Metabolic Fate of *N*-γ-Phenylpropyl-*N*-benzyloxy Acetamide (W-1372) in Rats, Dogs, and Monkeys

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Abstract □ The absorption, distribution, and metabolic fate of *N*-γ-phenylpropyl-*N*-benzyloxy acetamide (W-1372) was studied in the rat, dog, and monkey. W-1372-^{14}C was readily absorbed by all three species. Following an oral dose, peak blood levels of radioactivity were attained within 1 hr. in the monkey and in 2 hr. in the dog. The blood half-life of radioactivity following oral administration was 11.5 hr. in the dog. In the rat, maximum blood concentration of ^{14}C was reached 1 hr. following intraperitoneal administration, and the half-life was about 2 hr. Distribution studies of labeled drug in the rat indicate that the administered radioactivity is largely excreted within 24 hr. The major metabolites of W-1372 are hippuric acid, benzoic acid, *N*-γ-phenylpropyl-*N*-benzyloxyamine, and carbon dioxide.

Keyphrases □ *N*-γ-Phenylpropyl-*N*-benzyloxy acetamide (W-1372)—metabolism □ Absorption, distribution, metabolic fate—W-1372-^{14}C □ Inverse isotope dilution—analysis □ Hippuric acid, benzoic acid, *N*-γ-phenylpropyl-*N*-benzyloxyamine, carbon dioxide—W-1372-^{14}C metabolites □ TLC—identification □ IR spectrophotometry—identification

N-γ-Phenylpropyl-*N*-benzyloxy acetamide (W-1372) is a new hypolipidemic agent that has been shown by Berger *et al.* (1, 2) to reduce the extent of atherosclerotic lesions and to lower serum cholesterol levels in animals maintained on a high-cholesterol diet. This manuscript describes studies from this laboratory on the absorption, distribution, and metabolic fate of W-1372 in the rat, dog, and squirrel monkey.

METHODS

Preparation of *N*-γ-Phenylpropyl-*N*-benzyloxy-benzyl-7-^{14}C Acetamide (W-1372-Benzyl-^{14}C)—*N*-γ-Phenylpropyl-*N*-hydroxy acetamide, 965 mg., was added to a solution of 115 mg. of sodium in 10 ml. of anhydrous ethanol. After 2 mc., 633 mg., of benzyl chloride-7-^{14}C was added, the reaction mixture was heated to reflux for 4 hr. and left at room temperature overnight. The solvent was removed *in vacuo* and the residue was stirred with 40 ml. of water and 40 ml. of ethyl ether. The organic phase was separated and the aqueous phase was again extracted with an equal volume of ether. The organic phases were combined and dried over anhydrous sodium sulfate. After removal of the solvent, the residue was molecularly distilled (90°/0.004 mm.) to yield 681 mg. of W-1372-benzyl-^{14}C, specific activity 1.69×10^6 d.p.m./mg.

The product gave only one radioactive spot in TLC in methanol–acetone–acetic acid (95:95:10), and its IR spectrum was identical to that of authentic W-1372.

Preparation of *N*-γ-Phenylpropyl-*N*-benzyloxy Acetamide-acetyl-1-^{14}C (W-1372-Acetyl-^{14}C)—Acetyl chloride-1-^{14}C, 1.0 mc., 791 mg., was added to a solution of 4.84 g. of *N*-γ-phenylpropyl-*N*-benzyloxyamine in 20 ml. of ethyl ether. The reaction mixture was heated to reflux for 30 min., and the solid which formed, *N*-γ-phenylpropyl-*N*-benzyloxyamine hydrochloride, was removed by filtration. The filtrate was washed consecutively with 10 ml. of 0.1 *N* hydrochloric acid, 10 ml. of 0.1 *N* sodium hydroxide, and 10 ml. of water. The remaining organic phase was dried and molecularly distilled, as previously described, to yield 2.73 g. of W-1372-acetyl-^{14}C, specific activity 8.24×10^5 d.p.m./mg. Its purity was verified by TLC as previously described.

Absorption and Blood Concentration—Three male Sprague-Dawley rats, weighing about 200 g. each, received intraperitoneally a solution containing 40 mg. of W-1372-benzyl-^{14}C in 0.5 ml. of polyethylene glycol 400. Blood samples were taken from the tail vein at appropriate intervals and assayed for radioactivity by liquid scintillation counting.[1]

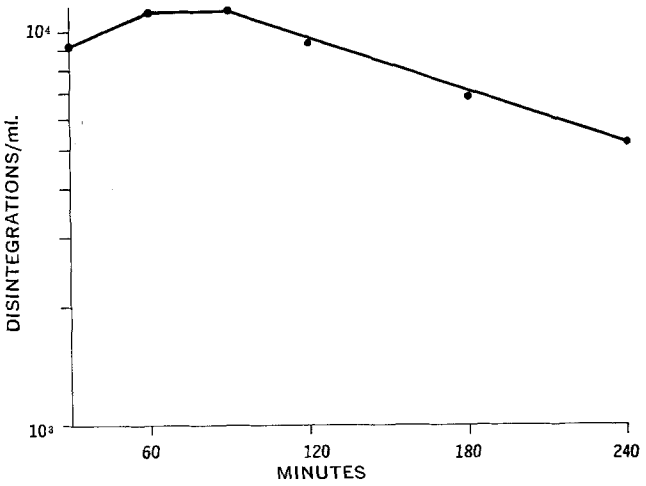

Figure 1—*Blood radioactivity after intraperitoneal administration of W-1372-benzyl-^{14}C to the rat.*

A male beagle hound, weighing 11 kg., was given a capsule containing 1.258 g. of W-1372-benzyl-^{14}C. Blood samples were taken from the vein to the toe nail at intervals and assayed for radioactivity.

Two 800-g., male squirrel monkeys received, by stomach tube, 50.8 mg. of W-1372-benzyl-^{14}C dissolved in 2.0 ml. of polyethylene glycol 400. At appropriate intervals, blood was removed from the jugular vein and assayed for radioactivity.

Tissue Distribution in the Rat—A male Sprague-Dawley rat, weighing 150 g., received intraperitoneally 42.2 mg. of W-1372-benzyl-^{14}C dissolved in 1.0 ml. of polyethylene glycol 400. Urine and feces were collected for 24 hr. The animal was then sacrificed and the organs were excised. The tissues and the remainder of the carcass were dissolved in 6 volumes of 1 *M* hydroxide of hyamine (Packard Instrument Co., Downers Grove, Ill.) by digesting for 18 hr. at 50°. An aliquot of each solution was taken and assayed for radioactivity in a liquid scintillation spectrometer.

A second rat, weighing 180 g., was given intraperitoneally a solution containing 51.2 mg. of acetyl-labeled W-1372 in 1.0 ml. of propylene glycol. The animal was placed in a metabolism chamber for 24 hr. and then sacrificed. The tissues were processed and analyzed for radioactivity. The carbon dioxide of respired air was trapped in 2.5 *M* sodium hydroxide, and the radioactivity of an aliquot of this solution was measured.

Inverse Isotope Dilutions—Typical inverse isotope dilution experiments were carried out as follows: a male Sprague-Dawley rat, weighing about 200 g., received intraperitoneally 12.7 mg. of W-1372-benzyl-^{14}C dissolved in 1.0 ml. of polyethylene glycol 400. Urine was collected for 24 hr. and its radioactivity measured. Isotope dilutions were carried out on aliquots of urine as follows.

1. Nonradioactive hippuric acid, 1000 mg., was added to the first 1.0-ml. aliquot, followed by the addition of 20 ml. of water and

[1] The scintillating fluid consisted of a solution of 7 g. of 2,5-diphenyloxazole, 250 mg. of 1,4-bis-2-(4-methyl-5-phenyloxazoyl)benzene, and 125 g. naphthalene in 1 l. dioxane.

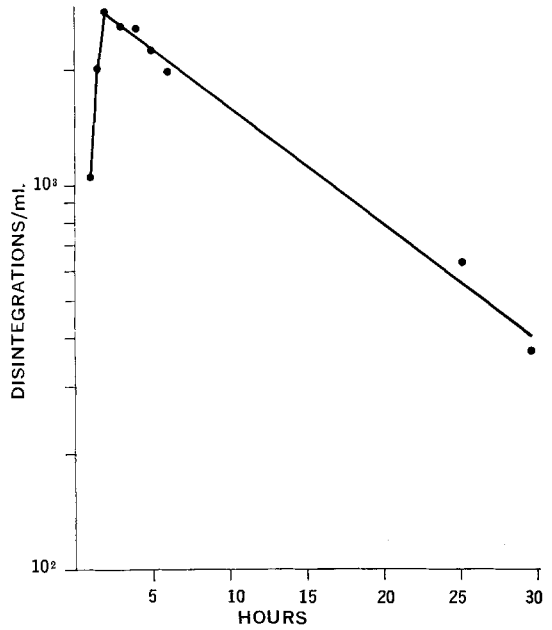

Figure 2—*Blood radioactivity after oral administration of W-1372-benzyl-^{14}C to the dog.*

sufficient 50% sodium hydroxide to yield a clear solution. Acidification of the mixture with concentrated hydrochloric acid precipitated the hippuric acid. The solid was removed and recrystallized from water to constant specific activity; m.p. 189–190°.

2. Nonradioactive benzoic acid, 1000 mg., was added to a second 1.0-ml. aliquot of urine and the mixture was diluted with water and heated until homogeneous. Upon cooling, benzoic acid crystallized and was removed by filtration. It was recrystallized from water to constant specific activity; m.p. 121–122°.

3. Nonradioactive *N*-γ-phenylpropyl-*N*-benzyloxyamine hydrochloride, 1000 mg., was added to a 2.0-ml. aliquot of urine. A few drops of concentrated hydrochloric acid were added, followed by sufficient hot ethanol to dissolve the solid. Water, 100 ml., was

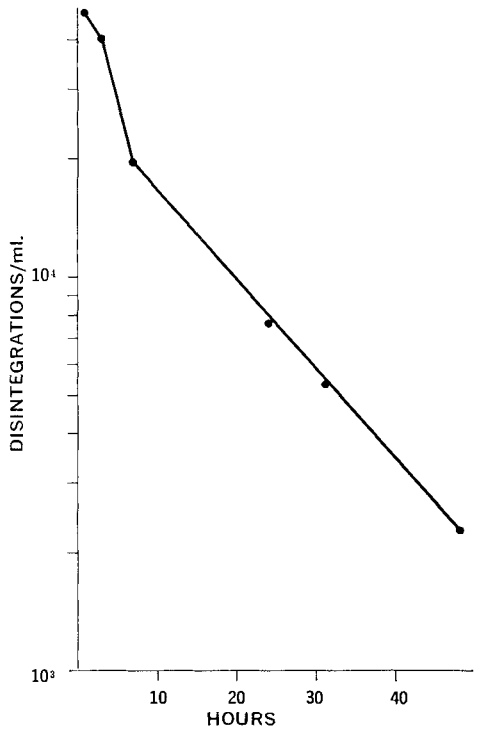

Figure 3—*Blood radioactivity after oral administration of W-1372-benzyl-^{14}C to the squirrel monkey.*

Table I—Tissue Distribution of ^{14}C in a Rat 24 hr. after Intraperitoneal Administration of 42.2 mg. W-1372-Benzyl-^{14}C

Organ	Radioactivity Recovered— d.p.m. × 10^3	Radioactivity Recovered— %
Urine	39,100	54.7
Carcass	23,250	32.7
Stomach and intestines	7,980	11.1
Liver	1,135	1.6
Kidney	661	0.9
Lung	71.4	0.1
Heart	27.9	0.04
Spleen	17.4	0.02
Total		101.3

added, and the mixture was made alkaline with 25 ml. of 10% sodium hydroxide and extracted with two 150-ml. portions of ether. The ether phase was dried over anhydrous sodium sulfate, and hydrogen chloride gas was bubbled into the ether solution to precipitate the hydrochloride of *N*-γ-phenylpropyl-*N*-benzyloxyamine. The solid was separated and recrystallized from ethanol to constant specific activity; m.p. 134–135°.

4. Nonradioactive benzaldehyde, 1000 mg., was added to a 2.0-ml. aliquot of urine; ethanol was added until the mixture was homogeneous. Sodium acetate, 1.5 g., and 1.0 g. semicarbazide hydrochloride were added, and the mixture was vigorously shaken and heated in a boiling water bath for 10 min. On cooling, the semicarbazone of benzaldehyde crystallized. The derivative was repeatedly recrystallized from 50% aqueous ethanol to constant specific activity; m.p. 221–223°.

To quantitate the various metabolites occurring in the urine in a combined form, an aliquot of urine was hydrolyzed by the addition of an equal volume of concentrated hydrochloric acid, and the mixture was heated on a steam bath for 10 min. Nonradioactive carrier was then added and the inverse isotope dilution carried out for total metabolites as described for the free metabolites.

RESULTS AND DISCUSSION

Absorption and Blood Concentration in the Rat, Dog, and Squirrel Monkey—After intraperitoneal administration to rats, benzyl-labeled W-1372 was rapidly absorbed, and a maximal blood concentration of radioactivity was reached after 1 hr. This level of blood radioactivity was maintained for 30 min. and was followed by a first-order decline. The half-life of ^{14}C in the blood was approximately 2 hr. (Fig. 1).

In the dog the drug was readily absorbed after an oral dose of W-1372-benzyl-^{14}C, and a peak blood level of ^{14}C was obtained within 2 hr. Under these conditions the disappearance of radioactivity from the blood followed first-order kinetics and the ^{14}C half-life was estimated to be 11.5 hr. (Fig. 2).

W-1372 was also rapidly absorbed by the squirrel monkey since the maximum blood concentration of radioactivity was reached during the 1st hour after oral administration of benzyl-labeled drug (Fig. 3). In this study the disappearance of radioactivity from blood did not follow the kinetics of a single-compartment model but occurred at two distinct rates. It is difficult to estimate the half-life during the initial depletion pattern. However, after 7 hr., the elim-

Table II—Tissue Distribution of ^{14}C in a Rat 24 hr. after Intraperitoneal Administration of 51.2 mg. W-1372-Acetyl-^{14}C

Organ	Radioactivity Recovered— d.p.m. × 10^3	Radioactivity Recovered— %
Respired CO_2	26,900	62.1
Carcass	5,320	12.3
Stomach and intestines	4,950	11.4
Urine	2,436	5.6
Liver	583	1.4
Kidney	135	0.3
Lung	52	0.1
Heart	22	0.05
Spleen	20	0.05
Brain	6	0.01
Total		93.4

Table III—Urinary Excretion of Hippuric and Benzoic Acids by Animals Given W-1372-Benzyl-^{14}C

Species	No. of Animals	Dose (mg.), Route	% of Urinary Radioactivity as Hippuric and Benzoic Acids
Rat, Sprague-Dawley	5	12–71, i.p.	85.6
Dog, beagle hound	5	25–520, i.p. and p.o.	65.8
Monkey, squirrel	3	16–32, i.p.	68.7

ination of blood radioactivity follows first-order kinetics with a half-life of approximately 13.5 hr.

Tissue Distribution in the Rat—Radioactivity from the intraperitoneal administration of W-1372-benzyl-^{14}C was principally excreted in the urine within 24 hr. Significant amounts of ^{14}C were also found in the carcass and gastrointestinal tract at this time (Table I).

Radioactivity from W-1372-acetyl-1-^{14}C was largely eliminated as respiratory carbon dioxide. Over 60% of the radioactivity of the dose administered was exhaled within 24 hr. while less than 6% was voided in the urine during this period. The distribution of the residual radioactivity is shown in Table II.

Identification and Quantitation of Urinary End Products—The major urinary end products of W-1372 in the three species studied, rat, dog, and monkey, were benzoic acid and hippuric acid. Quantitatively these compounds accounted for at least 65% of the urinary radioactivity. Table III shows the average values obtained with a number of animals of each species. In a single experiment on dog urine, the authors found an additional 1.9% of the urinary radioactivity present as benzoate conjugated with glucuronic acid. This value was determined by the increase in the amount of free benzoic acid present after hydrolysis with β-glucuronidase.

These conversions of W-1372 in the animal body would be anticipated from the metabolic fate of the related compound, benzyl *N*-benzyl carbethoxyhydroxamate (W-398), which is converted into benzoic acid and hippuric acid in both the rat and man (3, 4).

The deacetylated derivative of W-1372, *N*-γ-phenylpropyl-*N*-benzyloxyamine, was also identified and quantitated in the urine of animals receiving W-1372-benzyl-^{14}C. The *N*-γ-phenylpropyl-*N*-benzyloxyamine was present in both the free and bound form since acid hydrolysis of the urine liberated additional free amine. This increase was particularly significant for the dog. The nature of the conjugate has not been elucidated. Trace amounts of benzaldehyde were also identified in acid-hydrolyzed urine, but this may have arisen from the degradation of some metabolite. The quantities of the various metabolites occurring in the urines of various animal species are summarized in Table IV.

Table IV—Excretion of W-1372-Benzyl-^{14}C Urinary Metabolites in the Rat, Dog, and Monkey

Urinary End Product	% of Urinary Radioactivity[a] Rat No. 185	Dog No. 192	Monkey No. 195
Hippuric acid	87.1	54.8	61.0
Benzoic acid	0.32	0.84	1.54
N-γ-Phenylpropyl-*N*-benzyloxyamine (uncombined)	2.04	0.55	6.22
N-γ-Phenylpropyl-*N*-benzyloxyamine (total)[b]	2.23	19.22	7.69
Benzaldehyde (uncombined)	0.18	0.042	1.54
Benzaldehyde (total)[b]	—	0.28	—
Total radioactivity recovered	89.83	75.14	71.77

[a] Values obtained by inverse isotope dilution. [b] Total metabolite after acid hydrolysis of urine.

REFERENCES

(1) F. M. Berger, J. F. Douglas, B. J. Ludwig, and S. Margolin, *J. Pharmacol. Exp. Ther.*, **170,** 371(1969).

(2) F. M. Berger, J. F. Douglas, G. Lu, and B. J. Ludwig, *Proc. Soc. Exp. Biol. Med.*, **132,** 293(1969).

(3) J. F. Douglas, *J. Pharmacol. Exp. Ther.*, **150,** 105(1965).

(4) J. Edelson, A. Schlosser, and J. F. Douglas, *Biochem. Pharmacol.*, **17,** 779(1968).

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