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A Nonmetabolized Analogue of Phenytoin[1]

James D. Henderson,* Peter G. Dayton, Zafar H. Israili, and Leon Mandell

Departments of Medicine and Chemistry, Emory University, Atlanta, Georgia 30322. *Received August 25, 1980*

Nine novel analogues of 5,5-diphenylhydantoin bearing a CF_3 groups(s) in the meta or para position of one or both rings were synthesized. Preliminary evaluation of all the analogues (performed by the ADD Program, NIH) indicated no significant anticonvulsant activity against electrical or chemical shock in mice at doses of ≤100 mg/kg. The analogue 5,5-bis[4-(trifluoromethyl)phenyl]hydantoin (1) was synthesized labeled with ^{14}C in the 4 position of the hydantoin ring. Certain physicochemical properties (pK_a, partition ratio, protein binding, etc.) and the LD_{50} of 1 in mice (40 mg/kg, ip; 100 mg/kg, po) were determined. The disposition of [^{14}C]1 was determined in rodents. The compound was excreted unchanged in rat feces (94% in 18 days), urinary excretion <0.5%. The half-life of elimination of [^{14}C]1 from plasma was 67-72 h (ip and iv) in rats and 115 h (ip) in mice. Studies of tissue distribution and biliary excretion of [^{14}C]1 indicate low tissue/plasma ratios (due to high plasma binding, 97%) and low biliary excretion. The lack of metabolism of [^{14}C]1 may possibly be explained by (1) the strong electron-withdrawing effects of CF_3 substituents, (2) the preemption of the primary metabolic sites, (3) the accompanying steric hindrance, and (4) the apparent inability of the CF_3 group to undergo the NIH shift.

It has been proposed that electron-withdrawing groups on aromatic rings inhibit in vivo and in vitro oxidation of the aromatic nucleus of a number of drugs.[2-5] As a further test of this hypothesis, we synthesized and studied the biological fate of a bis(trifluoromethyl) analogue of phenytoin (Dilantin, diphenylhydantoin, DPH), i.e., 5,5-bis-[4-(trifluoromethyl)phenyl]hydantoin (1). Strong deactivation toward electrophilic substitution in 1 should markedly reduce aromatic hydroxylation (the major route of biotransformation of DPH). The combination of electronic and steric factors was expected to result in a more slowly metabolized and eliminated compound compared to DPH. Further, increased activity of 1 was anticipated, since CF_3 or F substitution of the aromatic rings has resulted in increased activity for certain other molecules.[6,7] Fluorinated phenytoins are active[8] as anticonvulsant agents but cause more gingival hyperplasia than DPH.[9] To facilitate measurement of physicochemical properties and studies of the diposition of 1 in rodents, [^{14}C]1 was synthesized. Besides compound 1, eight other novel analogues of DPH were synthesized; all were screened for anticonvulsant activity.[10,11]

Chemistry. The trifluoromethyl-substituted analogues of DPH 1-9 (Table I) were synthesized by the procedure of Bucherer and Berg, as modified by Henze and Isbell.[12] Grignard reagents were condensed with the appropriate aldehydes to give substituted benzhydrols, which were oxidized with pyridinium chlorochromate[13] to the corresponding benzophenones. The latter were converted to the hydantoins by heating with KCN and ammonium carbonate in acetamide in a sealed tube. The labeled analogue, [^{14}C]1, was obtained by the same procedure.

Results

Physicochemical Properties. Physicochemical properties for 1 were determined; these data and the corresponding values for DPH are given in Table II. The K_p for 1 was ten times that reported for DPH[14]. Previously reported vaues for the pK_a of DPH range from 8.1 to 8.3 under different experimental conditions.[15-23]

* Address correspondence to: Department of Pharmacology, University of South Alabama, Mobile, Alabama 36688.

Table I. Substituted Diphenylhydantoins[a]

compd	R_1	R_2	mp, °C	% yield	activity,[b] mmol/kg MES	scMET
3	3-CF_3	3-CF_3	225-226	45	c	2.6
4	4-CF_3	H	207-209	88	0.94	c
5	3-CF_3	H	216-218	74	0.31	c
DPH	H	H			0.038	inact

[a] The following compounds were inactive against either maximal electroshock (MES) or subcutaneous pentylenetetrazol (scMET) induced seizures in mice at 0.5 or 4 h after ip administration at doses ≤0.77-0.90 mmol/kg: 1, R_1 = 4-CF_3, R_2 = 4-CF_3, mp 280-282 °C (an independent synthesis has also been reported[11]); 2, R_1 = 3-CF_3, R_2 = 4-CF_3, mp 240-241; 6, R_1 = 4-CF_3, R_2 = 4-CH_3, mp 226-228; 7, R_1 = 3-CF_3, R_2 = 4-CH_3, mp 219-220; 8, R_1 = 4-CF_3, R_2 = 3-CH_3, mp 203-204; 9, R_1 = 3-CF_3, R_2 = 4-CH_3, mp 194-195. Yields were between 52 and 90%. Satisfactory spectra (IR, NMR, and mass) were obtained for all compounds. All compounds were analyzed for C, H, F, and N (Midwest Microlabs, Ltd., Indianapolis, IN); analyses were found to be within ±0.4% of theoretical values. [b] Dose required for protection against MES- or scMET-induced seizures 4 h after ip administration. [c] Inactive in this test at 0.5 or 4 h after ip administration at doses ≤0.77-0.90 mmol/kg.

Plasma Half-life. The values for plasma $t_{1/2}$ (β phase) of [^{14}C]1 in rats, after a dose of 5 mg/kg given ip (group

(1) Portions of this work were presented at the 28th ACS Southeast Regional Meeting; see "Abstracts of Papers", 28th ACS Southeast Regional Meeting, Gatlinburg, TN, Oct 27-29, 1976; American Chemical Society: Washington, DC, 1976; Abstr 216; Dayton, P. G.; Henderson, J. D.; Israili, Z. H.; Mandell, L. *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **1979**, *38*, 742 (Abstr 2711).

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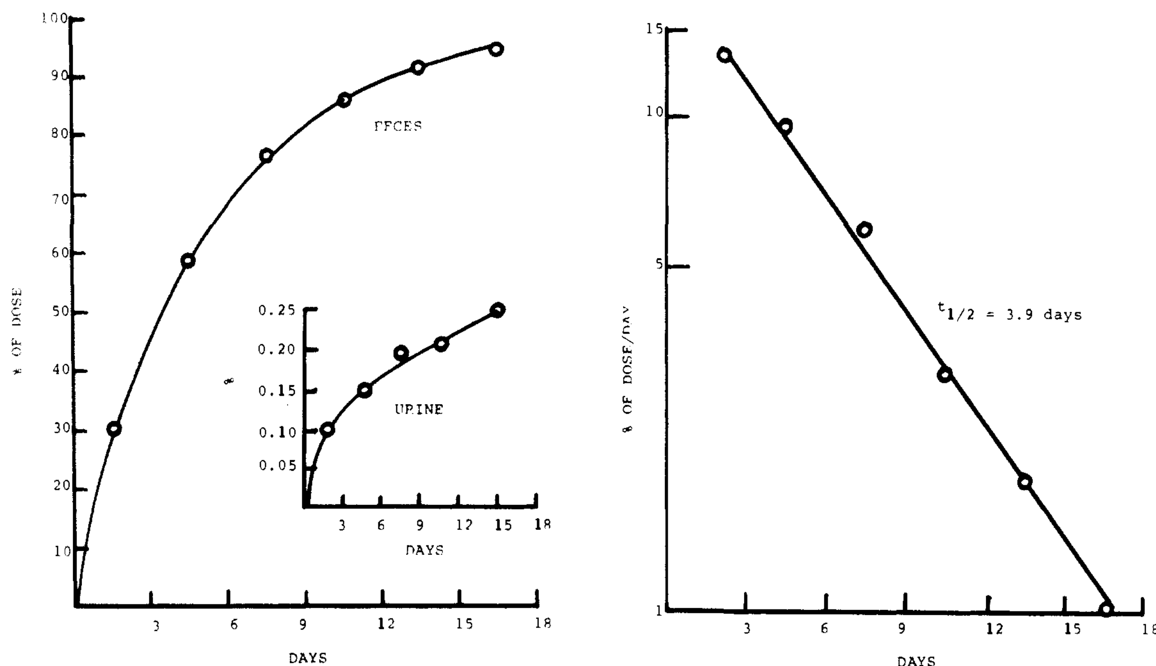

Figure 1. Excretion of ^{14}C after administration of [^{14}C]1 (10 mg/kg, ip) to rats (group B). Left panel: cumulative excretion of ^{14}C in feces and urine. Right panel: excretion rate of ^{14}C in feces. The percent of dose per day value for the 0–3 day collection interval has been corrected for gastrointestinal transit time (about 18 h in the rat).[4]

Table II. Physicochemical Properties of 1 and DPH

property	compound		ref for data on DPH
	1	DPH	
pK_a (H_2O)	8.0	8.1, 8.3	15, 16
binding[a]	3 ± 0.6	13-28	17-20
solubility,[b] µg/mL	3	20.5	16
K_p[c]	80	8	14
brain/plasma ratio[d]	0.14	0.4-1.3	17, 21-23

[a] Expressed as percent free drug plus or minus SD in rat plasma (inside concentration of 1, 3.3 µg/mL). [b] Sörensen buffer, pH 7.4; in rat plasma, the solubility of 1 is 208 µg/mL. [c] Peanut oil/buffer, pH 7.4. [d] Plasma concentration of [^{14}C]1: rats, 6.75-13.9 µg/mL; mice, 15.6-27.1 µg/mL.

C) and iv (group D), were 71.7 and 66.7 h, respectively. The distribution phase was rapid [$t_{1/2}$ (α) = 0.2 h]. Average plasma levels of [^{14}C]1 at 24 h, after ip and iv doses, were 11.0 and 9.4 µg/mL, respectively. The average apparent volume of distribution (iv dose) was 0.42 L/kg.

In other studies, Swiss Webster mice (20–30 g) were given [^{14}C]1 at a dose of 10 mg/kg (ip) and were sacrificed at 12, 18, 24, 36, 48, and 72 h (four animals at each time period) after dose. Levels of [^{14}C]1 were measured in the plasma and brain. The plasma $t_{1/2}$ of [^{14}C]1 in the mouse was 115 h. The maximum levels of [^{14}C]1 in the plasma (27.0 µg/mL) and brain (3.5 µg/mL) were achieved at 18 and 24 h after dosing, respectively. The brain/plasma concentration ratio of [^{14}C]1 remained fairly constant (0.14) up to 72 h. The $t_{1/2}$ of DPH has been reported to be about 0.5 h in the rat (10 mg/kg, iv)[24,25] and 16.5 h in the mouse (40 mg/kg, ip).[26]

Metabolism and Excretion. Fecal excretion was the major route of elimination of [^{14}C]1 in rats. In four rats (group A) about 67% of the dose (10 mg/kg, ip) was excreted in feces and only 0.3% in urine in 7 days. Residues from the EtOAc extracts (93–100% recovery of [^{14}C]1) of the fecal homogenates were chromatographed in two solvent systems. In every case, only one major radioactive zone (94–98% of ^{14}C) was observed, the R_f of which corresponded to that of 1; small amounts (0.5–2%) of ^{14}C remained at the origin.

Since only 67% of the dose was accounted for in excreta in 7 days (group A), the cumulative excretion of [^{14}C]1 was studied in four other rats (group B, 10 mg/kg, ip) for 18 days. In that time an average of 94% of the dose was recovered in feces (Figure 1) and only 0.25% of the dose

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appeared in the urine. The percent of dose excreted per day was calculated for each collection period and plotted against the midpoint of that interval; the resulting semilogarithmic plot is shown in Figure 1. Analysis of the data gave a $t_{1/2}$ of elimination of [^{14}C]1 in feces of 3.9 days, which is essentially the $t_{1/2}$ of total body elimination. Isotope dilution analysis of the fecal homogenates indicated that 97–100% of the excreted ^{14}C was associated with unchanged 1.

The biliary excretion of [^{14}C]1 in three rats (group E), after a dose of 5 mg/kg (iv), averaged about 0.5% of the dose in 4 h (rate = 0.06–0.15%/h). Inaba and Umeda[27] have reported excretion of 54 and 28% of DPH in the bile of anesthetized rats in 6 h after iv doses of 1 and 10 mg/kg, resp. The average bile flow rate in group E rats was 1.2 mL/h.

Tissue Distribution. Tissue levels of [^{14}C]1 were determined in rats (group D) at 1, 8, and 24 h after administration of the compound (5 mg/kg, iv). In all tissues except fat, highest concentrations of [^{14}C]1 occurred 1 h after injection; tissue/plasma ratios for [^{14}C]1 followed the sequence: liver (0.96), heart (0.66), lungs (0.62), kidneys (0.61), gastrointestinal tract (0.48), muscle (0.41), brain (0.14), red cells (0.12). Except for fat, these ratios did not change significantly at 8 and 24 h after dosing; the concentration in fat increased progressively.

Toxicity. The 7-day LD_{50} of 1 in mouse was 40 mg/kg (ip) and 100 mg/kg (po). At doses ≥100 mg/kg, noted side effects were ataxia and convulsions. The marked drowsiness observed at all doses was probably mostly due to the vehicle. The 72-h LD_{50} for DPH in mouse had been reported to be 200 mg/kg (ip).[28]

The neurotoxicity of 1 was evaluated in mice by the rotorod ataxia test.[10] No neurological deficit was observed for up to 48 h after doses of ≤500 mg/kg (ip). However, significant neurotoxicity was found at 24 and 48 h after doses of 750 and 1000 mg/kg. Compounds 2–9 did not show neurotoxicity up to 4 h after doses of ≤300 mg/kg (ip). The reported median toxic dose of DPH in mouse is 65 mg/kg (ip).[10]

Anticonvulsant Activity. One of the objectives of molecular modification designed to prevent drug metabolism was the retention of activity. Compound 1 was screened for anticonvulsant activity by the Antiepileptic Drug Development (ADD) Program, Epilepsy Branch, NINCDS, NIH. Based on methods developed by Swinyard et al.,[10] two assays for anticonvulsant activity in mice (Carworth Farm No. 1) were employed, maximal electroshock (MES) seizure test and subcutaneous pentylenetetrazol (metrazol) seizure threshold test (scMET). Compound 1 did not have significant activity against either MES or scMET seizures at doses of up to 1000 mg/kg and at time periods of up to and including 48 h after ip administration of the compound in 30% aqueous PEG 400 (0.01 mL/g).

Compound 1 was further tested in the "timed intravenous pentylenetetrazol infusion test" (ivMET), which provides a somewhat more sensitive measure of a compound's ability to modify the threshold for minimal seizures and the threshold for seizure spread.[29] Two end points are determined in this test. The "time to first twitch" is a measure of minimal excitation, and prolongation of this time correlates reasonably well with inhibition of subcutaneous pentylenetetrazol seizures. The "time to tonic extension" is a measure of the spread of seizure activity, and prolongation of this time correlates well with inhibition of maximal electroshock seizures. As shown by the data in Table III, there was minimal, if any, change at any of the doses in the time to first twitch. In contrast with the maximal electroshock data, however, the time to tonic extension is markedly prolonged with doses as low as 100 mg/kg at all time periods tested. At present, there is no explanation for the observed differences in the anticonvulsant activity of 1, as determined by the ivMET and MES tests.

Compounds 2–9 were also screened by the MES and scMET tests. Activity was observed at 0.5 and 4 h after ip dose. The minimum doses of compounds required to protect mice against MES- or scMET-induced seizures at 4 h after dosing are given in Table I. Compounds **2** and **6–9** were inactive in both tests at doses ≤300 mg/kg (compound **3** was active against scMET-induced seizures at 1000 mg/kg after 4 h). Compound **4** protected mice from MES-induced seizures at 100 mg/kg, 4 h after administration; **5** was active at 300 mg/kg (4 h) in this test. By contrast, DPH is active against MES-induced seizures at 10 mg/kg but inactive in the scMET test.[10] Thus, introduction of one or more CF_3 substituents on the aromatic rings of the DPH molecule greatly diminished activity; two CF_3 groups caused at least a 10- to 100-fold reduction. A CF_3 in one ring and a CH_3 on the other also gave inactive compounds.

Discussion

The mechanism of action of the anticonvulsant DPH has been studied extensively but is still obscure; the same is true for the mechanism of toxicity.[30–32] It is not even known whether at the receptor DPH mimicks diaminobutyric acid (open DPH structure),[33] phenylalanine peptide (N-terminal amide),[34] or a cannabinoid,[35] or acts on active-transport processes, etc. Frequently, studies of structure–activity combined with investigations of drug disposition and physicochemical properties aid in this area of research. To elaborate on these questions, we sought a longer-acting, nonmetabolized analogue of DPH.

We achieved one of our objectives by preparing a CF_3-substituted analogue of DPH which is not metabolized. However, the activity of compound 1 was found to be considerably less (about 10%) than that of DPH.

Consistent with its binding to plasma protein (free-fraction, 3%), glomerular filtration of 1 in the kidney should be significantly less than for DPH. Due to high lipid solubility, its tubular reabsorption should be extensive. Indeed, urinary excretion of 1 was far less than for DPH.[30] Brain/plasma concentration ratios in rodents reflected the difference in binding—0.14 and 1.3[21–23] for 1 and DPH, respectively. However, in vivo distribution by itself cannot explain the low order of activity of 1. Thus, steric factors may be the major determinants of the decreased activity of 1.

In the rat, the fate of 1 is markedly different from that of DPH. The major route of elimination of 1 is via feces

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(>94%). Analysis by radiochromatographic and isotope dilution techniques established that 1 was not metabolized (<3%). In contrast, DPH undergoes extensive biotransformation in the rat;[36,37] oxidation of the aromatic nucleus occurs mainly via an arene oxide pathway.[38] Accordingly, the CF_3 groups in 1, which are strongly deactivating to electrophilic aromatic substitutions ($\alpha = 0.54$),[39] should inhibit epoxide formation. Indeed, this is in agreement with the observed long plasma $t_{1/2}$ of 1 (about 140 times that of DPH) and the fecal excretion of unchanged drug. It is possible that mechanisms other than those involving epoxide formation may contribute to the hydroxylation of DPH.[40] However, in the case of 1, a mechanism such as direct substitution of an electrophilic species would necessitate removal or migration (by an "NIH shift"[41]) of the CF_3 moiety; such reactions for an aromatic CF_3 substituent have not been reported. An *N*-glucuronide metabolite is known for DPH.[42] No evidence for an *N*-glucuronide of 1 was found in the isotope dilution studies; however, it is doubtful that such a conjugate could have survived the alkaline extraction procedure or the subsequent acidification step. In the TLC experiments a small amount (0.3–3%) of ^{14}C remained at the origin; this could possibly represent an *N*-glucuronide conjugate of 1 or some other polar biotransformation product.

Besides the longer $t_{1/2}$, the fate of 1 differs from that of DPH in other respects. The fate of 1, in contrast to DPH, resembles that of halogenated lipid-soluble compounds (e.g., 3,3′,5,5′-tetrachlorobiphenyl) for which multicompartmental flow-dependent models have been constructed[43,44] (long $t_{1/2}$, slow but extensive fecal excretion, high binding, minimal urinary excretion, etc.). The biliary excretion of 1 (about 0.1%/h) is much less than that of DPH (and metabolites),[27] possibly due to the higher binding of 1. Based on the hypothesis of R. T. Williams involving molecular weight and phenyl rings,[45] significant biliary excretion of 1 was expected. Consequently, a major route of elimination of 1 could be passive diffusion (with perhaps some trapping in feces) from plasma to the intestinal lumen. Recent studies of the toxic organochlorine chlordecone[46] in a patient with a cholecystectomy and in rats illustrate this process. Several other compounds, including bilirubin and dieldrin, and handled by this excretory mechanism.[46,47]

Although a large number of hydantoins have been preparded and tested, only limited structure–activity relationships could be applied to man. The reason is the difficulty in interpreting animal data and applying them to man without sufficient knowledge of the mechanism(s) of action. Various parameters of 1, the inactive compound, are similar to those of DPH, but the bulkiness of the CF_3 groups might prevent a good fit with the receptor. It has been suggested, based on crystallographic evidence, that DPH and diazepam, though not obviously related chemically, have similar molecular conformations and, thus, fit the same receptor.[48] These conclusions have been criticized on the basis of divergent distances between donor atoms and aromatic rings among antiepileptic drugs and ring to donor atom distances.[49] In the anticonvulsant benzenesulfonamide series (which have a smaller molecular volume than DPH), a CF_3 group potentiates activity.[50]

In conclusion, we have demonstrated that compound 1 is not metabolized in the rat due to the deactivating effects of the aromatic CF_3 substituent. The altered pharmacokinetics of 1 compared to DPH include extensive fecal excretion, minimal urinary and biliary excretion, a greatly prolonged plasma $t_{1/2}$, and decreased tissue/plasma ratios. The slow but extensive fecal excretion and insignificant biliary excretion of 1 in the rat suggests a nonbiliary route for diffusion of 1 from the plasma into the gastrointestinal lumen. Although the in vivo distribution characteristics of 1 may have a role in its lack of anticonvulsant activity, it is more likely that the inactivity of 1 and the other DPH analogues **2–9** is due to an unfavorable receptor interaction.

Experimental Section

Melting points (uncorrected) were determined on a Thomas-Hoover Unimelt apparatus. Solvents were removed with a Büchi rotary evaporator, normally at room temperature. Ultraviolet spectra were obtained on a Beckman Model 25 spectrophotometer. pH was measured with a Radiometer 26 pH meter. Thin-layer chromatography (TLC) was performed on Anasil GF prescored glass plates (0.25 mm, Analabs, New Haven, CT). Plates were developed to 15 cm in the following solvent systems: A, benzene–dioxane (5:2, v/v); B, benzene–dioxane–acetone (1:1:1); C, benzene–Et_2O (1:1). Spots were visualized under UV light. Bromobenzotrifluoride (para and meta) and *p*-(trifluoromethyl)benzaldehyde were purchased from PCR, Inc. (Gainesville, FL); *m*-(trifluoromethyl)benzaldehyde and pyridinium chlorochromate were from Aldrich Chemical Co. All aldehydes were distilled under reduced pressure immediately before use.

5,5-Bis[4-(trifluoromethyl)phenyl]hydantoin-*4*-^{14}*C* ([^{14}C]1). A mixture of 4,4′-bis(trifluoromethyl)benzophenone[51] (0.6 g, 1.9 mmol), $(NH_4)_2CO_3$ (0.8 g, 6.9 mmol), KCN (0.2 g, 2.5 mmol), and $K^{14}CN$ (1 mCi, New England Nuclear) was heated in acetamide (8 g) in a sealed tube for 40 h at 135 °C. After the tube cooled, the seal was broken and the contents were suspended in hot water (200 mL). The mixture was acidified with 6 N HCl (hood) and filtered. The solid obtained was triturated with 1 N NaOH. After filtration, crude [^{14}C]1 was precipitated by acidification of the filtrate with 6 N HCl. The product was filtered and recrystallized (EtOH–H_2O), affording white needles (0.55 g, 74%). Radiochemical purity (>99%) was shown by TLC (systems A and B) and isotope dilution. The R_f values of 1 in systems A, B, and C were 0.52, 0.75, and 0.32, respectively. The specific activity of [^{14}C]1 was 0.77 μCi/mg (radiochemical yield from $K^{14}CN$, 41%).

Measurement of Radioactivity. A Beckman Model LS-255 liquid scintillation spectrometer was used to measure ^{14}C (efficiency, 90%). Radioactivity in organic solutions and certain biological fluids (up to 2 mL) was measured directly after adding 15 mL of scintillation fluid (prepared by mixing 3 L of Mallinckrodt Dilufuor and 600 mL of Beckman Biosolv BBS-3). Radioactivity in homogenates of feces and tissues was measured after combustion in a Biological Material Oxidizer (Harvey In-

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Table III. Intravenous Pentylenetetrazol Test of 1 in Mice[a]

dose, mg/kg	time of test, h:	time to first twitch,[b] s				
		24	30	48	72	96
100			23.1	24.3	27.1	27.5
150			28.6	26.7	24.1	23.4
200			29.6	24.5	23.6	34.5
300			22.0	32.4	24.9	
500		27.3		28.7		
750		26.6		71.0[c]		
1000		25.5				

[a] A solution of pentylenetetrazol (0.5% in heparinized saline) was infused (0.34 mL/min) into the tail vein of Carworth Farm no. 1 mice (av wt 20 g) at the designated time after the ip dose of **1**; results represent individual mice. [b] "Normal" time to first twitch is 22.7-29.0 s; "normal" time to tonic extension is 59.5-97.7 s; in all mice given **1**, the time to tonic extension was greater than 240 s. [c] Died before 240 s.

strument Corp., Hillsdale, NJ). The evolved $^{14}CO_2$ was trapped in Harvey Carbon-14 cocktail (counting efficiency, 80%; recovery of standard, 70–90%).

Physicochemical Properties. The partition ratio (K_p, concentration in organic phase/concentration in aqueous phase) of [^{14}C]**1** between peanut oil and Sörensen buffer (pH 7.4) was determined at room temperature.[14] The binding of [^{14}C]**1** to rat plasma proteins was measured by equilibrium dialysis[4] at pH 7.4 and 37 °C. The solubility of [^{14}C]**1** was determined in rat plasma and Sörensen buffer at pH 7.4 and at room temperature.

Dissociation Constant (pK_a). Aliquots (0.25 mL) of an ethanolic solution of [^{14}C]**1** (0.5 mg/mL) were pipetted into 15-mL centrifuge tubes and the EtOH was removed with a N_2 stream (N-Evap, Organomation Associates, Shrewsbury, MA). Two milliliters of *n*-heptane was added to each tube, and to the first tube 0.01 M Tris buffer, pH 7.5 (2.0 mL), was added. To the remaining tubes were added 0.01 M Tris buffers of pH 7.7, 7.9, 8.0, 8.1, 8.2, 8.3, 8.5, 8.7, 8.9, and 9.1, respectively. The tubes were stoppered, shaken vigorously for several minutes, and centrifuged. Aliquots (0.25 mL) of each phase were counted, and the K_p was calculated. Data for K_p and pH were fitted to polynomial equations of degrees 1–5 by using a basic polynomial regression program. Optimum reduction of the sum of squares [K_p(obsd) − K_p(est)] occurred with a third-degree polynomial. The second derivative of this polynomial was set equal to 0 and the value of K_p (at pK_a) was determined. Substitution of this value into the third-order polynomial gave the value of pK_a (pH at the inflection point on the curve).

Disposition of [^{14}C]1 in the Rat. Male Wistar rats (Camm Research Institute, Wayne, NJ) were maintained on Purina Chow and water ad lib and housed in separate metabolic cages. The rats used in experiments A–D weighed between 150 and 330 g. The following experiments were performed.

(A) Four rats received [^{14}C]**1** (10 mg/kg, ip) in propylene glycol (5 mL/kg). Urine and feces were collected for 7 days. Urine was diluted with EtOH and aliquots were counted directly. Feces were homogenized in EtOH (100 mL) in a commercial Waring blender; 0.5-mL aliquots of the homogenate were combusted after drying. For two rats, aliquots (0.1 mL) of the pooled homogenate were counted. Other aliquots (10 mL) were concentrated with a stream of N_2; EtOAc (10 mL) and Sörensen buffer, pH 7.4 (10 mL), were added to the residue. The mixture was shaken for 1 h and centrifuged (2 h, 500*g*), and 0.1-mL aliquots of the organic layer were counted. The aqueous layer was reextracted with EtOAc (10 mL); this resulted in essentially quantitative extraction of ^{14}C (93–100%). Aliquots of the combined extracts were evaporated to dryness. The residues were dissolved in EtOH (0.2 mL) and subjected to radiochromatography in systems A and C; 0.5-cm zones of the silica gel were scraped and transferred (Autozonal Scraper, New England Nuclear) into counting vials, and EtOH (0.5 mL) and scintillation fluid (15 mL) were added. The solutions were allowed to stand for 2 h before counting.

(B) Four rats received [^{14}C]**1** (10 mg/kg, ip) in propylene glycol (5 mL/kg). Urine and feces were collected at 3-day intervals for 18 days. Feces were homogenized in EtOH (250 mL) and 0.5-mL aliquots were combusted. For three rats, the 3-day fecal sample containing the largest percentage of the dose was subjected to isotope dilution analysis as follows: A solution of a known amount of **1** (200–250 mg) in EtOH was added to an aliquot (30 mL) of the homogenate and the mixture was shaken for 1 h. Most of the EtOH was then removed by evaporation (N_2). The residue was shaken (1 h) with 15 mL of 1 N NaOH and centrifuged (1 h, 500*g*). The extraction was repeated with a second 15-mL portion of 1 N NaOH. Aliquots of the combined NaOH extracts were acidified (6 N HCl). The resulting suspension was shaken for 15 min and centrifuged for 1 h (500*g*), and the supernatant was decanted. The residual solid was washed with H_2O (10 mL), dried under vacuum at 60 °C for 18 h, and recrystallized (Norit) from EtOH–H_2O to constant specific activity.

(C) Rats were given [^{14}C]**1** (5 mg/kg, ip) in propylene glycol (2 mL/kg). Blood was collected in heparinized syringes by cardiac puncture after ether anesthesia at 1, 2, 3, 5, and 7 days after administration of [^{14}C]**1**; six animals were studied at each time period. Plasma was separated by centrifugation (500*g*, 30 min), and the radioactivity in the plasma was measured to determine the half-life of elimination ($t_{1/2}$) of ^{14}C.

(D) The tissue distribution of [^{14}C]**1** in rats was studied at 1, 8, and 24 h after an iv dose (5 mg/kg) in propylene glycol–0.1 N NaOH (1:1, v/v). Anesthesia was achieved with sodium pentobarbital (25 mg/kg, ip). For 1- and 8-h studies, the jugular vein was cannulated with Intramedic polyethylene tubing (Clay Adams, Parsipanny, NJ), size PE-50, and the dose solution was administered by slow infusion (2 mL/kg, 0.05 mL/min). For measurements at 24 and 48 h, the dose was administered via tail vein (only plasma ^{14}C levels were measured at 48 h). Blood was collected as above; four animals were studied at each time period.

Selected tissues were removed, rinsed with saline, blotted dry, and weighed. Samples of fat, muscle, and red cells were combusted directly. Other organs (liver, intestine, kidneys, lungs, heart, and brain) were homogenized in EtOH (Brinkman Polytron homogenizer) and 0.5-mL aliquots were combusted. The total volume of the homogenate was 100 mL for liver and intestine and 25 mL for all other tissues.

(E) The biliary excretion of [^{14}C]**1** was studied in three rats (340–390 g). After anesthesia (as above), the jugular vein and common bile duct were cannulated with polyethylene tubing (sizes PE-50 and PE-10, respectively). Body temperature was monitored with a rectal thermometer and maintained at 37–38 °C by using a heating pad. Fifteen minutes after completion of surgery, [^{14}C]**1** (5 mg/kg) was administered (3–5 min infusion) through the jugular cannula; the vehicle was propylene glycol–ethanol–0.1 N NaOH (4:1:5, v/v, 1 mL/kg). Bile collections were for two 30-min periods and then for 1-h intervals up to 4 h. Aliquots (50 μL) of bile were counted, and the remaining sample was diluted with EtOH to 3 mL; 0.5-mL aliquots were counted for volume determination. Heparinized blood samples (0.3 mL) were withdrawn at 0.5, 1, 2, 3, and 4 h postinfusion; 50-μL aliquots of plasma were counted.

Toxicity Studies. The LD_{50} of **1** was determined in male Swiss Webster mice (20–40 g, Camm Research Institute). Compound **1** was administered at doses of 10, 20, 50, 100, and 200 mg/kg (ip) and 100 and 200 mg/kg (po) in propylene glycol (10–20 mL/kg). Ten mice were used at each dosage level, and the animals were observed for toxicity for 1 week.

The neurotoxicity of compounds **1**–**9** was evaluated by the rotorod ataxia test[10] (performed by the ADD program) in Carworth Farm No. 1 mice. The compounds were administered ip in aqueous 30% polyethylene glycol (PEG) 400.

Calculations. Statistical calculations were performed on a Olivetti Underwood Programma 101 computer using standard programs. Values for plasma $t_{1/2}$ were obtained from a basic computer program using the method of least squares. Both one and two compartment open models were considered.

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