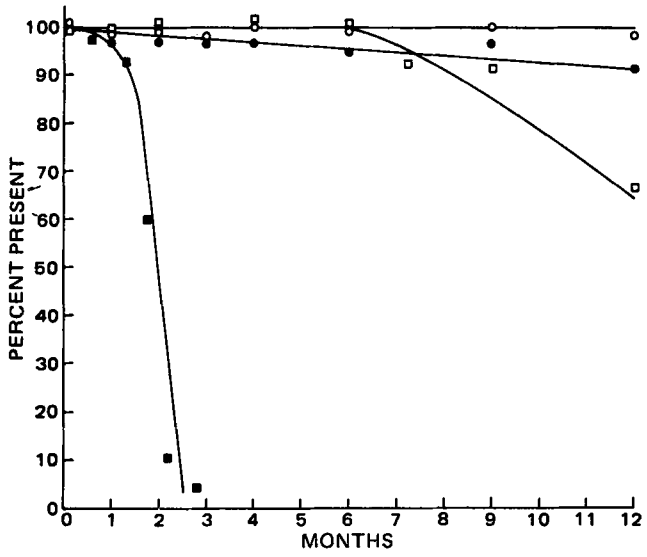


**Figure 1**—*Stability of I in the solid state and triacetin solutions. Data for solutions are average values for theoretical concentrations from 0.10 to 12 mg/ml. Key: □, 25° bulk drug; ■, 47° bulk drug; ○, 25° triacetin solution; and ●, 47° triacetin solution.*

## EXPERIMENTAL

**Materials**—Compounds I (9) and II (10) were synthesized[1] as described previously. Triacetin[2] was purchased.

**Stability Studies**—Both I and II were tested for stability as bulk drug and in triacetin solution. Stability[3] was monitored at 4, 25, and 47° for up to 12 months (Table I and Fig. 1).

[1] At The Upjohn Co.
[2] Aldrich Chemical Co. or Union Carbide Chemicals Co., New York, N.Y.
[3] Analyses were performed by the Control Division, The Upjohn Co., using high-pressure liquid chromatography.

## RESULTS AND DISCUSSION

The solid-state stability of I is shown in Fig. 1. Degradation occurred at 47 and 25°. At these temperatures, the drug was stable at early times and then rapidly decomposed. As the prostaglandin degraded, a physical transformation of the solid resulted, producing an oil phase. The marked change in the slopes at 25 and 47° may have been due to subsequent catalysis by the decomposed liquid phase. In sharp contrast to the stability of bulk drug, I was quite stable in triacetin solutions at 25°, and there was a marked improvement of stability at the elevated temperature of 47° (Fig. 1).

Table I shows the stability of another E series prostaglandin drug in triacetin. Analysis of these data further substantiates the stability-enhancing property of this solvent. Triacetin dosage forms prepared with II were clinically effective as inhibitors of simulated gastric secretion in humans, indicating that the drug is bioavailable from these dosage forms (11).

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# Fluorinated Phenytoin Anticonvulsant Analogs

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Received March 20, 1978, from the *Department of Pharmaceutical Sciences, School of Pharmacy, University of Washington, Seattle, WA 98195.* Accepted for publication June 8, 1978.

**Abstract** □ Six ring-fluorinated phenytoin analogs were synthesized, and their anticonvulsant activity in the maximal electroshock seizure and subcutaneous pentylenetetrazol assays was determined. 5-(4-Fluorophenyl)-5-phenylhydantoin, 5-(3-fluorophenyl)-5-phenylhydantoin, and 5,5-bis(4-fluorophenyl)hydantoin were active in the maximal electroshock seizure assay. The compounds were much less potent than phenytoin but showed an extremely long duration of action.

**Keyphrases** □ Phenytoin analogs, fluorinated—synthesized, evaluated for anticonvulsant activity in mice □ Anticonvulsant activity—fluorinated phenytoin analogs evaluated in mice □ Fluorinated phenytoin analogs—synthesized, evaluated for anticonvulsant activity in mice □ Structure-activity relationships—fluorinated phenytoin analogs evaluated for anticonvulsant activity in mice

Phenytoin (5,5-diphenylhydantoin) has been used as an effective agent for the treatment of many different seizure disorders for about 40 years. The principal route of metabolism in humans and dogs is *para*-hydroxylation, affording optically active 5-(4-hydroxyphenyl)-5-phenylhydantoin (1, 2). This compound is eliminated as a urinary conjugate, making up 60–70% of the daily dose (2). Other metabolites of aromatic hydroxylation also have been reported, including 5-(3-hydroxyphenyl)-5-phenylhydantoin (3), 5-(3-dihydroxyphenyl)-5-phenylhydantoin (4, 5), 5,5-bis(4-hydroxyphenyl)hydantoin (6), 5-(3-methoxyphenyl)-5-phenylhydantoin (4, 5), and the dihydrodiol 5-(3,4-dihydroxy-1,5-cyclohexadienyl)-5-phenylhydantoin (7). Further oxidized phenytoin conjugates, a trihydroxyphenytoin glucuronide and a dihydroxymethoxyphenytoin glucuronide, were identified as metabolites of 5,5-bis(4-hydroxyphenyl)hydantoin (6).

Since the primary route of metabolism, aromatic hydroxylation in the *para*-position, provides inactive me-

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**Table I—Anticonvulsant Activity and Neurotoxicity**

| Compound | MES 0.5 hr | MES 4 hr | $ED_{50}$ | Toxicity[a] | Additional Notes |
|---|---|---|---|---|---|
| I | – | +[b] | 61 (44–71)[c] at 6 hr | 1190 (982–1712) at 24 hr | Onset of MES activity was 2–4 hr. Doses of 133 mg/kg showed protection at 24 hr. Doses of 1000–1500 mg/kg showed a slow onset of neurotoxicity (8–20 hr), lasting more than 48 hr. |
| II | – | – | nd[d] | nd | — |
| III | +++ | +++ | 23 (20–26) at 4 hr | 158 (117–183) at 6 hr | — |
| IV | – | – | See note | Nontoxic at 2000 mg/kg | At 2000 mg/kg, 4/8 animals were protected at 192 hr. At 1000 mg/kg, maximum protection was at 24 hr, and 4/8 remained protected at 48 hr. |
| V | – | – | nd | nd | — |
| VI | – | – | 270 (227–321) at 8 hr | Nontoxic at 2000 mg/kg | — |

[a] A description of the assay methods appears in the *Experimental* section. [b] The +++, ++, and + signify activity at 30, 100, and 300 mg/kg, respectively; – indicates no activity was observed. [c] The 95% confidence limits. [d] Not done.

tabolites, it has a significant influence on blood levels and the duration of action of phenytoin. It was reasoned that by addition of fluorine atoms to the aromatic ring, it might be possible to slow or block this metabolic pathway and thus provide compounds of a longer duration of action than phenytoin. This change also offered the potential of providing compounds with a different spectrum of anticonvulsant activity.

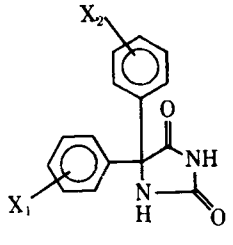
The addition of fluorine atoms offered the advantage of being the smallest possible change to the phenytoin molecule next to isotopic substitution. Also, it was thought that these compounds could serve as standards for work related to $^{18}F$-phenytoin derivatives as potential organ-scanning agents (8, 9). The synthesis of six different fluorinated phenytoin analogs is reported here, with results of initial screening in anticonvulsant assays.

## RESULTS AND DISCUSSION

Fluorinated phenytoin analogs I–IV and VI were prepared by a Bucherer synthesis, modified by the method of Henze and Isbell (10), from the corresponding benzophenone, potassium cyanide, and ammonium carbonate with acetamide as solvent. Compound V was prepared from urea and 3,3-difluorobenzil, available through the benzoin condensation of 3-fluorobenzaldehyde.

The results of maximum electroshock seizure (MES) assays of I–VI appear in Table I. The compounds were not active in subcutaneous pentylenetetrazol (sc-Met) assays. The observed restriction of activity to the maximum electroshock seizure assay was similar to that of phenytoin. Activity was noted in I, IV, and VI, which contain a fluorine atom in at least one 4-position, and in 5-(2-fluorophenyl)-5-phenylhydantoin (III). The compounds were considerably less active than phenytoin [$ED_{50}$ = 9.5 mg/kg for phenytoin (11)]. Although not potent, I, IV, and VI were of an extremely long duration of action: up to more than 7 days duration for IV and 8–48 hr for the doses of I and VI tested. Additionally, the compounds were very slow in onset of activity, requiring several hours to reach peak activity.

If the substitution of a fluorine atom at the *para*-position in phenytoin yielded a compound protected from metabolic conversion to an inactive metabolite, I and IV would be less readily hydroxylated and would be more potent than phenytoin, but this behavior was not observed. Additionally, since IV has fluorines at both *para*-positions, it should be more active than I since I retains one possible site for metabolic inactivation. This hypothesis is also contrary to the results observed.




I: $X_1$ = 4-F, $X_2$ = H
II: $X_1$ = 3-F, $X_2$ = H
III: $X_1$ = 2-F, $X_2$ = H
IV: $X_1$ = 4-F, $X_2$ = 4-F
V: $X_1$ = 3-F, $X_2$ = 3-F
VI: $X_1$ = 4-F, $X_2$ = 3-F

Since the addition of fluorines in the *para*-position results in compounds with reduced activity compared to phenytoin, factors other than metabolic deactivation (*e.g.*, steric, electronic, and partitioning) must contribute to the observed low activity. The reported absence of activity of other *para*-substituted phenytoin analogs, especially halogenated or methyl-substituted analogs (12, 13), is similar to that observed with these fluorinated analogs.

The slow onset of action is difficult to explain satisfactorily. A latent period before onset of pharmacological activity is usually interpreted to indicate that activity may be mediated by formation of an active metabolite or alteration of enzyme levels by alteration of the rate of synthesis or degradation, which requires some time for these changes to occur. These interpretations assume that drug absorption is not rate limiting, which seems to be valid in these cases since the compounds are very similar to phenytoin. Although these possibilities potentially exist for the fluorinated phenytoin derivatives, no evidence in this work supports such an explanation.

The presence of activity in *para*-substituted compounds and not in *meta*-substituted compounds (II and V) is also puzzling. However, in view of the low potency of the *p*-fluoro-substituted compounds, this difference is not worthy of protracted speculation. Too few *meta*-substituted systems have been studied to allow valid comparison of II and V with other related compounds.

Only III, 5-(2-fluorophenyl)-5-phenylhydantoin, showed reasonable activity, being slightly less than half as potent as phenytoin on a molar basis and less than half as neurotoxic ($TD_{50}$ = 158 *versus* 65 mg/kg).

In conclusion, the long duration of activity of I, IV, and VI is notable. However, the low potency of these compounds suggests that preparation of other compounds is probably of higher priority than intensive investigation of the behavior of these phenytoin analogs. The significant decrease in activity of the analogs suggests that there may be only a very limited number of successful modifications of the aromatic ring possible, at least at the *meta*- and *para*-positions.

## EXPERIMENTAL

**5-(4-Fluorophenyl)-5-phenylhydantoin (I)**—A mixture of 2.00 g (0.010 mole) of 4-fluorobenzophenone, 0.75 g (0.011 mole) of potassium cyanide, 1.0 ml of water, and 3.00 g (0.032 mole) of powdered ammonium carbonate in 20.0 g of acetamide was heated in a Parr bomb at 125–130° for 24 hr. After cooling, the bomb was opened and 300 ml of water was added. The resulting mixture was acidified (hood, hydrogen cyanide evolved), and the precipitate removed by filtration. Recrystallization from ethanol–water afforded I as off-white crystals, 2.70 g (52% yield), mp 274–276°.

*Anal.*—Calc. for $C_{15}H_{11}FN_2O_2$: C, 66.67; H, 4.07; N, 10.35. Found: C, 66.42; H, 4.01; N, 10.29.

**5-(3-Fluorophenyl)-5-phenylhydantoin (II)**—3-Fluorobenzophenone was prepared from 3-fluorobenzoyl chloride, which was prepared by refluxing 3-fluorobenzoic acid with 50% molar excess of thionyl chloride for 1 hr followed by distillation, and benzene in the presence of anhydrous aluminum chloride. 3-Fluorobenzophenone was obtained in a 50% yield, bp 112–114°/0.9 mm, mp 52–54° [lit. (14) mp 53°]. 3-Fluorobenzophenone was converted to II by the procedure described for the preparation of I. Compound II was obtained in a 48% yield, mp 246–251° (ethanol–water).

*Anal.*—Calc. for $C_{15}H_{11}FN_2O_2$: C, 66.67; H, 4.07; F, 7.04; N, 10.35. Found: C, 66.39; H, 3.98; F, 7.31; N, 10.41.

**5-(2-Fluorophenyl)-5-phenylhydantoin (III)**—By a procedure analogous to that for the preparation of I, the title compound was prepared from 2-fluorobenzophenone in a 27% yield, mp 264–266°.

*Anal.*—Calc. for $C_{15}H_{11}FN_2O_2$: C, 66.67; H, 4.07; N, 10.35. Found: C, 66.15; H, 3.99; N, 10.51.

**5,5-Bis(4-fluorophenyl)hydantoin (IV)**—By a procedure analogous to that for the preparation of I, the title compound was prepared from 4,4′-difluorobenzophenone in a 50% yield, mp 310–312°.

*Anal.*—Calc. for $C_{15}H_{10}F_2N_2O_2$: C, 62.50; H, 3.47; F, 13.16; N, 9.72. Found: C, 62.03; H, 3.46; F, 13.37; N, 9.59.

**5,5-Bis(3-fluorophenyl)hydantoin (V)**—This compound was prepared from 3,3′-difluorobenzil and urea by the general hydantoin synthesis described by Adams *et al.* (15). A mixture of 1.95 g (0.03 mole) of potassium cyanide, 15 ml of water, 30 ml of ethanol, and 18.60 g (0.15 mole) of 3-fluorobenzaldehyde was refluxed for 30 min and cooled in an ice bath to effect crystallization. The crude product was collected, washed with cold 50% aqueous alcohol and water, and dried, affording 11.40 g (66% of theory) of the benzoin, mp 77–79°.

A mixture of 9.93 g (0.04 mole) of the crude benzoin, 40 ml of acetic acid, and 2.80 g (0.34 mole) of concentrated nitric acid was heated on a steam bath for 3 hr. Then water, 150 ml, was added. On cooling, the crude benzil was deposited, 9.20 g (93% yield).

A mixture of 1.80 g (4.06 mmoles) of the benzil, 0.50 g (8.33 mmoles) of urea, 15 ml of ethanol, and 13 ml of 30% aqueous potassium hydroxide was refluxed for 2 hr. Then 25 ml of water was added, and the solution was filtered. Acidification afforded the desired hydantoin, 325 mg (28% yield), mp 238–240°.

*Anal.*—Calc. for $C_{15}H_{10}F_2N_2O_2$: C, 62.50; H, 3.47; F, 13.16; N, 9.72. Found: C, 62.04; H, 3.41; F, 12.89; N, 9.85.

**5-(3-Fluorophenyl)-5-(4-phenyl)hydantoin (VI)**—This compound was prepared from 3,4′-difluorobenzophenone by a procedure similar to that for the preparation of I. The product was obtained in 58% yield, mp 279–280° (dioxane–water).

*Anal.*—Calc. for $C_{15}H_{10}F_2N_2O_2$: C, 62.50; H, 3.47; F, 13.16; N, 9.72. Found: C, 62.21; H, 3.59; F, 13.08; N, 9.48.

**Pharmacological Testing**[1]—All tests were performed on male Carworth Farms No. 1 mice. All compounds were tested initially at three dosage levels (30, 100, and 300 mg/kg) at 30 min and 4 hr after their intraperitoneal administration. Four animals were injected with each dose. Thirty minutes later, each animal was examined for toxicity in the rotorod test. Immediately thereafter, anticonvulsant activity was evaluated by subjecting one mouse to the protection against the maximal electroshock seizure test and another to the subcutaneous pentylenetetrazol (sc-Met) test.

The same tests were repeated 4 hr later on the two remaining mice. All compounds were solubilized in 30% polyethylene glycol 400 and administered intraperitoneally in a volume of 0.01 ml/g. The $ED_{50}$ and $TD_{50}$ values and their confidence limits were determined by probit analysis and/or by the method of Litchfield and Wilcoxon (16).

Maximum electroshock seizure activity is defined as abolition of the hindlimb tonic extensor component of the maximal electroshock seizure elicited in mice with a 60-Hz alternating current of 50 mamp delivered for 0.2 sec *via* corneal electrodes. Subcutaneous pentylenetetrazol activity is defined as failure to observe even a threshold seizure (a single episode of clonic spasms of at least 5 sec). Neurologic toxicity is defined as failure of an animal to remain for 1 min on a 2.54-cm diameter knurled plastic rod rotating at 6 rpm.

[1] Compounds were tested for anticonvulsant activity by the Anticonvulsant Screening Project, Antiepileptic Drug Development Program, Epilepsy Branch, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, MD 20014.

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