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Disposition and Metabolism of 4-Methyl-2-(4-phenylbenzyl)-2-oxazoline-4-methanol in the Rat and Dog

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Following oral administration of [2-^{14}C]-4-methyl-2-(4-phenylbenzyl)-2-oxazoline-4-methanol (1), a synthetic anti-inflammatory substance, to rats and dogs the drug is eliminated rapidly in the urine and feces. The major metabolite (50–70%) in the rat is 4-(4-hydroxyphenyl)phenylacetic acid (6), whereas the dog eliminates primarily the unusual taurine conjugate of biphenylylacetic acid 4 and biphenylylacetic acid 5. Six minor metabolites have been characterized. The synthesis of the ^{14}C-drug sample and three of the metabolites 2–4 is described.

The biphenylylacetic acid derivative, 4-methyl-2-(4-phenylbenzyl)-2-oxazoline-4-methanol (**1**, AHR-5318), has recently been developed and studied in this laboratory as an antiinflammatory substance. A study of its disposition and metabolic fate is the subject of this article and its pharmacology and more detailed chemistry will be reported at a later date. The metabolic fate of this ring system has not been reported in detail previously although Cressman[1] has reported the use of a ^{14}C-labeled sample of 2-amino-5-phenyl-2-oxazoline (Aminorex) in a pharmacokinetic study. Turnbull[2] and Morrison[3] describe the metabolism of 2-oxazolidinone derivatives in rats and dogs; however, this ring system is stable *in vivo* and its fate quite different compared with the 2-oxazoline system.

Experimental Section

Radioassay Methods. Analysis for ^{14}C activity was carried out in a Packard Tri-Carb liquid scintillation spectrometer in an aqueous phosphor mixture of diphenylyloxazole (5 g, PPO) dissolved in 1 l. of toluene–dioxane–fotocol (200 proof), 4:4:2.5. Freeze-dried feces samples were assayed on a Packard sample oxidizer, Model 305 Tri Carb.

Chromatography. Thin-layer chromatography was performed on a precoated, 250-mm thickness, silica gel plate (Analtech, Inc., Silica GF). The following systems were used to develop the plates: A, formic acid (88%)–ethanol–chloroform, 5:5:90; B, methanol–chloroform, 1:9; C, acetic acid–water–1-butanol, 11:25:50; D, ammonium hydroxide (concentrated)–methanol–chloroform, 2:30:70; E, formic acid (88%)–ethanol–chloroform, 2:2:96; F, ammonium hydroxide (concentrated)–methanol–chloroform, 1:10:90; G, 2 *N* ammonium hydroxide–ethanol–1-butanol, 1:1:4; H, methanol–benzene, 1:9. For radioactivity assay the active zones were scraped from the plates and extracted with methanol or 5% ammoniacal methanol and aliquots were counted. The spots were visualized as quenching zones under short-wave uv light.

Mass Spectrometry. Analyses were performed on a Hitachi RMU-6 mass spectrometer using the solid inlet system. Samples obtained from thin-layer plates were first extracted from the scraped off silica with methanol. After evaporation of the solvent the solid residue, consisting of a small amount of silica that had dissolved in the methanol plus the sample, was reextracted with chloroform. After removal of the chloroform the resulting sample gave satisfactory fragmentation patterns. Usually 20-μg samples or larger were extracted from the thin-layer plates in this way.

Hydrolysis of Conjugates. Potential glucuronide and sulfate conjugates were hydrolyzed by 24-hr incubation with Glusulase (Endo Laboratories) of urine at 37°, pH adjusted to pH 5.0 with acetic acid, and 0.1 ml of enzyme concentrate to 10 ml of urine. Tlc experiments with rat urine indicated that the metabolite spectra were the same before and after Glusulase treatment. Rat feces and dog urine and feces were not examined before Glusulase hydrolysis.

Rats. Three 200-g female rats housed in metabolism cages were given the drug, 200 mg/kg, as a sonified oral suspension in one dose and the urine and feces were collected at intervals for 3 days.

Dog. A 14.2-kg female mongrel dog housed in a metabolism cage was given a single oral dose, 50 mg/kg, in a gelatin capsule. Urine and feces were collected daily and analyzed.

Isolation of Metabolites. Urine samples before or after Glusulase treatment were passed through columns of XAD-2 resin, bed volume approximately one-third the urine volumes, and after a water wash the columns were eluted with methanol. By this procedure 92–98% of the drug-related material (radioactivity) could be concentrated into a fraction suitable for thin-layer chromatography directly or for other fractionation procedures.

Homogenized and freeze-dried rat feces were extracted by overnight refluxing and stirring with ethanol, giving about 50–55% of the radioactivity in the extract. Partition between methanol containing 10% water and low-boiling petroleum ether removed fat and after removal of the methanol the residue in water was passed onto an XAD-2 column. All of the radioactivity was then removed by methanol elution.

The freeze-dried sample of dog feces (24–48-hr collection containing 17% of the dose) was run through a Wiley mill and preextracted with petroleum ether to remove fat. It was then extracted successively with acetonitrile, hot water, and refluxing 1:1 ethanol–water to finally extract 91% of the radioactivity in the feces. The total extracts were combined and treated by the same procedure as the rat feces extract to obtain an XAD-2 resin eluate which contained substantially all of the radioactivity in the dog feces extract.

Dog Gastric Juice Incubation. Samples of 1 were dissolved in dog gastric juice at concentrations of 0.5 and 1.0 mg/ml and incubated at 37° for periods of 0.5 and 1 hr. The products were isolated on an XAD-2 resin column and examined by tlc in systems C, E, F, and G.

†This paper is dedicated to my former professor, Alfred Burger.

Table I. Excretion of ^{14}C-AHR-5318 by a Dog That Received a Single Oral Dose of 50 mg/kg in a Capsule

Time, hr	Feces, % of dose	Urine, % of dose
0–24	3.7	26.2
24–48	12.6	9.7
48–72	4.2	7.2
72–96	2.7	4.2
96–120	2.3	2.7
120–144	1.0	1.2
144–168		0.7
Total[a]	26.5	51.9

[a] Total recovery in urine and feces = 78.3%.

Table II. Excretion of ^{14}C-AHR-5318 Following a Single Oral Dose of 200 mg/kg as a Suspension

Time, hr	Urine, % of dose		
	Rat 1	Rat 2	Rat 3
0–24	50.0	47.0	47.0
24–48	10.0	18.0	18.0
48–72	0.0	2.0	1.0
Total	60.0	67.0	66.0
	Feces, % of dose		
0–48	24.2	24.2	24.2
48–72	1.5	5.9	6.9
Total	25.7	30.1	31.1
Total (urine + feces)	85.7	97.1	97.1

[*Carboxyl*-^{14}C]**biphenylylacetic Acid.** Essentially the method of Dauben[4] was used. An ether solution, 50 ml, containing 12 mmol of the Grignard reagent of chloromethylbiphenylyl was carbonated with $^{14}CO_2$ from 1.970 g, 10 mmol, of ^{14}C-barium carbonate, 250 mCi/m*M*. This gave 2.08 g, 22.2 mCi, of crystalline [^{14}C]biphenylylacetic acid.

[2-^{14}C]-**4-Methyl-2-(4-phenylbenzyl)-2-oxazoline-4-methanol** (1). The 2.08-g sample of [^{14}C]biphenylylacetic acid, 9.83 mmol, along with 1.93 g, 18.4 mmol, of 2-amino-2-methyl-1,3-propanediol was refluxed overnight in 50 ml of xylene in a Dean-Stark apparatus. Benzene was added and the organic phase was washed several times with dilute ammonia. Removal of the solvent and recrystallization gave 2.19 g: 18.1 mCi; mp 121–123.5°; 1.84 × 10^7 DPM/mg; yield 77.5% from barium carbonate. *Anal.* ($C_{18}H_{19}NO_2$) C, H, N.

N-[1,1-**Bis(hydroxymethyl)ethyl]-4-biphenylylacetamide** (3, **AHR-5456**). To 150 ml of cold 2-propanol, 2.5 g (0.00109 mol) of 4-biphenylylacetyl chloride and 5 g (0.05 mol) of 2-amino-2-methyl-1,3-propanediol were added. The resulting mixture was stirred at 35° for 2 hr and then was allowed to stand at room temperature overnight. The white crystalline solid which formed in the mixture solution after standing in the refrigerator for 2.5 hr was filtered. Recrystallization with 2-propanol yielded 2 g of the product which melted at 124–125.5°. *Anal.* ($C_{18}H_{21}NO_3$) C, H, N.

2-Amino-2-(hydroxymethyl)propyl 4-Biphenylylacetate Hydrochloride (2, **AHR-5678**). A 2.5-g sample of 4-methyl-2-(4-phenylbenzyl)-2-oxazoline-4-methanol was dissolved in 30 ml of 2-propanol and to this was added 0.001 *N* hydrochloric acid to pH 5.0–4.5. After standing at room temperature 20 min anhydrous ether was added to cloudiness. The while crystalline solid which separated was recrystallized from 2-propanol–isopropyl ether producing 2.4 g (80.6%) of crystalline solid, mp 136–138°. *Anal.* ($C_{18}H_{22}ClNO_3$) C, H, N.

N-**Biphenylylacetyltaurine.** A 50-mg sample of taurine in 1 ml of 10% sodium bicarbonate was shaken in a sealed vial at 25° with 2 ml of an ether solution containing 90 mg of biphenylylacetyl chloride. The reaction solution was diluted and acidified by the addition of 2 *N* hydrochloric acid. Filtration removed a small amount of flocculent precipitate and the filtrate was extracted several times with ether. The aqueous phase was then passed through a small XAD-2 resin column and, after a water wash elution of the column with methanol, produced 30 mg of crystalline product which was not recrystallized: ir 6.12 μ characteristic for secondary amides and 8.35 μ characteristic of sulfonates; tlc gave a single zone in systems C, D, and G; positive Cl_2–starch iodide test[5] on the tlc plate; mass spectrum 319 *m/e* parent ion. *Anal.* ($C_{16}H_{17}NO_4 \cdot 0.5H_2O$) C, H, N, S.

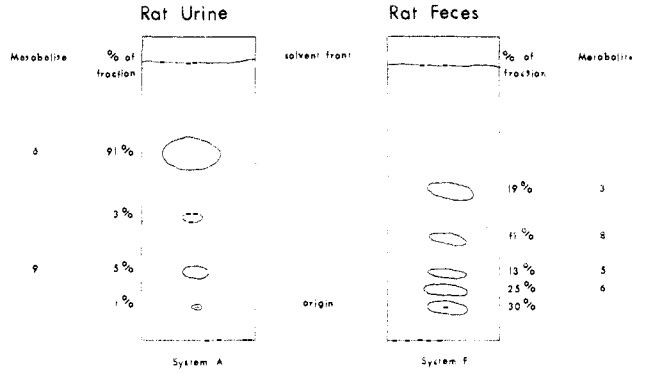
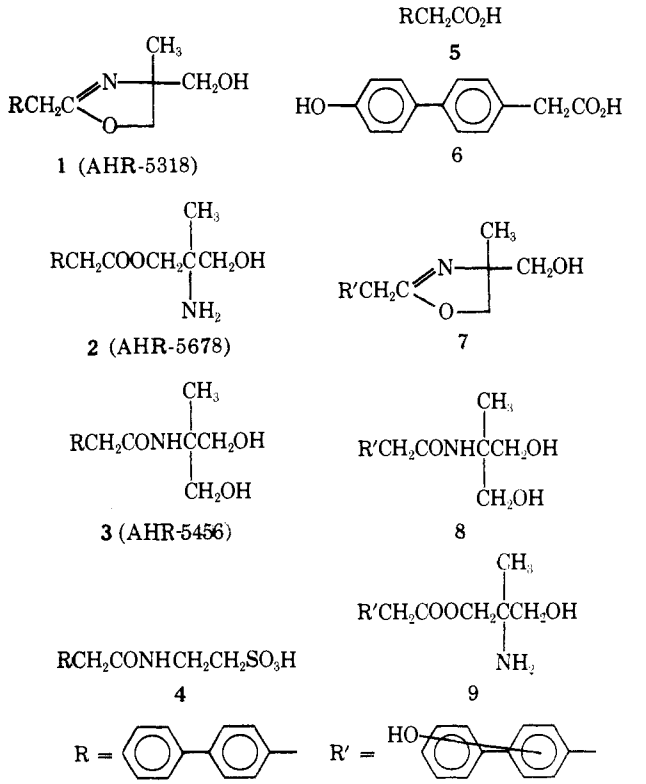


Figure 1. Idealized tlc radioautograms (rat). The fractions chromatographed are methanol eluates of XAD-2 resin columns.

Results

Excretion. Tables I and II give the results of the excretion of the ^{14}C drug in dog and rat urine and feces. Adequate absorption of the drug is indicated by the levels of approximately 35% in the dog urine by 48 hr and 65% in the rat urine by 48 hr. The account of the isolation and identification of the drug-related material found in the urine and feces of the two animals follows, and the metabolites which represent most of the dose to the animals are shown below.



Rat Urine. Thin-layer chromatography, system A, of the rat urine XAD-2 fraction containing essentially all of its radioactivity, Figure 1, shows one main zone and two minor zones. The mass spectrum of the 91% zone gave a parent ion at *m/e* 228, 16 units higher than that for biphenylylacetic acid. Treatment of the fraction with diazomethane in methanolic ether gave a derivative *m/e* 28 units higher at *m/e* 256, R_f about 0.7 in system H, a rather nonpolar system. This evidence indicates a ring-hydroxylated biphenylylacetic acid for the metabolite and a methoxy methyl ester for the diazomethane product. Con-

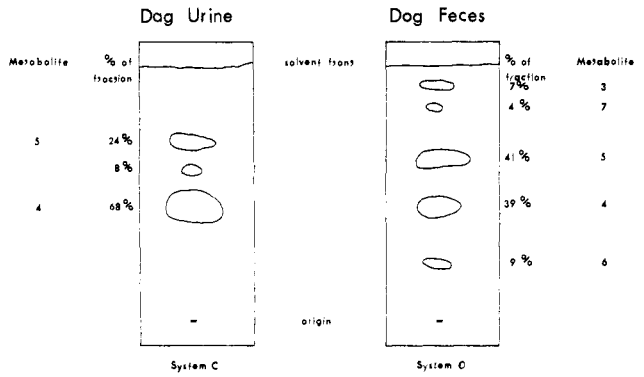


Figure 2. Idealized tlc radioautograms (dog). The fractions chromatographed are methanol eluates of XAD-2 resin columns.

firmation of this was established by isolation of a larger sample of metabolite from nonradioactive rat urine. The same XAD-2 resin fraction of urine was treated with excess diazomethane directly and the product was chromatographed on a 10-g silicic acid column. Elution with a benzene–ethanol gradient produced a 75-mg crystalline fraction of metabolite. Nmr and ir confirmed the mass spectra assignment as a monomethoxy biphenylylacetic acid methyl ester and the nmr spectra located the substitution as para in the terminal ring which gives for the metabolite structure 4-(4-hydroxyphenyl)phenylacetic acid (**6**).

The 5% zone was isolated from a preparative plate and rechromatographed in system B which resolved two additional minor components. The small sample of the main component from this resolution gave a mass spectrum similar to that of both **2** and **3** but m/e 16 units higher at m/e 315. Compounds **2** and **3** give almost identical fragmentation patterns. The sample gave a carbonyl band at 5.77 μ in the ir characteristic of an ester and is thus assigned structure **9**, analogous to the ring hydroxylation of biphenylylacetic acid. However, the position of the hydroxyl group could not be established.

Rat Feces. Tlc of the rat feces metabolite fraction, Figure 1, in system F gave four zones of radioactivity and a fifth zone at the origin. The 19% zone was isolated and its mass spectrum, ir, and R_f values in systems A and B were identical with compound **3**, the ring-opened amide. The 13 and 25% zones were isolated and gave mass spectra for biphenylylacetic acid **5** and its parahydroxylated derivative **6**. The identities were confirmed by ir and R_f comparison in system A as well as system F. The 11% zone gave a m/e 315 parent ion m/e 16 units higher than **3** which would indicate a hydroxylated analog **8** but no other confirming data established this.

When the ester **2** is thin-layer chromatographed in the basic system F it is converted to the amide isomer **3** (see Discussion) by the reagents in the system. Thus, the 19% zone for the rat feces could also be represented by compound **2**. The reverse of this is not true and tlc of the amide **3** in the acidic system A does not produce the ester **2**.

Dog Urine. The Glusulase hydrolyzed XAD-2 fraction, Figure 2, contained 90–95% of the urine radioactivity and when thin-layer chromatographed in system C it showed three zones containing 68, 24, and 8% of radioactivity, respectively. An isolated sample of the 24% zone gave the same mass spectrum as compound **5** and its diazomethane derivative gave an identical mass spectrum to derivatized compound **5**. The radioactivity of the 8% zone was not characterized.

A sample of the 68% zone from a preparative plate failed to give a stable derivative with diazomethane or by permethylation,[6] and treatment with acetic anhydride and pyridine did not give a new compound. However, hydrolysis with hot 6 N hydrochloric acid for 2 hr gave radioactive biphenylylacetic acid **5** as one product. The other hydrolysis component taurine was identified by cochromatography with an authentic sample in systems C, D, and G visualizing the spots by the chlorine–starch iodide test.[5] Glutamic acid, glutamine, glycine, ornithine, and arginine did not cochromatograph with the second hydrolysis product in these systems. A satisfactory mass spectrum on the conjugate itself was obtained after a sample was retained on XAD-2 resin from an acid solution and eluted with methanol, parent ion at m/e 319. Synthetic N-biphenylylacetyltaurine (**4**) (Experimental Section) gave the identical mass spectrum.

Table III. Antiinflammatory Activity[a]

Biphenylylacetic acid	1	Phenylbutazone	2	3
36	25		24	
	20			0
	24	27		

[a] Expressed as the per cent decrease in volume of pleural fluid over that of the control group at $p < 0.05$. The substances were tested in parallel at 50 mg/kg orally using six fasted rats per group.

Dog Feces. A series of extractions of Wiley mill treated dog feces gave 91% of the feces radioactivity (see Experimental Section). After a petroleum ether partition to remove fat and an XAD-2 resin fractionation this fraction was thin-layer chromatographed in system D which resolved the radioactivity into five zones. The 41 and 39% zones, Figure 2, are the two major metabolites in the feces, biphenylylacetic acid (**5**) and N-biphenylylacetyltaurine (**4**).

The 7% zone was taken off the plate and tlc'd against **3** in system F. They cochromatographed and a mass spectrum of **3** was identical with the metabolite.

The mass spectrum for the 4% zone gave a parent ion at m/e 297, 16 mass units higher than **1**. Its fragmentation pattern was similar to that for **1**, and it was assigned structure **7** on this basis.

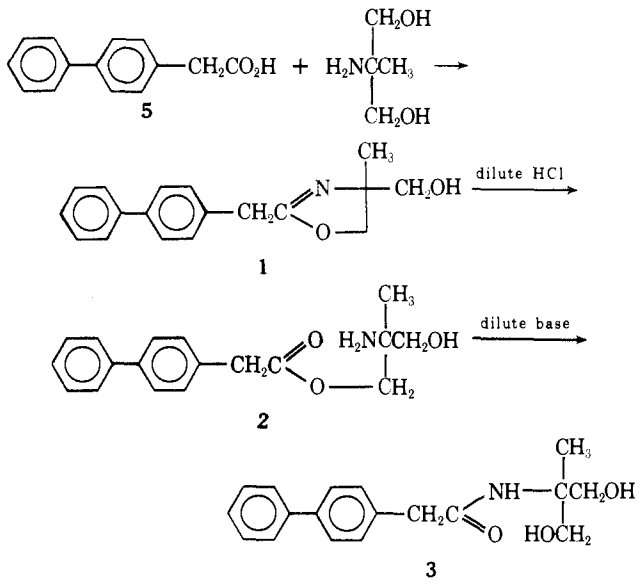
The 9% zone was para-hydroxylated biphenylylacetic acid **6**, the most abundant transformation product in the rat.

Antiinflammatory Activity. Initial evaluation of the antiinflammatory activity of the parent compound and certain metabolites was made using the Evans Blue carrageenan pleural effusion technique.[7] The order of antieffusive activity of the substances tested was biphenylylacetic acid > **1** = phenylbutazone = **2** > **3**. These relative potencies are expressed in Table III.

Discussion

The oxazoline ring of **1** results from the reaction of 2-amino-2-methyl-1,3-propanediol with **5**,[8] Scheme I. Treatment of the compound with dilute acid opens the ring up to the corresponding amino ester **2** which can then be converted by weak base to the O,N acyl migration product, the amido alcohol **3**. This O,N acyl migration has been frequently noted.[9-11] That the drug is opened up to the amino ester **2** in the stomach was shown by incubation in dog gastric juice (pH 1.1). After 0.5-hr incubation tlc analysis of the juice indicates virtually complete conversion to the amino ester **2**, no amido alcohol **3**, and a trace of unchanged drug **1**.

Scheme I



It is likely then that the amino ester **2** passes into the gut where it is hydrolyzed by esterases either before or after absorption or both. The resulting biphenylylacetic acid **5** is then the substrate for liver metabolism and the major metabolites **4** and **6** arise from the metabolism of biphenylyl acetic acid **5**.

During the course of the studies on this compound, glc methods were developed for the determination of **1**, **2**, and biphenylylacetic acid. With this method it is possible to determine each of these compounds in the presence of the others with sensitivities of less than 1 γ/ml of blood. Analysis of the blood of dogs after receiving oral doses of 50 mg/kg of the drug revealed that only biphenylylacetic acid was present. A second dog was then administered an iv dose of 5 mg/kg and blood samples were taken at 2, 10, and 30 min and 1 and 1.5 hr and then at hourly intervals for 6 hr. None of these compounds were found in the 2-min sample where the maximum concentration of drug should have appeared. The 10-min sample showed the presence of biphenylylacetic acid only. The concentration of biphenylylacetic acid increased through the 1.5-hr sample to 2 γ/ml at which level it remained through the 6-hr period. It therefore seems possible that the drug is very rapidly absorbed into the tissues where it is metabolized and biphenylylacetic acid slowly released into the blood stream.

Conjugation of biphenylylacetic acid **5** with taurine as a significant route of elimination is an infrequently observed pathway of elimination. A familiar and long-known step in the bile elimination of steroid acids, it has been reported only seldom for exogenous substances.[12] Boyland has reported a taurine derivative of glutathione conjugated with a naphthalene metabolite[13] and James[14] has recently reported the conjugation of phenylylacetic acid with taurine as a significant route of elimination in nine species.

This compound affords another example by which to compare the extent of aromatic hydroxylation in different species. Here ring hydroxylation is the primary metabolic route in rats whereas in dogs only a minor fraction is hydroxylated while the major portion is conjugated, which falls in line with the predominant para hydroxylation of amphetamine in the rat *vs.* deamination or excretion unchanged in other species.[15]

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