ed seven times with 250 ml of 20% benzene–petroleum ether. The solvent was removed *in vacuo* from the combined extracts to yield 11.2 g of material. The crude extract was dissolved in 50 ml of benzene and placed on a chromatography column (2.5 cm diameter × 55 cm long, neutral alumina, activity I). The column was eluted with, in order, petroleum ether, 20% benzene–petroleum ether, 40% benzene–petroleum ether, and pure benzene (200 ml/fraction).

Fractions 1–7 of the 40% benzene–petroleum ether elution were purified by preparative TLC [9.75 mm thickness, silica gel GF-254, acetone–benzene–chloroform (1:2:17) as the solvent, and chloroform as the extracting solvent]. Lupeol was obtained as a white crystalline solid (11 mg), mp 206–208° [lit. (7) mp 215–216°]. The IR and mass spectra of lupeol were completely in agreement with those of authentic lupeol.

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Potential Organ or Tumor Imaging Agents XV: Radioiodinated Phenytoin Derivatives

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Abstract □ Three radioiodinated phenytoin analogs were synthesized, and tissue distribution studies were conducted in rats. Except for a greater retention of radioactivity following administration of the radioiodinated derivatives, the tissue distribution patterns were qualitatively similar to those found for ^{14}C phenytoin. In nearly all cases, the adrenals, heart, kidneys, and liver displayed the greatest capacity to retain radioactivity. The high uptake of radioactivity observed for the thyroid was attributed to *in vivo* metabolic deiodination of the radioiodinated derivatives.

Keyphrases □ Phenytoin—radioiodinated derivatives, potential tumor imaging agents, tissue distribution studies, rats □ Radiopharmaceuticals—radioiodinated phenytoin derivatives, potential tumor imaging agents, tissue distribution studies, rats □ Tissue distribution—radioiodinated phenytoin derivatives, potential tumor imaging agents, rats □ Tumor imaging agents—radioiodinated phenytoin derivatives, tissue distribution studies, rats

In the search for radiopharmaceuticals that will selectively concentrate in various tumors and organs, several reports concerning the tissue distribution of phenytoin were of interest. Although early studies with this drug focused on only a few tissues, the concentration of phenytoin in the liver, kidneys, skeletal muscle, and brain was found to exceed plasma levels (1–3). Moreover, higher concentrations of phenytoin were reported in human primary brain tumors than in normal brain tissue (4).

More recently, autoradiographic studies (5) revealed that the concentration of phenytoin in the brain of cats was one to three times greater than in plasma at 0.5–6 hr following an intravenous dose. Similar studies in pregnant mice (6) revealed an intense and persistent concentration of phenytoin in the heart. This finding suggested that the antiarrhythmic action of phenytoin may be related to its affinity for myocardial tissue.

Several ^{11}C-hydantoins were synthesized (7), and an accumulation of radioactivity in the liver and, to a lesser extent, heart was noted within 5–10 min following intravenous administration to dogs. The uptake was not specific for cardiac muscle, however, since skeletal muscle contained a comparable concentration of radioactivity. Radioactivity was also high in the pancreas and mesenteric fat.

The purposes of the present study were to synthesize several radioiodinated analogs of phenytoin and to evaluate their potential utility as brain or myocardial scanning agents. Although several radiopharmaceuticals are currently employed in nuclear medicine for localization of brain tumors, these agents do not concentrate selectively in brain tissue or tumors. Instead, their selective localization arises from their passive diffusion from the bloodstream to the tumor as a result of a breakdown in the blood–brain barrier (8). As a result, positive uptake of radiopharmaceuticals is shown by hematomas, abscesses, and infarcts as well as tumors. A more tumor-specific agent is needed. Moreover, no radiopharmaceutical is currently available for imaging the myocardium, but several are undergoing evaluation for this purpose.

Since none of the elements of phenytoin has a useful γ-emitting nuclide, it was thus necessary to intro-

Table I—Distribution of Radioactivity in Rats at 2 hr following Subcutaneous Administration of ^{14}C-Phenytoin and ^{125}I-Phenytoin Analogs[a]

Tissue	^{14}C-Phenytoin	^{125}I-III*a*	^{125}I-III*b*	^{125}I-III*c*
Adrenal	0.39 ± 0.02	1.52 ± 0.10	0.35 ± 0.06	1.20 ± 0.16
Brain	0.14 ± 0.01	0.36 ± 0.04	0.05 ± <0.01	0.55 ± 0.10
Blood	0.15 ± 0.01	0.40 ± 0.05	0.08 ± 0.01	0.36 ± 0.07
Heart	0.26 ± 0.01	1.02 ± 0.09	0.43 ± 0.04	0.76 ± 0.13
Kidney	0.37 ± 0.02	0.88 ± 0.05	0.23 ± 0.02	0.77 ± 0.10
Liver	0.48 ± 0.03	1.66 ± 0.14	0.33 ± 0.01	1.08 ± 0.16
Lung	—	0.81 ± 0.09	0.18 ± 0.01	0.78 ± 0.13
Ovary	0.21 ± 0.01	0.88 ± 0.05	0.19 ± 0.02	0.69 ± 0.13
Pancreas	0.29 ± 0.01	0.74 ± 0.07	—	—
Pituitary	0.26 ± 0.02	0.70 ± 0.07	0.16 ± 0.01	0.72 ± 0.14
Thyroid	—	1.40 ± 0.12	0.87 ± 0.04	4.74 ± 0.31

[a] Values are expressed as the mean percentage of administered dose per gram of tissue ± *SEM* from three rats. The percent dose per gram was calculated as follows:

$$\frac{\text{dpm/mg} \times 1000 \times 100}{2.22 \times 10^6 \times \mu\text{Ci administered dose}}$$

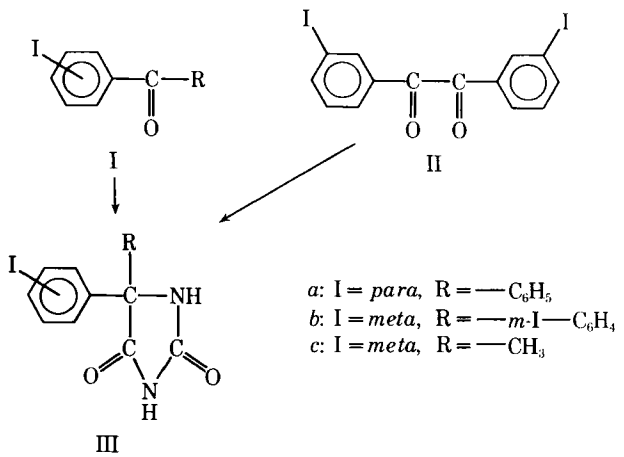
duce this feature into the molecule. Three iodinated analogs of phenytoin were synthesized for radiolabeling (Scheme I). Compounds III*a* and III*b* were prepared by standard methods for the synthesis of hydantoins, and III*c* had been synthesized previously in this laboratory (9).

Iodine-125 was selected as the radiolabel for preliminary studies because its half-life (60 days) and low radiation energy (35 kev) simplified synthesis and storage. Radioiodine was incorporated at the final synthetic step by heating the iodinated analogs with ^{125}I-sodium iodide in ethylene glycol at temperatures above 165°. Each radioiodinated analog was purified by recrystallization and gave a single peak of radioactivity when analyzed radiochromatographically.

EXPERIMENTAL

Preparation of 3,3′-Diiodobenzil (II)—A solution of *m*-iodobenzaldehyde (2.3 g, 0.01 *M*) and potassium cyanide (0.2 g) in ethanol–water (1:1, 25 ml) was refluxed for 1.5 hr. The mixture was cooled, water (25 ml) was added, and the solution was extracted with ether. The ether extract was dried (sodium sulfate) and evaporated. The residual crude oil was dissolved in pyridine (20 ml) and water (10 ml), and cupric sulfate pentahydrate (2.0 g) was added.

The mixture was refluxed for 2 hr, allowed to cool, and poured into water. Ether extraction and workup as already described afforded II (0.5 g, 11%). Recrystallization from ethanol gave the purified compound, mp 133–134°. The IR and NMR spectra were as expected.



Scheme I—Synthesis of iodinated analogs of phenytoin

Anal.—Calc. for $C_{14}H_8I_2O_2$: C, 36.36; H, 1.73. Found: C, 36.55; H, 1.93.

Preparation of 5-Phenyl-5-(*p*-iodophenyl)hydantoin (III*a*)—A mixture of 50% ethanol (10 ml), *p*-iodobenzophenone[1] (250 mg), sodium cyanide (50 mg), acetamide (93 mg), and ammonium carbonate (250 mg) was placed in an ampul. The ampul was sealed and kept in an autoclave at 140–150° for 7 days. Then the ampul was broken, and the contents were extracted with 10% NaOH solution. The clear alkaline solution was made slightly acidic with 10% H_2SO_4. The solid which separated was collected, washed with water, and recrystallized from methanol–water to give pure III*a* (60 mg, 28%), mp 235–237° dec. The IR and NMR spectra were as expected.

Anal.—Calc. for $C_{15}H_{11}IN_2O_2$: C, 47.60; H, 2.91. Found: C, 47.75; H, 3.03.

Preparation of 5,5-Di(*m*-iodophenyl)hydantoin (III*b*)—Sodium (0.2 g) was dissolved in ethanol (15 ml), and urea (0.1 g) was added to the boiling solution. Small portions of II (0.2 g) were added, and the mixture was refluxed for 50 min. Ethanol was removed by distillation, water (5 ml) was added, and the solution was refrigerated. Acidification of the solution afforded a white precipitate which, after collection and recrystallization from methanol, gave pure III*b* (0.11 g, 30%), mp 245–248°. The IR and NMR spectra were as expected.

Anal.—Calc. for $C_{15}H_{10}I_2N_2O_2$: C, 35.71; H, 1.98. Found: C, 35.99; H, 2.25.

Preparation of Radioiodinated Hydantoins—A solution of ^{125}I-sodium iodide (4 mCi) in water (0.3 ml) was added to a solution of III*a* (50 mg) in ethylene glycol (2.0 ml). The solution was heated with stirring at 165° for 28 hr under a nitrogen atmosphere. Then the solution was allowed to cool, water (20 ml) was added, and the mixture was extracted with ether. The ether extract was carefully washed with water to remove ^{125}I and dried (sodium sulfate). Removal of the solvent *in vacuo* and trituration of the resulting residue with a small amount of water gave essentially pure ^{125}I-III*a* in crystalline form.

Further purification was achieved by recrystallization from ethanol–water to give 30 mg with a specific activity[2] of 20 μCi/mg. TLC using two solvent systems, benzene–ethyl acetate (2:1) and benzene–ethanol (4:1), displayed a single radioactive peak on scanning (R_f 0.34 and 0.40, respectively). The peak was coincident with the single spot observed visually. Treatment of III*b* (100 mg) with ^{125}I-sodium iodide (3 mCi) in a similar manner gave 60 mg of ^{125}I-III*b* with a specific activity of 13.3 μCi/mg, R_f 0.73 and 0.85 in benzene–ethyl acetate (2:1) and benzene–ethanol (4:1), respectively.

Similarly, treatment of III*c* (9) (80 mg) with ^{125}I-sodium iodide (6 mCi) gave 27 mg of ^{125}I-III*c* with a specific activity of 5.5 μCi/mg, R_f 0.52 and 0.60 in benzene–ethyl acetate (2:1) and benzene–ethanol (4:1), respectively.

[1] Prepared according to the method of Koopal (10).

[2] Determined using Picker isotope calibrator, Picker Nuclear, White Plains, N.Y.

Table II—Tissue to Blood Ratios 2 hr Postinjection of ^{14}C-Phenytoin and ^{125}I-Phenytoin Analogs

Tissue	^{14}C-Phenytoin	^{125}I-III*a*	^{125}I-III*b*	^{125}I-III*c*
Adrenal	2.8 ± 0.11	3.94 ± 0.60	4.42 ± 0.53	3.39 ± 0.26
Brain	0.98 ± 0.03	0.92 ± 0.09	0.59 ± 0.08	1.51 ± 0.05
Heart	1.83 ± 0.02	2.59 ± 0.21	5.48 ± 0.86	2.12 ± 0.08
Ovary	1.51 ± 0.06	2.24 ± 0.14	2.38 ± 0.15	1.90 ± 0.15
Pancreas	2.03 ± 0.03	1.89 ± 0.24	—	—

Tissue Distribution Studies—All subjects were Spartan Sprague–Dawley female rats, 190–275 g. The animals were housed in air-conditioned quarters under normal lighting conditions. Tap water and food[3] were available *ad libitum*.

The hydantoins were dissolved in dimethyl sulfoxide and administered subcutaneously into the nape of the neck using a 1-ml tuberculin syringe and a 23-gauge 2.54-cm (1-in.) hypodermic needle. Three rats were used for each evaluation, and the doses were as follows: ^{14}C-phenytoin[4], 7 μCi/0.23-ml injection, specific activity 19.3 μCi/mg; III*a*, 30 μCi/0.30-ml injection, specific activity 20 μCi/mg; III*b*, 26 μCi/0.20-ml injection, specific activity 13.3 μCi/mg; and III*c*, 20 μCi/0.30-ml injection, specific activity 5.5 μCi/mg.

The subjects were anesthetized with ether in a desiccator jar and sacrificed by exsanguination from the heart ventricle at 0.25, 2, and 24 hr postinjection. The major organs such as brain, heart, kidneys, liver, lungs, pancreas, spleen, and stomach were excised, washed thoroughly with isotonic saline to remove blood, and minced with surgical scissors to a paste-like consistency. Smaller organs such as adrenals, ovaries, pituitary, and thyroid were weighed intact.

Several weighed samples of minces, heparinized blood, and the smaller organs were placed in microfuge tubes and radioassayed[5] using a tetrabutyltin liquid scintillation cocktail (11). Counts were accumulated to attain a 3% counting error with 95% confidence intervals. All counts were corrected for system efficiency using calibrated ^{125}I-sodium iodide solution[6] or liquid scintillation ^{14}C quenched standards[7].

RESULTS AND DISCUSSION

The tissue distribution of radioactivity at 2 hr after administration of radioiodinated hydantoins is compared with that shown for ^{14}C-phenytoin (Table I). In all cases, high levels of radioactivity were found in the liver, kidneys, and heart, as expected from previous studies.

Although not previously noted by other investigators, the adrenal gland also demonstrated high levels of radioactivity. No attempt was made to ascertain whether the radioactivity resided mainly in the cortex or the medulla. However, it is believed to be the former on the basis that other polycyclic aromatic compounds such as 1,1-dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane are taken up well by the adrenal cortex (12, 13). Despite the known hyperglycemic action of phenytoin and its ability to impair secretion of insulin from pancreatic β-cells (14, 15), there appeared to be no extraordinary uptake of radioactivity by the pancreas following administration of ^{14}C-phenytoin or the radioiodinated derivative of III*a*.

While there were quantitative differences among all compounds, the adrenal to blood ratios for the radioiodinated hydantoins were very close to one another and ranged between 3.4 and 4.4. These values were slightly higher than those shown for phenytoin itself. The tissue to blood ratios at 2 hr for the adrenal and other organs of interest appear in Table II. Except for minor differences, all radiolabeled phenytoin derivatives displayed a similar tissue distribution pattern.

The tissue distribution of only ^{14}C-phenytoin was examined at 15 min following administration. In contrast to previous studies in cats (5), no selective uptake of radioactivity was noted for the brain. Moreover, the general distribution pattern was similar to that noted in the 2-hr experiments.

Distribution analysis at 24 hr demonstrated that radioactivity associated with ^{14}C-phenytoin had essentially cleared from most tissues by this time. Similar analysis of the radioiodinated hydantoins, however, revealed only a modest decrease in radioactivity associated with the tissues. The increase in lipid solubility caused by introduction of iodine could account for the apparent increase in retention for the radioiodinated hydantoins.

Despite the ability of the radioiodinated hydantoins to concentrate in the heart, the heart to blood ratio was not sufficiently encouraging to pursue these agents as potential myocardial scanning agents, particularly in light of recent studies showing the selective localization of certain radioiodinated bretylium analogs in myocardial tissue (16, 17).

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