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THE PHYSIOLOGICAL DISPOSITION OF THE URICOSURIC-SALURETIC AGENT (6,7-DICHLORO-2-METHYL-1-OXO-2-PHENYL-5-INDANYLOXY)ACETIC ACID (MK-196) IN THE RAT, DOG, AND MONKEY

A. G. ZACCHEI AND T. I. WISHOUSKY

Merck Institute for Therapeutic Research

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ABSTRACT

The physiological disposition of a new saluretic-uricosuric agent, (6,7-dichloro-2-methyl-1-oxo-2-phenyl-5-indanyloxy)acetic acid (MK-196), was studied in the rat, dog, and monkey. MK-196 was well absorbed and showed minimal metabolism in these species. Peak plasma levels of radioactivity and drug occurred 0.5–2 hr after oral administration at a dose of 2.5 mg/kg. Essentially all of the radioactivity present in the plasma during the first day was intact MK-196. Following a single dose, a long terminal half-life for plasma radioactivity was observed in the dog (~68 hr) and monkey (~105 hr). The chronic administration of MK-196 to dogs resulted in a dose-related plasma profile and showed no tendency to increase or decrease with dosing. However, upon repeated drug administration to monkeys, the plasma levels of drug increased and then decreased, possibly due to hypochloremia and secondary metabolic alkalosis.

Fecal excretion was the predominant route of tracer elimination in the dog (~80%) and rat (~94%), whereas the monkey eliminated the majority of the dose (~60%) *via* the urine. Minimal metabolism was noted in the three lower species; most of the urinary, plasma, and fecal radioactivity was accounted for as intact drug and its glucuronide conjugate. Three minor metabolites, which were present in dog bile, plasma, and urine, were characterized as: (6,7-dichloro-1α-hydroxy-2-methyl-2-phenyl-5-indanyloxy)acetic acid, **I**; (6,7-dichloro-2-(4-hydroxyphenyl)-2-methyl-2-oxo-5-indanyloxy)acetic acid, **II**; and 2-methyl-2-phenyl-5-hydroxy-6,7-dichloro-1-indanone, **III**. The monkey urine and plasma also contained small amounts of **II**.

The recent disclosure (1, 2) of several series of new orally active indanyloxyacetic acids with saluretic and uricosuric activity led to the selection of (6,7 - dichloro - 2 - methyl - 1 - oxo - 2 - phenyl - 5-indanyloxy)acetic acid, MK-196, for further investigation. MK-196 has been shown to have pronounced saluretic activity in the dog and rat (3, 4) and both uricosuric and saluretic activity in the chimpanzee (5, 6). The efficacy and tolerability of single and multiple doses of MK-196 and furosemide were compared in normal volunteers (7). The effects of MK-196 and furosemide on human uric acid levels have also been reported (8).

The biotransformation of this potent uricosuric-saluretic agent in the chimpanzee has been described (9). This report deals with the absorption, metabolism, and excretion of MK-196-^{14}C in the rat, dog, and monkey. Comparisons of the metabolic profiles will be discussed as related to the various species. Such information may provide insight into the design and interpretation of continuing pharmacological studies on this drug.

A preliminary report of these studies was presented at the 59th Annual Meeting of the Federation of American Societies for Experimental Biology, Atlantic City, N. J., 1975 [*Fed. Proc.* 34, 802 (1975)].

Send reprint requests to: Dr. A. G. Zacchei, Merck Institute for Therapeutic Research, West Point, Pa. 19486.

Methods and Materials

The materials and reagents used in these studies are described in the preceding paper (9).

Animal Studies. Twenty-one male Sprague-Dawley rats weighing 150 ± 10 g were given MK-196-^{14}C orally

in a water solution at a dose of 2.5 mg/kg (sp. act. = 11,400 dpm/μg); 24 additional rats weighing 235 ± 5 g were given the drug iv in a saline solution (2.5 mg/kg, 6300 dpm/μg). At each of the indicated time intervals, three rats were anesthetized with ether and blood was removed *via* cardiac puncture into a heparinized syringe. The blood was immediately centrifuged and the plasma was separated and removed. The rats were killed and the tissues were excised, rinsed with saline (intestine washed with water), blotted, and weighed. The weighed sections of the tissues were digested in alcoholic KOH followed by subsequent decolorization with H_2O_2 and radiometric analysis. Urine and feces were collected separately from animals placed in metabolism cages, the urine in Dry Ice-cooled containers.

Two male and two female beagle dogs weighing 7–10 kg were given a single oral dose of 2.5 mg of MK-196-^{14}C per kg as a solution (1.25 mg/ml) in water. The specific activity of the solution was 1343 dpm/μg. Blood was sampled from the jugular vein and excreta were collected by the same technique used for rats. In a 14-day subacute study, beagle dogs received MK-196 in capsule form at doses of 2.5, 5, 10, 20, and 40 mg/kg/day. Blood specimens were obtained at 1, 2, 4, 6, and 24 hr after dosing on day 1 and 14. Bile was obtained from these animals on day 15 (24 hr after last doses).

Four male Rhesus monkeys weighing 3–4 kg were anesthetized lightly with phencyclidine and placed in restraining chairs. The next day, the monkeys received an oral dose (2.5 mg/kg) of MK-196-^{14}C by gavage at a concentration of 1 mg/ml (sp. act. = 2925 dpm/μg). One month later, the same monkeys were given the same dose iv at a concentration of 2.5 mg/ml (sp. act. = 2741 dpm/μg). Blood was obtained from a femoral vein. Excreta were collected at 24-hr intervals in Dry Ice-cooled containers. Rhesus monkeys also received the drug again chronically at oral doses of 2.5, 5, 10, and 20 mg/kg. Blood samples (where possible) were monitored for drug for the duration of the study.

In all experiments except the toxicity studies, the animals were deprived of food for 16 hr before dosing. Animals were given food *ad lib.* 6 hr after drug and were allowed free access to water.

Radioactivity Measurements. The radioactivity content present in the various samples was measured as previously described (9, 10).

Measurement of MK-196 in Biological Fluids. The amount of intact drug in the plasma, urine, and bile specimens was determined by the highly specific GLC methods previously described (11). The procedure involves the addition of an internal standard to the biological specimen, followed by extraction of the acids into benzene at pH 1. The extracted acids are back-extracted into NaOH solution. The aqueous layer is made acidic and the materials extracted into methylene chloride. The acids are subsequently converted to the methyl esters for GLC analysis by reaction with ethereal diazomethane.

The sensitivity of the method is such that 1.0 μg of MK-196 per ml plasma can be analyzed with a flame-ionization dectector (FID).[1] The sensitivity of the method is increased to 2.5 ng per ml of plasma when a ^{63}Ni-ECD is used.

Gas-liquid Chromatography. GLC analyses and separations were carried out in: 1) a Hewlett-Packard model 5830A gas chromatograph equipped with a FID and a ^{63}Ni-ECD and 2) a Packard model 7400 gas chromatograph equipped with a FID and a ^{63}Ni-ECD. A 1.2-m × 4-mm (i.d.) glass column packed with 3% OV-210 on 100-120 mesh Gas-Chrom Q was used in both instruments. The operating conditions were similar to those described previously (9, 11).

Mass Spectrometry. Mass spectrometric analyses were performed on the same instrument and under conditions similar to those described in the preceding paper (9). The derivatization reactions for GLC and GLC/MS analyses were carried out in a manner analogous to those previously described (9).

Results

Plasma Levels. The plasma-level data for total radioactivity and for unchanged MK-196 after administration of a single dose of MK-196 to rats and dogs are presented in table 1. The data obtained after oral or iv administration to monkeys are presented in table 2. Radioactivity and MK-196 levels in monkeys after an oral dose were approximately three times higher than those in dogs and about six times greater than those in rats. The drug was well absorbed in all species, as seen by comparisons of the plasma levels (and excretion data) after oral and iv doses. In the rat, the highest levels of radioactivity and drug were found at the first time period after iv (10 min) or oral (30 min) dosing. An apparent triphasic rate of elimination of radioactivity from the plasma was observed during the first 4 hr after iv dosing, $(t_{1/2})_\alpha \sim 0.5$ hr, $(t_{1/2})_\beta \sim 1.1$ hr, and $(t_{1/2})_\gamma \sim 3.9$ hr. In the dog, peak levels of radioactivity and drug occurred about 1 hour after dose. A triphasic rate of elimination of radioactivity was observed in the plasma, $(t_{1/2})_\beta \sim 3$ hr, $(t_{1/2})_\gamma \sim 68$ hr. Peak levels of drug and radioactivity occurred 2 hr after oral administration to monkeys. Similar triphasic rates of elimination for drug and total radioactivity were observed after iv or oral administration. The half-lives, $(t_{1/2})_\beta$, for intact drug were 27.8 hr (po) and 25.4 hr (iv). The radioactivity half-lives were longer: $(t_{1/2})_\beta \sim 43$ hr (po) and 39 hr (iv); $(t_{1/2})_\gamma \sim 105$ hr (po) and 103 hr (iv). In the three species, essentially all of the plasma radioactivity (0–6 hr)

[1] The abbreviations used are: GLC, gas-liquid chromatography; GLC/MS, combined gas-liquid chromatography/mass spectrometry; ECD, electron-capture detector; FID, flame-ionization detector; T_r, retention time; BSA, bis(trimethylsilyl)acetamide; TMSi, trimethylsilyl.

was accounted for as unchanged drug. At 96 hr after drug in the monkey, 30–40% of the plasma radioactivity was accounted for as unchanged drug. These data suggest that MK-196 is slowly metabolized in these species.

Plasma levels of MK-196 obtained in the dog subacute study (table 3) were dose-related but showed no tendency to increase or decrease during the course of the study. The 24-hr plasma levels indicated no accumulation of drug for doses of 20 mg/kg/day or lower. These data suggest no drug induction in this species. The concentration of the compound in the bile was higher than in plasma. The mean bile/plasma ratios varied from 393 to

TABLE 1

Plasma levels of radioactivity and intact MK-196 in rats and dogs after administration of a single dose of MK-196-^{14}C (2.5 mg/kg)

The values listed represent the means ± SD for three rats and four dogs at each time period. The radioactivity values are expressed as μg-equivalents of MK-196 per ml.

Time	Rat iv		Rat po		Dog po	
	^{14}C	MK-196	^{14}C	ML-196	^{14}C	MK-196
hr			*μg/ml*			
0.17	5.29 ±0.68	5.12 ± 0.68				
0.25					3.74 ± 2.93	3.65 ± 3.18
0.5	3.74 ± 0.85	3.37 ± 0.81	2.82 ± 1.61	2.88 ± 1.54	5.36 ± 2.75	5.33 ± 2.96
1.0	2.94 ± 0.47	2.50 ± 0.37	1.28 ± 0.35	1.25 ± 0.36	5.79 ± 2.47	5.85 ± 2.73
1.5					4.44 ± 1.72	4.40 ± 1.89
2	1.54 ± 0.19	1.26 ± 0.18	1.55 ± 0.90	1.59 ± 0.87	3.47 ± 1.15	3.43 ± 1.24
3					2.50 ± 0.81	2.25 ± 0.75
4	1.08 ± 0.29	0.89 ± 0.30	0.53 ± 0.08	0.49 ± 0.10	1.97 ± 0.83	1.78 ± 0.73
6					1.54 ± 0.64	1.33 ± 0.54
24	0.11 ± 0.09		0.04 ± 0.01		0.23 ± 0.09	0.12 ± 0.06
48	0.04 ± 0.06		0.02 ± 0.01		0.075 ± 0.005	
72	0.08 ± 0.07		0.01 ± 0.01		0.061 ± 0.006	
96					0.051 ± 0.003	

TABLE 2

Plasma levels of MK-196 and radioactivity in monkey after oral and iv administration of MK-196-^{14}C (2.5 mg/kg)

Values are expressed as μg-equivalents of MK-196 per ml and represent the mean ± SD for four monkeys at each time period.

Time	Oral Dose		Intravenous Dose	
	^{14}C	MK-196	^{14}C	MK-196
hr		*μg/ml*		
0.08			39.9 ± 3.1	38.1 ± 3.1
0.27			33.6 ± 2.8	31.8 ± 2.7
0.5	5.8 ± 1.2	6.4 ± 1.6	28.5 ± 4.0	27.0 ± 3.9
1	10.3 ± 3.1	11.0 ± 3.2	22.4 ± 3.2	20.7 ± 3.0
2	14.3 ± 5.6	15.1 ± 6.1	17.8 ± 2.4	15.8 ± 2.2
4	13.7 ± 4.4	14.2 ± 4.7	14.7 ± 2.9	12.9 ± 3.1
6	12.6 ± 3.7	12.8 ± 3.8	12.6 ± 2.3	10.8 ± 2.4
24	7.0 ± 2.1	5.6 ± 2.3	6.4 ± 2.2	4.3 ± 1.7
48	5.0 ± 2.2	3.4 ± 2.1	3.8 ± 1.7	2.3 ± 1.3
72	3.2 ± 1.9	1.8 ± 1.6	2.5 ± 1.6	1.2 ± 1.1
96	2.3 ± 1.7	0.96 ± 1.1	1.7 ± 1.2	0.53 ± 0.70
120	1.9 ± 1.4	0.64 ± 0.85	1.4 ± 1.1	
144	1.7 ± 1.3	0.50 ± 0.63	1.24 ± 0.92	
168	1.41 ± 1.08	0.41 ± 0.72	1.07 ± 0.76	
336	0.59 ± 0.09	0.008 ± 0.006	0.46 ± 0.04	
672	0.23 ± 0.05		0.19 ± 0.02	

TABLE 3

Plasma levels of MK-196 obtained from the various dose levels of MK-196 administration to dogs

Comparisons between day 1 (D-1) and day 14 (D-14) are made. Data represent averages of two animals per time period, except where noted.

Time	MK-196 Plasma Levels									
	2.5[a]		5.0[a]		10.0[a]		20.0[a]		40.0[a,b]	
	D-1	D-14	D-1	D-14	D-1	D-14	D-1	D-14	D-1	D-14[b]
hr	*μg/ml*									
0	0.0	0.4	0.0	0.2	0.0	0.4	0.0	1.9	0.0	75.0
1	2.0	0.7	6.5	5.7	24.0	30.2	44.0	35.6	20.5	94.5
2	2.1	3.2	8.8	7.4	29.8	29.2	58.3	40.2	66.8	127.5
4	1.5	4.1	5.1	6.7	12.5	11.1	35.0	41.7	116.0	135.0
6	2.7	3.7	3.4	5.0	8.6	8.0	20.6	21.3	113.0	107.5
24	0.7	0.7	0.1	0.2	0.4	1.7	2.9	2.9	8.4	51.3
Bile/plasma[c]		563		963		476		393		

[a] Dose, mg/kg/day.
[b] Results from one dog only.
[c] Mean value; samples obtained 24 hr after last dose.

TABLE 4

Plasma levels of MK-196 after chronic administration of MK-196 to monkeys at doses of 2.5 and 10 mg/kg

Asterisks represent values of <1 μg/ml.

Dose and Monkey Number	MK-196 Plasma Levels											
	Day 1			Day 7	Day 14				Day 21			
	2 hr	6 hr	24 hr	0 hr	0 hr	2 hr	6 hr	24 hr	0 hr	2 hr	6 hr	24 hr
	μg/ml											
2.5 mg/kg/day												
75-0009F	4.1	6.8	3.2	9.4	*	9.0	7.4	*	*	7.0	6.2	*
75-0070M	2.0	2.7	1.3	8.0	*	9.2	8.8	*	*	8.8	11.0	*
75-0005F[a]	2.5	4.0	2.4	12.6	*	*	1.0	*	*	*	*	*
75-0106M[a]	6.0	7.1	4.3	*	*	*	*	*	*	*	*	*
10.0 mg/kg/day												
75-0047F	10.4	20.2	21.4	74[b]								
75-0114M	15.0	23.0	10.8	55.0	53.6	76.0	58.8	28.6	*	*	7.2	*
75-0045F[a]	14.8	35.4	34.4	14.1	*	10.6	12.2	*	*	14.2	11.0	*
74-0072M[a]	22.8	27.8	50.8	56.8[b]								

[a] Animals supplemented with electrolyte solution.
[b] Animals died during 2nd week.

963 in samples obtained 24 hr after the last dose (table 3). The actual concentration of MK-196 in the bile ranged from 146 to 1419 μg/ml. However, in the chronic monkey study at a dose of 2.5 mg/kg/day (table 4), the levels of MK-196 in the plasma at first increased as expected (3–4 times on day 7) due to the long terminal half-life, but then decreased with continued administration. Inasmuch as the animals became hypochloremic[2] (a situation which would induce alkalosis), it appears as if the rapid excretion of MK-196 after 14 days of drug dosing resulted from induced alkalosis. Clearance studies in chimpanzee (9) and dogs (4) have indicated enhanced elimination of MK-196 under conditions of induced alkalosis.

Excretion. Marked species differences in the excretion of radioactivity were observed (table 5). The rat and dog eliminated the radioactivity predominantly in feces (>80%), whereas urinary excretion (~60%) was favored in the monkey. The

[2] W. Bagdon, unpublished observations.

TABLE 5

Comparison of the urinary and fecal excretion of radioactivity following single-dose administration MK-196-^{14}C to rats, dogs, and monkeys

All animals received a dose of 2.5 mg/kg. Values represent means for four monkeys, four dogs, and nine rats (day 1), six rats (day 2), and three rats (day 3).

Specimen and Day	% Dose Excreted				
	Rat		Dog	Monkey	
	iv	po	po	iv	po
Urine					
1	4.9	5.9	7.1	42.8	35.6
2	1.5	0.5	3.6	7.3	10.4
3	1.2	0.4	0.4	4.0	6.0
4			0.1	2.7	3.6
5-7	—	—	—	2.2	3.3
Total	7.6	6.8	11.2	59.0	58.9
Feces					
1	49.4	87.0	44.4	0.1	0.0
2	30.7	7.4	33.1	2.6	2.6
3	14.3	0.4	2.4	11.6	5.8
4			0.6	7.8	7.6
5-7				4.4	5.7
Total	94.4	94.8	80.5	26.5	21.7
Grand total	102.0	102.4	91.7	85.5	80.6

route of drug administration (iv or po) had no effect on the excretion pattern of radioactivity in a particular species, indicating that absorption was essentially complete. More than 80% of the dose was recovered in the excreta of all animals, the monkey excreting the radiolabel more slowly than rat or dog. These results are consistent with the plasma level profiles. The amount of MK-196 in the urine and feces (table 6) was determined by the GLC method previously described (11). About 60–70% of the rat fecal radioactivity was identified as intact MK-196, and an additional 10% was present as the glucuronide conjugate. Most of the rat urinary radioactivity (~50%) was in intact drug, and its glucuronide conjugate accounted for an additional 5–10% of the material. In the dog about 80% of the urinary radioactivity was in unchanged drug; the fecal specimens also contained 76% of the radioactivity as intact MK-196, an additional 20% of the fecal radioactivity was accounted for as MK-196 glucuronide. In the monkey, however, only about 30% of the radioactivity in the feces was in intact drug. Little difference in the excretion profile of MK-196 was noted between the iv or oral route of drug administration for a given species. The data suggest extensive biliary secretion in the rat and dog.

Tissue Distribution in the Rat. A summary of the tissue distribution of radioactivity in rats after a single dose (2.5 mg/kg) of MK-196 (iv or po) is presented in table 7. All tissues (with the exception of liver, kidney, and small intestine) exhibited tissue/plasma ratios of less than 1, regardless of route of drug administration. The biliary secretion of the drug is illustrated by the percentage of dose present in the intestinal contents (as a function of time after iv administration). These data actually represent the passage of drug from the liver to the small intestine and then to the large intestine.

Metabolism. *Rat.* GLC analysis of the plasma, urine, and fecal specimens from the rats which received a single dose of MK-196 (iv or po) indicated that minimal metabolism occurred in this species. More than 82% of the plasma radioactivity was attributed to MK-196 (table 1). The 24-hr urine specimens contained about 5% of the administered radioactive dose. Most of the radioactivity was present as intact MK-196 (~ 50–60%) and its glucuronide conjugate (5–10%) (table 6). The fecal specimens, which contained about 94% of the administered dose, contained mainly intact MK-196 and its glucuronide conjugate. These substances account for 70–80% of the fecal radioactivity. Traces of another component were observed in the GLC profiles; however, no quantitation was possible. This minor component, which was present in the plasma and urine samples obtained from rats receiving the drug chronically, behaved chromatographically similar to the ma-

TABLE 6

Summary of GLC analysis for intact MK-196 in rat, dog, and monkey urine and fecal specimens

The values are expressed as the mean percentage of urinary or fecal radioactivity accounted for as MK-196. The samples were analyzed with (K) and without (NK) Ketodase treatment.

Specimen	Rat[a]		Dog[b]	Monkey	
	iv	po	po	iv	po
Urine NK	48.5	57.6	76.6	50.1[b]	43.9[b]
K	57.6	62.7	80.1		
Feces NK	58.3	73.7	75.6	22.6[c]	33.1[c]
K	68.9	78.5	94.2		

[a] 0–24 hr samples.
[b] 0–48 hr samples.
[c] 48–72 hr samples.

TABLE 7

Tissue distribution of radioactivity in rats given a single dose of MK-196-^{14}C (2.5 mg/kg; iv or po)

Values are the means for three rats at each time period, expressed as μg-equivalents of MK-196 per g or per ml. Dashes indicate that values were less than 0.01 μg/g.

Tissue	Radioactivity in Tissues						
	0.5 hr	1 hr	2 hr	4 hr	24 hr	48 hr	72 hr
Oral							
Plasma	2.82	1.28	1.55	0.53	0.04	0.02	0.01
Adrenals	—	—	—	—	—	—	—
Brain	0.05	0.03	0.04	0.02	—	0.01	0.01
Fat	0.31	0.14	0.20	0.12	0.02	—	—
Heart	0.61	0.32	0.44	0.17	0.03	—	—
Small intestine	13.39	7.17	13.11	5.39	0.28	0.07	0.02
Kidney	5.33	3.88	4.64	3.04	0.18	0.07	0.04
Liver	20.21	12.11	17.43	10.20	0.30	0.09	0.04
Lung	2.30	1.34	1.73	0.54	0.04	0.02	—
Muscle	0.15	0.10	0.10	0.05	0.02	—	—
Spleen	0.41	0.26	0.30	0.18	0.02	0.06	—
Intravenous							
Plasma	3.74	2.94	1.54	1.08	0.11	0.04	0.08
Adrenals	0.63	—	0.40	—	—	—	—
Brain	0.09	0.04	0.03	0.02	0.01	—	0.01
Fat	0.25	0.11	0.10	0.05	0.02	—	—
Heart	0.82	0.60	0.32	0.22	0.02	—	—
Small intestine	12.03	13.14	16.81	10.76	2.08	3.66	1.23
Kidney	16.12	12.63	8.43	5.54	0.65	0.46	0.57
Liver	53.48	60.69	37.01	33.41	3.49	1.89	1.43
Lung	3.68	3.66	1.59	.410	0.08	0.03	0.05
Muscle	0.23	0.29	0.15	0.10	0.01	—	—
Spleen	0.63	0.48	0.23	0.27	0.08	—	—
Small intestine contents[a]	7.1	26.4	39.2	33.4	1.8	1.3	1.8
Large intestine contents[a]	0.1	0.4	9.0	29.4	22.8	8.6	2.6

[a] Calculated as percentage of dose

jor metabolite previously identified in the chimpanzee (9), namely, [6,7-dichloro-2-(4-hydroxyphenyl)-2-methyl-1-oxo-5-indanyloxy]acetic acid (II).

Dog. As in the rat, little metabolism was noted in this species, as evidenced by the high levels of intact MK-196 in the plasma, urine, and feces. In the dog, about 80% of the dose was excreted *via* the feces, 94% of which was intact MK-196 and its glucuronide conjugate. Samples of dog plasma, bile, and urine, which were obtained from the chronic study, were analyzed by GLC/MS techniques after the samples were carried through the procedure to analyze for intact drug. Analysis of the plasma extracts after derivatization with diazomethane, BSA, or diazomethane/BSA showed no drug-related components other than MK-196 for all dogs except one that received 40 mg/kg/day. GLC/MS analysis of the esterified extract gave rise to a peak (metabolite I) at 1.8 min which resulted in a mass spectrum that exhibited characteristic peaks at *m/e* 380 (M), 362 (M − 18) and 276 (M − 104). The base peak that occurred at *m/e* 105 resulted from the expulsion of the C^2 carbon atom and its substituents, with a concomitant H-transfer. The data indicate reduction of the ketone group to the alcohol, inasmuch as a net increase in two mass units over MK-196 was obtained. Fig. 1 shows a comparison of the mass spectrum obtained from metabolite I (lower) with that obtained from authentic I (upper). The same sample after esterification was treated with BSA in pyridine. GLC/MS analysis of this derivative gave rise to a peak at 0.9 min which produced a mass spectrum (fig. 2, upper) that exhibited a molecular

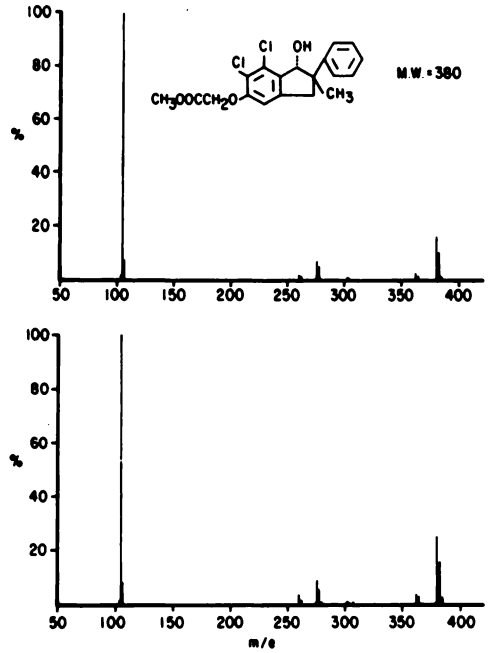

FIG. 1. *Comparison of the mass spectrum of metabolit* **I** *with that of authentic* **I**.

Upper spectrum is of authentic (6,7-dichloro-1α-hydroxy-2-methyl-2-phenyl-5-indanyloxy)acetic acid and lower spectrum is that of dog plasma metabolite **I**. Both were analyzed as the methyl esters.

ion at *m/e* 452. Other characteristic ions were observed at the following *m/e* values: 437 (loss of methyl radical, confirmed by metastable ion at 422.5), 363 (loss of TMSiO), 362 (loss of TMSi-OH) and 327 (loss of TMSiOH and Cl). These data add support for the aforementioned structure. Further confirmation of structure of metabolite **I** was obtained when the extracted metabolite was derivatized with BSA directly. The derivative exhibited a peak at 1.2 min. Mass spectral analysis (fig. 2, lower) of this component indicated a molecular ion at *m/e* 510. Characteristic ions were observed at the following *m/e* values: 495 (loss of methyl radical from M, confirmed by the metastable ion at 480.4), 433 (M − phenyl radical), 421 (M − TMSiO), and 420 (M − TMSiOH). These data establish the structure of metabolite **I** as (6,7-dichloro-1-hydroxy-2-methyl-2-phenyl-5-indanyloxy)acetic acid. Synthesis (2) of the 1α-(*cis* with respect to the 2-phenyl moiety) and 1β-hydroxy isomers established that the metabolite was of the α-configuration, based upon similarities in retention time on a 3% OV-210 column. At a column temperature of 250°, both metabolite **I** and the synthetic 1α-hydroxy isomer, as the methyl esters, had a retention time of 1.5 min, whereas the synthetic 1β-isomer had a retention time of 1.1 min.

In the urine samples, only one dog exhibited drug-related (chlorine isotope pattern) GLC peaks other than that of the parent compound. Following methylation and trimethylsilylation, two minor peaks were observed at 1.8 and 3.5 min, corresponding to metabolites **III** and **II**, respectively. Mass spectrometric analysis of the first peak (T_r = 1.8 min) indicated that metabolite **III** had a molecular ion at *m/e* 320 and that no additional derivatization was noted after subsequent reaction with BSA. The fragmentation pattern is consistent with the following structure: 2-methyl-2-phenyl-5-hydroxy-6,7-dichloro-1-indanone. A comparison of the mass spectrum of the authentic material with that of metabolite **III** is presented in fig. 3. The second peak (metabolite **II**), upon mass spectrometric analysis, gave rise to a molecular ion at *m/e* 466. The fragmentation pattern was similar to that of the authentic *p*-hydroxy metabolite previously characterized in chimpanzee urine (9). Similar data were obtained from the bile extract of the same dog. The first metabolite peak gave rise to a molecular ion at *m/e* 380 after methylation. This component, following direct trimethylsilylation, exhibited a molecular ion at *m/e* 510, corresponding to the addition of two TMSi groups. The metabolite was identified as the 1α-hydroxy isomer of MK-196 (same as metabolite **I** in plasma). The second peak produced a molecular ion at *m/e* 320 after methylation. After treatment with BSA only, this component yielded a molecular ion at *m/e* 378 containing one TMSi group. The fragmentation pattern was essentially the same as that of metabolite **III** previously identified in the urine.

Monkey. The monkey, like the dog and rat, exhibited little metabolism. The only drug-related component detected in the plasma and urine other than MK-196 were small amounts of **II**. These data are consistent with the plasma-level data for total radioactivity and intact MK-196 (table 2).

Discussion

The physiological disposition of the novel saluretic-uricosuric agent, MK-196, was studied in rat, dog and monkey. The drug was well absorbed and showed minimal metabolism in the three species. Following oral administration, peak levels of drug and radioactivity occurred at about 0.5 hr in the rat, about 1 hr in the dog, and about 2 hr in the monkey. Essentially all of the radioactivity circulating in the plasma during the 1st day was in

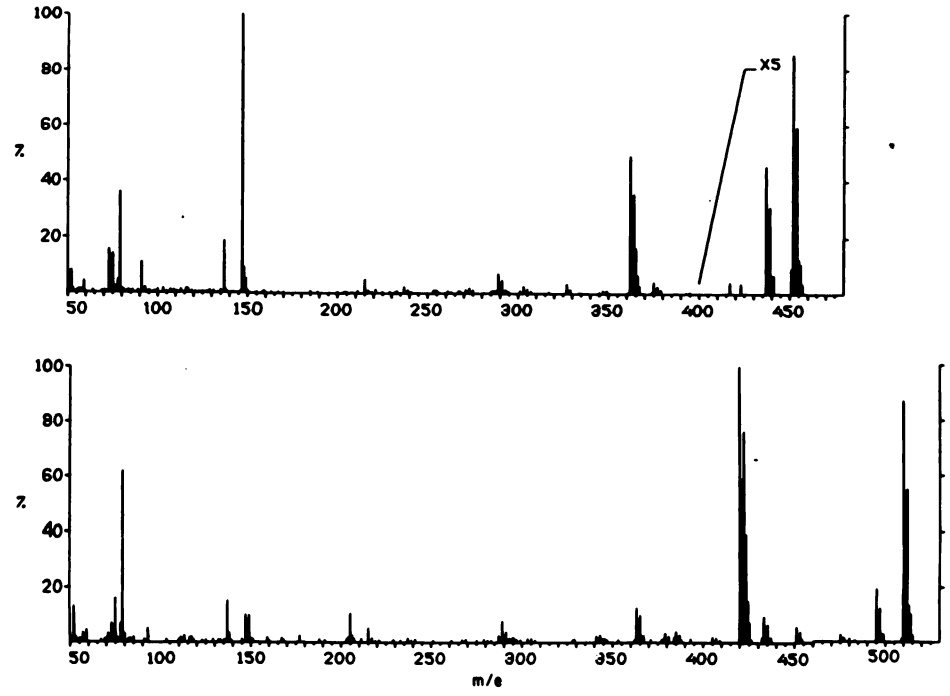

FIG. 2. *Mass spectra of metabolite* I.

The upper spectrum was obtained after methylation and trimethylsilylation of the plasma extract. The lower spectrum was obtained after direct trimethylsilylation.

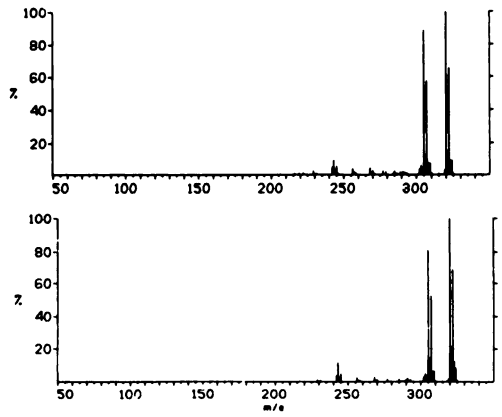

FIG. 3. *Comparison of the mass spectrum of metabolite* III *with that of authentic* III.

Lower spectrum is of authentic 2-methyl-2-phenyl-5-hydroxy-6,7-dichloro-1-indanone and upper spectrum was of a dog urinary metabolite. Both samples were analyzed as the methyl esters.

intact MK-196. Triphasic rates of elimination of drug and radioactivity were observed in the three species. The half-lives, $(t_{1/2})_\beta$ for plasma radioactivity in the rat and dog were relatively rapid (~ 3-4 hr), whereas in the monkey, a $(t_{1/2})_\beta$ of about 40 hr was observed. In the dog, the terminal half-life was estimated to be about 68 hr, whereas the monkey again exhibited a longer terminal half-life (~ 105 hr). The long terminal half-life of this compound in the plasma following a single dose may be due in part to the binding of MK-196 to plasma proteins. We have reported previously (9) that MK-196 and its major chimpanzee metabolite are extensively bound to human and chimpanzee plasma. However, upon repeated administration of the drug to monkeys, we have shown that the clearance of drug is increased, presumably because of an induced metabolic alkalosis resulting from hypochloremia. Clearance studies in the chimpanzee (9) and dog[3] have shown that under conditions of induced metabolic alkalosis (sodium bicarbonate) the plasma concentrations of drug decreased with a concomitant increase in urinary elimination of drug.

Comparison of the excretion profiles for total radioactivity and intact MK-196 after oral and iv doses demonstrate that the compound is well absorbed after oral administration. The major

[3] L. S. Watson and A. G. Zacchei, unpublished results.

route of radioactivity elimination is *via* the feces for the rat (~94%) and dog (~80%). In contrast, the monkey eliminates the majority of the dose (~60%) *via* the urine in a manner analogous to the chimpanzee (9) and man.[4] The difference in route of drug elimination between rat and dog on the one hand and monkey, chimpanzee, and man on the other may be explained in part by the extensive biliary excretion of the drug as observed in the dog. These findings are in agreement with previous observations by others (12, 13) that substances with molecular weights of 300 or greater are excreted in the bile.

Minimal metabolism of MK-196 was observed in the rat, dog, and monkey, whereas the chimpanzee and man showed extensive biotransformation, with the major metabolite characterized as [6,7-dichloro-2-(4-hydroxyphenyl)-2-methyl-1-oxo-5-indanyloxy]acetic acid. Evidence was presented for the existence of minor metabolites in dog plasma, urine, and bile after doses of 40 mg/kg/day for 14 days. These minor metabolites were identified as: (6,7-dichloro-1α-hydroxy-2-methyl-2-phenyl-5-indanyloxy)acetic acid, [6,7-dichloro-2-(4-hydroxyphenyl)-2-methyl-1-oxo-5-indanyloxy]acetic acid and 2-methyl-2-phenyl-5-hydroxy-6,7-dichloro-1-indanone.

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[4] A. G. Zacchei, unpublished results.

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