Iodine Heterocycles: [^{125}I] and [^{131}I] Ortho-Iodosophenylphosphoric Acid

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Since organic molecules tagged with radioiodine are often subject to dehalogenation, techniques are needed for "protecting" the iodine. A suggested approach was the incorporation of iodine directly into a heterocyclic compound as one of the ring's heteroatoms. Such a compound, orthoiodosophenylphosphoric acid, was synthesized with I-125 and I-131. Upon i.v. administration to dogs and rabbits, most of the radiolabel was excreted in the urine. There was no evidence of the appearance of free iodide. The renal elimination of orthoiodosophenylphosphoric acid was contrasted with the biliary excretion of another iodine heterocycle, diphenyleneiodonium. Iodine heterocycles, with appropriate substituents, may represent a useful class of compounds for biologic studies.

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Iodination of molecules has been a major tool for the introduction of a radioactive label. The iodine, however, is often in an exposed position, leaving the compound subject to chemical or enzymatic dehalogenation. To circumvent this, some investigators have placed the iodine in a more "protected," or less rapidly attacked, position on aliphatic or aromatic molecules—for example, it may be attached at the terminal end of a fatty acid (*1*), or in 19-iodocholesterol (*2*). There is, however, an additional approach to placement of the iodine in a invulnerable position. This involves placing iodine *in* the ring of the molecule, rather than as a substituent *on* the ring. In other words, a heterocyclic compound is produced, with iodine as one of the heteroatoms. Initial studies with the iodine heterocycle diphenyleneiodonium revealed rapid hepatic accumulation and biliary excretion of this hypoglycemic agent (*3*). We have extended the study of iodine heterocycles to a second compound, ortho-iodosophenylphosphoric acid (OIPA), which contains the grouping –I–O–P–O (Fig. 1).

MATERIALS AND METHODS

Compound preparation. The OIPA was synthesized with both I-131 and I-125, by the methods of Leffler and Jaffe (*4*). The essential steps were the radioiodination of ortho-iodophenol, the phosphorylation of the hydroxyl group by $POCl_3$, and cyclization of the phosphoric acid and iodine moieties by peracetic acid. The orthoiodophenol was labeled via exchange with $Na^{131}I$ or $Na^{125}I$ by reflux in alkalinized acetone. The radiolabeled ortho-iodophenol was purified by chromatography on a thin-layer silica plate with benzene as the solvent. The radioactive spot was extracted from the plate with petroleum ether (b.p. 30–60°C) and filtered. After drying, it was dissolved in benzene, and the reported synthetic method was followed after addition of carrier ortho-iodophenol.

Animal studies. The initial syntheses, with I-131, were used for preliminary studies on two mice. Subsequent studies of OIPA were performed with the I-125-labeled compound. Six rabbits (3 Kg) and two dogs (25 Kg) were studied by i.v. administration of labeled OIPA in saline. Enough carrier was added to administer 4.5 μg/g body weight in dogs and rabbits, and 0.33 and 0.17 μg/g in rabbits (two rabbits for each quantity). Blood samples were obtained from the rabbits up to 2 hr. Since the related compound, diphenyleneiodonium, is a known hypoglycemic agent (*5*), blood specimens were examined for their glucose content by the Health Center Labora-

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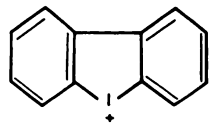
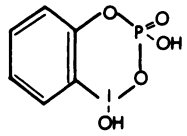
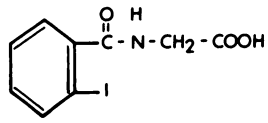


DIPHENYLENEIODONIUM



ORTHO - IODOSOPHENYLPHOSPHORIC ACID



ORTHO - IODOHIPPURIC ACID

FIG. 1. Structural formulae of compounds related to ortho-iodosophenyl-phosphoric acid.

TABLE 1. BLOOD GLUCOSE DETERMINATIONS AFTER I.V. ADMINISTRATION OF [^{131}I] OIPA THE COMPOUND WAS NOT HYPOGLYCEMIC

		Blood glucose mg/dl
Rabbit 1	Before experiment	97
	Mean of values at 10, 30, 130 min	92
Rabbit 2	Before experiment	95
	Mean of hourly blood samples (to 5 hr)	128
Two dogs	Before experiment (mean)	90
	Mean of blood samples at 1 and 2 hr	105

tory, to determine whether OIPA had lowered the blood glucose level. Urine was collected in the dogs by catheter, and in the rabbits by aspiration of the bladder and by counting a mulch made from absorbent paper that had collected voided urine. Postsacrifice blood, urine, and tissue samples were weighed and radioactivity determined in a well counter. An internal standard was used to correct for dependence on the sample size. By comparison with radioactive standards, both the percentages of dose per organ and dose per gram of tissue were calculated. Urine specimens were also chromatographed on thin-layer silica gel using benzene-methanol (50:50 v/v).

RESULTS

Compound. Thin-layer silica-gel chromatography with benzene as the solvent showed that ortho-iodophenol had an R_f of 0.59, as judged by the absorption of ultraviolet light (254 nm). After exchange with $Na^{131}I$, the major radioactive spot had an identical R_f value. After conversion to OIPA, the compound had a melting point of 143°C and gave the following elemental analysis (percent).

	Calculated	Found
C	22.81	22.86
H	1.91	1.94
P	9.80	9.90
I	40.16	40.28

Vapor-pressure measurement of 65°C showed that the compound was a monomer and had not polymerized. Specific activity of the [^{125}I] OIPA was 50 μCi/mg. No decomposition was noted after 3 months of storage at 5°C in the dark. When stored at room temperature, a slight discoloration developed after several weeks. The solubility of the compound in saline was less than 0.8% at room temperature.

Animal studies. In dogs and rabbits, OIPA was not hypoglycemic (Table 1). Chromatography of urine from injected dogs and rabbits did not reveal any radioactivity corresponding to free iodide. In two rabbits kept for 5 hr after i.v. administration of OIPA, 98% of the injected radioactivity was recovered in the urine. In two catheterized dogs, over 80% of the activity was recovered from the bladder in 4 hr.

In two mice given 0.1 mg of the [^{131}I] OIPA intraperitoneally, the radioactivity present at 2 hr in various organs was as follows: liver, 6.9%; spleen, 0.3%; kidneys, 1.8%; heart, 0.15%; lungs, 0.35%; and blood, 9%. In dogs and rabbits, the blood disappearance curves were complex, with a rapid ini-

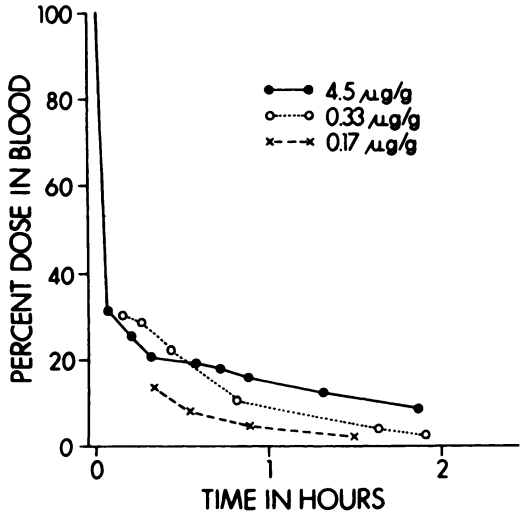


FIG. 2. Blood clearance of radioactivity in rabbits after i.v. administration of [^{125}I] OIPA (mean of two rabbits at each point). The three levels of carrier used are shown.

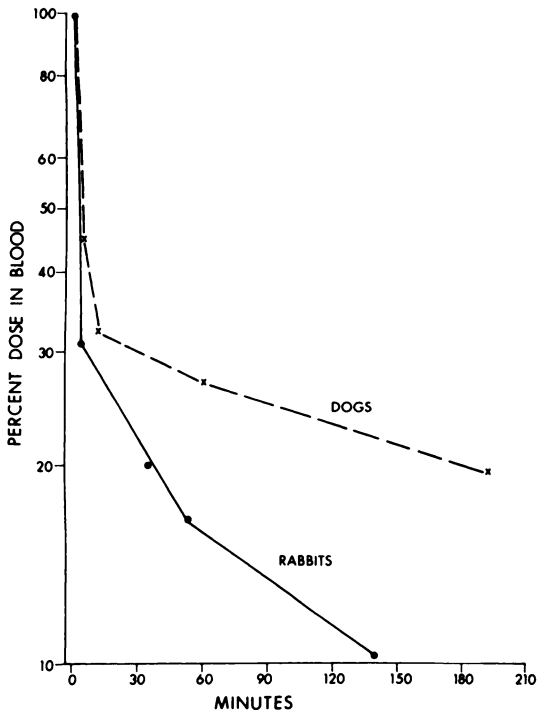


FIG. 3. Blood radioactivity (semilog) in two dogs and two rabbits after i.v. administration of [^{125}I] ortho-iodosophenylphosphoric acid. The dogs had 0.33 μg carrier/g body weight, the rabbits 4.5 μg/g.

tial component (Figs. 2 and 3). The tissue distribution of radioactivity is shown in Tables 2 and 3. Chromatography in silica gel (benzene:methanol, 50:50 v/v) showed OPIA to have an R_f of 0.5, whereas iodide migrated near the solvent front. Chromatograms of urine did not show a spot corresponding to free iodide.

DISCUSSION

Since the iodine heterocycle diphenyleneiodonium is known to be a hypoglycemic agent, we used OIPA in milligram amounts to determine whether it, too, would lower blood glucose levels. There was no resulting hypoglycemia in dogs or rabbits. The compound passed into the urine without the appearance of free iodide. The nearly quantitative accumulation of radioactivity in urine meant that there was little concentration in tissues (Tables 2 and 3). In dogs there was slight accumulation of OIPA in the bile. This may bear an analogy to the major biliary excretion of another iodine heterocycle, diphenyleneiodonium (*3*). The rapid renal excretion of OIPA alerts us to the structural resemblance between OIPA and another compound undergoing renal transport, ortho-iodohippuric acid (Fig. 1). However, it is still uncertain whether OIPA is secreted by a tubular mechanism or whether it can be used to evaluate renal function.

The apparent biologic inertness of the two heterocyclic iodine compounds—diphenyleneiodonium and ortho-iodosophenylphosphoric acid—suggests that other heterocycles may form the basis for synthesizing diagnostic or therapeutic compounds by introduction of relevant substituents. That is, appropriate heterocyclic rings might substitute for biologically important structures when appropriate side groups are introduced. Alternatively, additional iodine heterocycles themselves might be found to be useful radiotracers.

ACKNOWLEDGMENT

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TABLE 2. DISTRIBUTION OF RADIOACTIVITY IN TWO RABBITS SACRIFICED 5 HR AFTER I.V. [^{125}I] ORTHO-IODOSOPHENYLPHOSPHORIC ACID (0.17 μg/g BODY WEIGHT)

	Percentage of administered dose	
Organ*	Per gram of tissue	Per organ
Urine	1.154	98.0
Kidneys	0.0037	0.07
Liver	0.0057	0.08
Heart	0.0012	0.01
Spleen	0.0017	0.002
Lung	0.0011	0.01

* Negligible activity in: pancreas, bone, abdominal contents, skin, thyroid, adrenals, or gallbladder bile.

TABLE 3. DISTRIBUTION OF RADIOACTIVITY IN TWO FEMALE DOGS, 4 HR AFTER I.V. ADMINISTRATION OF [^{125}I] ORTHO-IODOSOPHENYLPHOSPHORIC ACID (4.5 μg/g BODY WEIGHT)

	Dose percentage	
	Per gram	Per organ
Liver	0.0121	3.6
Bile (gallbladder)	0.2949	5.3
Spleen	0.0065	1.6
Stomach	0.0102	1.2
Lungs	0.0148	1.6
Heart	0.0063	0.5
Pancreas	0.0061	0.4
Kidneys	0.0057	0.3
Thigh muscle	0.002	—
Femur	0.001	—
Marrow (femur)	0.0018	—
Ovaries	0.0164	0.04
Duodenum (+ contents)	0.009	—
Urine	1.033	80.

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