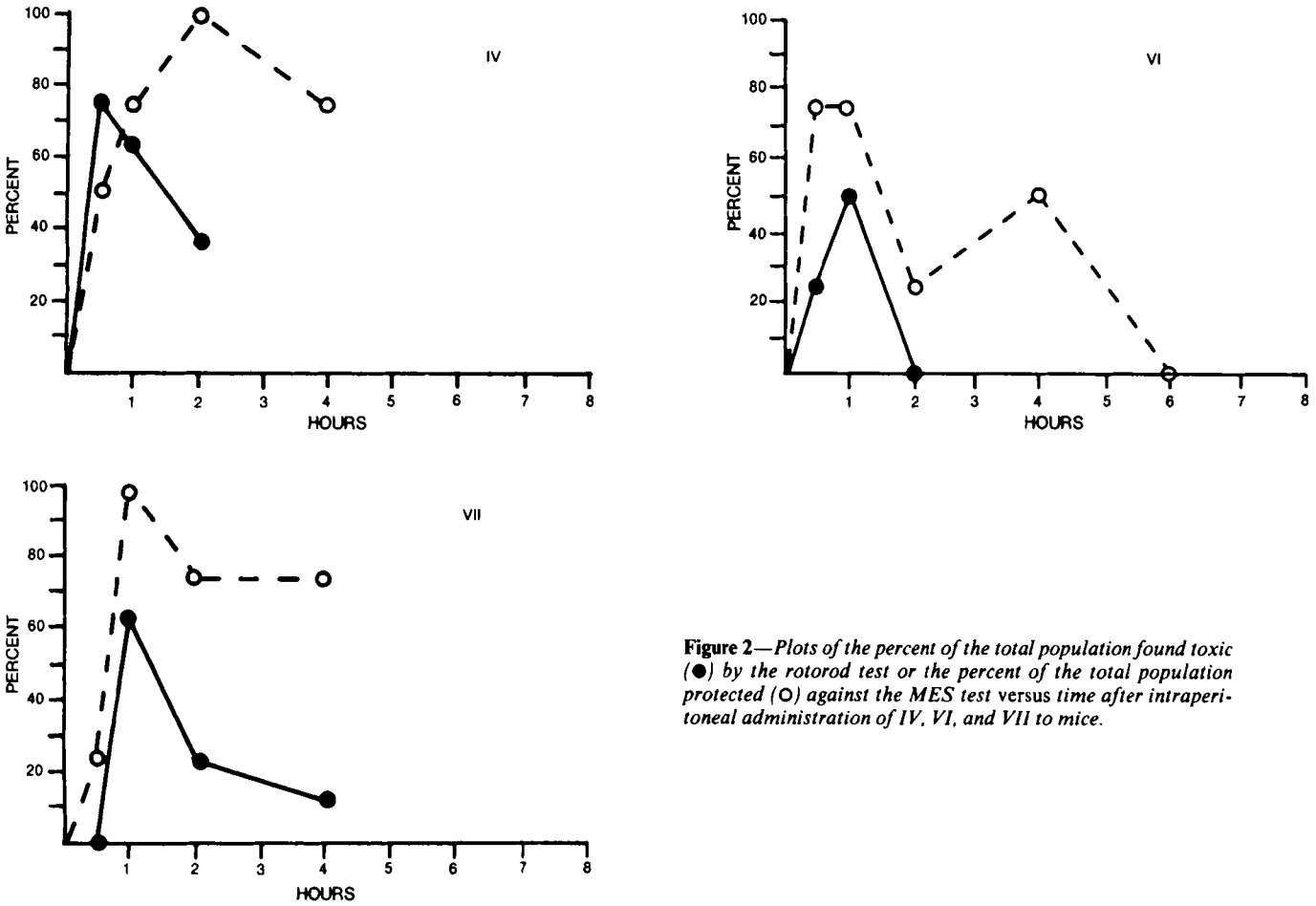


Figure 2—*Plots of the percent of the total population found toxic (●) by the rotorod test or the percent of the total population protected (○) against the MES test* versus *time after intraperitoneal administration of IV, VI, and VII to mice.*

of the sodium salts in methanol a certain amount of degradation took place and the final product may have been contaminated with small quantities of phenytoin.

The solubility of II in aqueous formaldehyde (1 M) and the solubilities of I and IV–VII in water at 25°C are shown in Table I. The increase in solubility of II over I of 8.5-fold may partly be attributed to the decreased intermolecular hydrogen bonding in the crystal lattice of II. This is reflected by the decrease in the melting point from 293°C for phenytoin (I) to 187–189°C for II. The reported solubilities of IV–VII are only approximate, as the prodrugs did begin to revert to phenytoin to some extent during the course of the solubility determinations, *i.e.*, the solid state is a mixture of prodrug and precipitated phenytoin. The methanesulfonate salts of IV, VI, and VII were prepared preferentially to the hydrochloride salts to prevent any common ion solubility problems on oral dosing with the prodrugs. Also, the hydrochloride salt of IV was very hygroscopic. The hydrochloride and methanesulfonate salts of V were prepared but were also found to be very hygroscopic; therefore, only the 2-naphthalenesulfonate salt was evaluated.

The solubilities of IX and X in water could not be determined because initial clear solutions rapidly began precipitating phenytoin acid, which was easily characterized on filtration by its melting behavior. This rapid precipitation of phenytoin from IX and X precluded the use of IX and X as parenteral forms of phenytoin and, therefore, IX and X were discarded as potentially useful prodrugs of I.

Compounds IV–VII were more soluble in water than phenytoin (Table I, last column). Based on this initial observation, IV, VI, and VII were considered possible candidates for parenteral and oral forms of phenytoin, while V was considered as a possible oral prodrug form of phenytoin.

For IV–VII to behave as true prodrugs of phenytoin, they must undergo cleavage to phenytoin under *in vivo* conditions. Therefore, the cleavage rates of IV–VII were studied in various rat, dog, and human tissues to give indications as to their *in vivo* behavior. The hydrolysis of esters IV–VI, 2×10^{-5} M, monitored by following the formation of phenytoin, exhibited pseudo-first-order kinetics in rat, dog, and human plasma. The half-lives are given in Table II. Esters IV–VI were quantitatively hydrolyzed to phenytoin in the plasma of all species studied. The hydrolysis of V and VI was determined in 10 and 0.5% rat plasma, respectively, because their cleavage rates were too fast to follow at higher plasma concentrations. Under comparable conditions in the absence of plasma, IV–VI hydrolyzed slowly to phenytoin; however, no attempts were made at this stage to unambiguously discriminate as to whether the acceleration in the cleavage rates in the presence of plasma was truly enzymatic in nature. Overall, the cleavage of the esters was slowest in dog plasma, consistent with findings of low overall esterase activity in various dog tissues relative to other animal species (37–39). The differences in the relative cleavage rates indicate that the spectrum of esterases present in plasma varies between the animal species.

An attempt was made to determine the hydrolysis of VII in the plasma of rats, dogs, and humans. No detectable cleavage was observed over a 3-h period. Since phosphatases are known to exist in red blood cells (40, 41), cleavage of

Table III—ED_{50} and TD_{50} of Phenytoin, IV, VI, and VII after Intraperitoneal and/or Oral Administration to Mice

Compound	Intraperitoneal Administration		Oral Administration	
	ED_{50} (MES)[a], mg/kg ± *SE*	TD_{50} (Rotorod)[a], mg/kg ± *SE*	ED_{50} (MES)[a], mg/kg ± *SE*	TD_{50} (Rotorod)[a], mg/kg ± *SE*
I[b]	9.50 (8.13–10.4)[c]	65.4 (52.5–72.1)[c]	9.04 (7.39–10.62)[c]	86.71 (80.39–96.09)[c]
IV	10.3 ± 2.35	46.2 ± 3.1	10.6 ± 1.1	80.7 ± 0.8
VI	7.9 ± 1.4	47.7 ± 1.02	—[d]	—[d]
VII	10.3 ± 2.2	42.1 ± 1.9	11.8 ± 1.4	81.6 ± 3.1

[a] All values are in phenytoin equivalents. [b] Reported by the Anticonvulsant Drug Screening program (36). [c] 95% confidence interval. [d] Not determined.

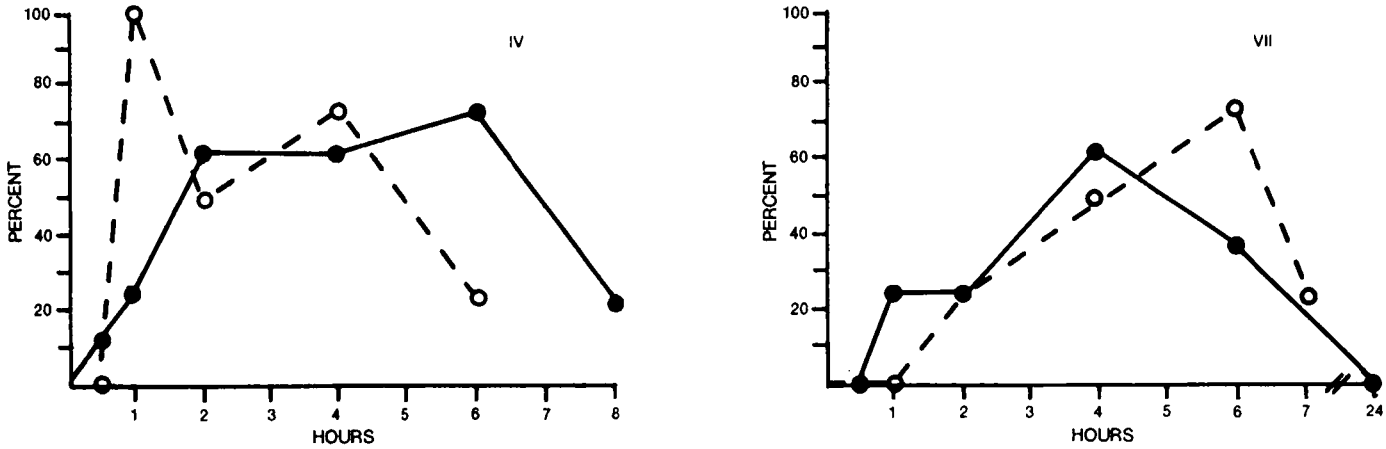
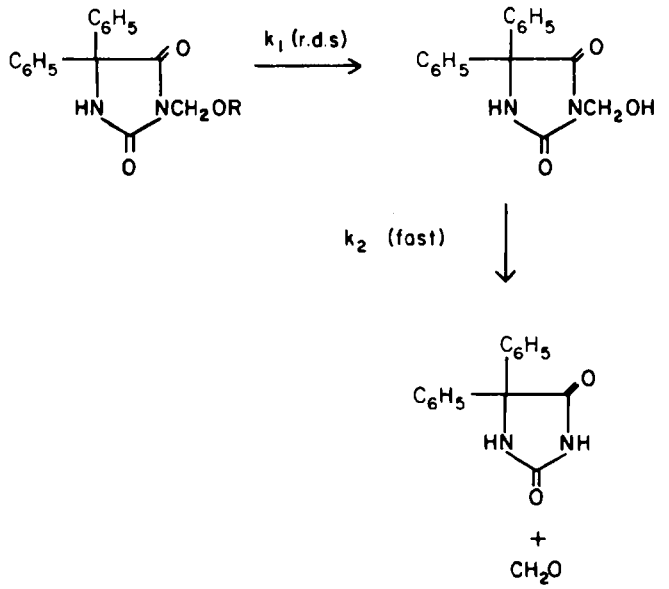


Figure 3—*Plots of the percent of the total population found toxic (●) by the rotorod test or the percent of the total population protected (○) against the MES test* versus *time after oral administration of IV and VII to mice.*

VII in rat, dog, and human whole blood was evaluated. Cleavage in rat whole blood was observed ($t_{1/2}$ = 2 h and 30 min at 25°C and 37°C, respectively). Slow cleavage in dog whole blood ($t_{1/2}$ > 24 h) was observed, but no significant cleavage was seen in human whole blood. These results tend to suggest that either the activity of the phosphatase enzymes was low in dog and human whole blood or that VII had poor accessibility to red blood cell phosphatases. The catalytic conversion of VII to phenytoin in the rat liver ($t_{1/2}$ < 30 s) and intestinal homogenates ($t_{1/2}$ < 30 s) and dog liver homogenate ($t_{1/2}$ = 57.5 min) were observed at 37°C.

It appears from these tissue studies that esters IV–VII would behave as prodrugs of phenytoin *in vivo* based on their reasonably facile cleavage *in vitro* in rat, dog, and human tissues. The only questionable candidate for quantitative conversion might be the phosphate ester VII, although there is adequate precedence in the literature for phosphate esters of various alcohols as prodrugs (42–50).

Mechanistically, the cleavage of esters IV–VII is expected to follow Scheme I, where k_1 represents the *in vitro* or *in vivo* cleavage of esters IV–X to II, which should then hydrolyze releasing formaldehyde[13]. The dehydroxymethylation of II to phenytoin was, therefore, studied in the pH range 4–7 at both 25°C and 37°C (μ = 0.5). As stated earlier, the hydrolysis followed pseudo-first-order kinetics, and no buffer catalysis was observed. Figure 1 shows the pH–rate profiles for the hydrolyses of II to phenytoin at 25°C and 37°C. The half-lives for the dehydroxymethylation extrapolated to pH 7.4 are also indicated in the figure. These data are quantitatively and qualitatively similar to those observed by Bundgaard and Johansen (5) who proposed a



Scheme I

mechanism involving an initial fast ionization of the hydroxyl group (p*Ka* > 12) followed by a slower nitrogen–carbon bond cleavage step producing phenytoin and formaldehyde. The very rapid breakdown of II to phenytoin ($t_{1/2}$ < 2 s at pH 7.4 and 37°C) precludes the accumulation of II when esters IV–VII are cleaved *in vitro* or *in vivo*.

Compounds IV–VII underwent pharmacological evaluation in the anticonvulsant drug screening program operated by National Institute of Neurological and Communicative Disorders and Stroke (36). In the phase I (initial screen) study all compounds showed good anticonvulsant (antiseizure) activity after intraperitoneal administration in the MES evaluation, but (as expected) showed no activity in the ScMET test[14]. The least active prodrug was V, which showed no protection in the MES test at 30 mg/kg; all the other compounds were active at 30 mg/kg. From the results of this initial screen, IV, VI, and VII were subjected to phase II analysis, where the anticonvulsant activity, MES, and rotorod toxicity were evaluated after intraperitoneal administration of the prodrugs in a larger number of animals. Compounds IV and VII were also evaluated in a phase IV study, which involves administering the prodrugs orally. Table III summarizes the ED_{50} and TD_{50} data for IV, VI, and VII after intraperitoneal and oral administrations to mice.

As can be seen in Table III, all the prodrugs had equivalent activity when compared with phenytoin, but all displayed slightly higher toxicity (lower TD_{50} values). This may be consistent with the more rapid absorption of phenytoin in the form of the prodrugs or an elevated intrinsic toxicity by the prodrugs exacerbated by their expected more rapid absorption relative to phenytoin, *i.e.*, the TD_{50} values probably should be compared with the TD_{50} of intravenous phenytoin. Figures 2 and 3 are plots of the percent of the total mice population studied found to be toxic by the rotorod test (toxicity–time profile) and the percent of the total population protected against the MES (activity–time profile) *versus* time after intraperitoneal and oral administrations, respectively. In Fig. 2 (intraperitoneal data) prodrugs VI and VII show an early peaking in the activity- and toxicity-time profile, suggesting rapid release of phenytoin, providing that the anticonvulsant activity is attributable to phenytoin. Ester IV, however, shows maximum activity at 2 h and the toxicity peaking at 0.5 h postdose. This tends to suggest that IV may be undergoing slow hydrolysis, compared with VI and VII, and that the early toxicity peaking may be due to the intact ester IV.

The lack of early toxicity in the oral study (Fig. 3) for IV compared with its activity suggests that after oral dosing, IV may be hydrolyzed during the absorption process. The delayed peaking of both toxicity and activity for VII after oral dosing (compared with the intraperitoneal data), suggests that the activity and toxicity of VII are probably due to phenytoin and that VII is only slowly absorbed from the intestine; alternatively, VII may be cleaved slowly in the GI tract, releasing phenytoin which was subsequently absorbed.

Based on the findings presented in this paper, IV, VI, and VII were evaluated further with respect to other physicochemical properties (51) and their pharmacokinetic behavior after oral and intravenous administration to dogs compared with phenytoin (52). Compound VII was also evaluated after intramuscular and intravenous administration to rats (53).

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[13] Formaldehyde toxicity may be raised as a problem with these types of prodrugs. Whether the quantity of formaldehyde generated here is sufficient to raise concerns will have to be evaluated.

[14] Phenytoin itself is inactive in the ScMET test.

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