Fate of the 8,9-Z Isomer of Avermectin B_{1a} in Rats

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The 8,9-Z isomer of avermectin B_{1a} is formed photolytically in the environment and on various crops after treatment with abamectin. Male rats were orally administered a single dose of 1.4 mg/kg ^{3}H-8,9-Z isomer of avermectin B_{1a} (8,9-Z-B_{1a}), and radioactive residues in the tissues and excreta were determined 1, 2, 4, and 7 days postdose. The initial residue in tissues was <1.5 ppm and essentially depleted within 7 days postdose. The depletion half-life of the total residue was approximately 1.5 days for liver, kidney, muscle, and fat. Most of the dose (99%) was recovered in the feces with <1% found in the urine. Unchanged 8,9-Z-B_{1a} levels in the liver tissue were 91 and 50% for 1 and 7 days postdose, respectively. The depletion half-life of the 8,9-Z-B_{1a} in the liver tissues was 1.2 days. Two metabolites were identified in the liver, kidney, muscle, and fat. These two metabolites and the 8,9-Z-B_{1a} accounted for 92–98% of the total tissue residue. The fate of 8,9-Z isomer of avermectin B_{1a} was similar to the fate of avermectin B_{1a} in rats.

Abamectin is the active ingredient in a commercial product that is being developed by Merck & Co. Inc. as an acaricide/insecticide. The major active component in abamectin is avermectin B_{1a} (Figure 1). The degradation of avermectin B_{1a} on cotton, celery, and citrus fruits results in the formation of the 8,9-Z isomer of avermectin B_{1a} (8,9-Z-B_{1a}). The photolysis of avermectin B_{1a} in aqueous or organic solution, or as a film, also results in the formation of 8,9-Z-B_{1a} (Ku et al., 1983; Mrozik et al., 1988). The 8,9-Z-B_{1a} is a geometric isomer of avermectin B_{1a} with a trans to cis olefin isomerization at carbons 8 and 9 (Figure 1). Since the 8,9-Z-B_{1a} is present at low levels in raw agricultural commodities, an investigation of the metabolism of this compound in rats was necessary. The major objective of this study was to determine the fate of 8,9-Z-B_{1a} in rats. The total residue in the tissues and excreta was measured at various times postdose. The level of unchanged 8,9-Z-B_{1a} and the characterization of the residue in selected tissues were conducted. The major metabolite and a minor metabolite present in the liver tissues were identified by cochromatography with standards generated by rat liver microsomal incubations of 8,9-Z-B_{1a}. This report describes the results from rat metabolism studies with [^{3}H]-8,9-Z-B_{1a}. Some of these data have been previously presented (Feely et al., 1987).

MATERIALS AND METHODS

Animal Treatment. Male CRCD rats from 212 to 235 g were obtained from Charles River, Wilmington, MA. After 13-days acclimation the rats were indiscriminately selected for grouping. All treated rats received a single oral (gavage) dose of ^{3}H-8,9-Z isomer of avermectin B_{1a} at 1.4 mg/kg. Three rats each were placed in groups 1–4, which were sacrificed at 1, 2, 4, and 7 days after dosing, respectively. The specific activity of the [^{3}H]-8,9-Z-B_{1a} was 350 μCi/mg with a radiochemical purity of >98.8%. Dosing solution contained 0.70 mg of [^{3}H]-8,9-Z-B_{1a}/mL of sesame oil. Control treated rats received the same volume of sesame oil. After dosing, each group of three rats was housed separately in metabolism cages. Urine and feces for each group were collected daily and frozen until analysis. Respired air was not collected for this study. Animals were given free access to food (Purina rodent chow) and water. There were no toxicological effects in the control and treated rats. At time of sacrifice, rats were anesthetized with ether followed by cardiac puncuture for blood samples. The following samples were taken, combined, and frozen until analysis: bone (hind leg), brain, fat (gonadal), gonads, heart, kidney, liver, GI tract (feces not removed), lung, muscle (hind leg), spleen, and residual carcass (less skin and tail).

Analysis. The radioactivity in the samples was determined by combustion (Packard B306 sample oxidizer) followed by liquid scintillation counting (LSC) (Packard Tri-Carb 460C or 4530). Radioactivity measurements were made with quench correction based on the external standard method. For urine samples, approximately 0.5-mL duplicate aliquots were taken and counted directly. For blood, approximately 0.4-g duplicate samples were combusted directly. For the remaining tissues except the GI tract, approximately a 0.4-g sample of a 1:4 tissue to water homogenate was weighed, combusted, and counted by LSC. GI tract was homogenized 1:3 tissue to water. Fat and bone samples were homogenized without water. Background radioactivity was determined by combustion and LSC of the appropriate control tissues. Radioactivity levels below the detection limit are reported as zero. All samples were combusted in duplicate and reported as the mean. The combustion efficiency for this study was >96%.

8,9-Z-B_{1a} Analysis. To quantitate the level of unchanged 8,9-Z-B_{1a} in the liver, kidney, muscle, and fat tissues from this study, a reverse isotope dilution assay (RIDA) was developed. The procedure developed for this study is shown in Figure 2. The RIDA procedure has been previously described (Chiu et al., 1985; Maynard et al., 1989) and was similar to the assay reported here. The reversed-phase HPLC column used was an IBM ODS (4.6 mm × 25 cm), and the mobile phase was 60:40 CH_3CN/H_2O at a flow of 1 mL/min for the first HPLC purification step and 85:15 CH_3OH/H_2O at 1 mL/min for the second HPLC step (Figure 2). Control tissues were spiked with a known amount of [^{3}H]-8,9-Z-B_{1a} and processed by the RIDA method. The RIDA values for the controls were >95%.

Characterization of the Tissue Residue. As part of the isolation procedure of 8,9-Z-B_{1a} for the RIDA analysis, the ethyl acetate eluate was injected on an HPLC system (Figure 2). The column effluent was monitored for radioactivity with a flow-through radioactivity detector (Flo-one Beta; RadioAnalytic Inc., Tampa, FL.). The 8,9-Z-B_{1a} was removed for subsequent RIDA analysis and is therefore not present on the radioactivity chromatograms. A radioactivity chromatogram of the metabolite residue was obtained for all samples analyzed. A major metabolite and a several minor metabolites were observed in all samples. Since the direct isolation and subsequent structure identification of these metabolites were not possible due to the low levels, the identification was accomplished by cochromatogra-

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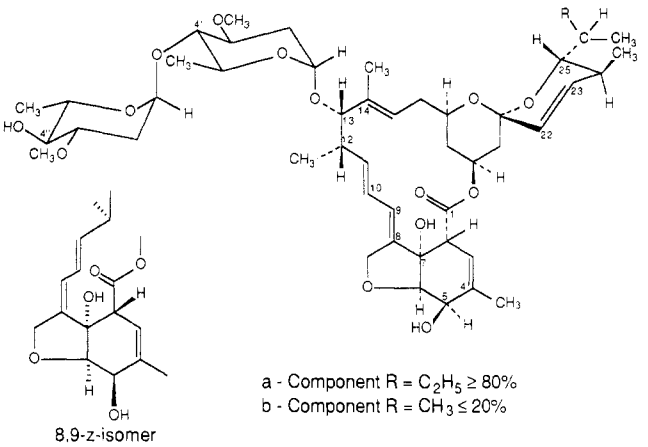

Figure 1. Structures of the two major components of abamectin and the 8,9-*Z* isomer.

phy with standards generated by rat liver microsomal incubations with 8,9-*Z*-B_{1a}.

Microsomal Incubations. An incubation of [^{3}H]-8,9-*Z*-B_{1a} with control rat liver microsomes was conducted to generate metabolite standards. The incubation and isolation procedure was similar to a previous report (Miwa et al., 1982). In the absence of an NADPH-generating system, no metabolites were formed from the microsomal incubations. The 24-(hydroxymethyl)-8,9-*Z*-B_{1a} (24-OHMe-8,9-*Z*-B_{1a}) metabolite was isolated from the microsomal incubations and identified by NMR and mass spectrometry (MS) by criteria used for identification of the 24-OHMe-B_{1a} (Miwa et al., 1982). The 3″-desmethyl-8,9-*Z*-B_{1a} (3″-DM-8,9-*Z*-B_{1a}) metabolite was also isolated from the microsomal incubations and identified by NMR and MS.

Metabolite Identification. The identification of the major metabolite observed in the liver, kidney, muscle, and fat tissues was accomplished by isolating the metabolite from the tissue sample by HPLC, mixing it with the metabolite standard that had the same retention time on the same HPLC system (3″-DM-8,9-*Z*-B_{1a}), and injecting the mixed sample on an HPLC system. The identification of the minor metabolite was accomplished by mixing a metabolite standard that had the same retention time on the same HPLC system (24-OHMe-8,9-*Z*-B_{1a}) with the extract from liver tissue and injecting the mixed sample on an HPLC system. The resulting peak that contained both the tissue metabolite of interest and the standard was analyzed for total radioactivity and elution purity. Furthermore, the radioactivity and absorbance retention times were determined. To ensure identity and purity, the well-defined eluting peak containing both the tissue metabolite and the standard was collected from one HPLC system and injected on a second HPLC system. Again the resulting peak was analyzed for purity.

The [^{3}H]-8,9-*Z*-B_{1a} standard was synthesized by Dr. A. Rosegay, Labeled Compound Synthesis Group, Department of Animal Drug Metabolism, Merck, Sharp and Dohme Research Laboratories, Rahway, NJ. All other solvents and chemicals were reagent or HPLC grade or better and were purchased commercially.

RESULTS AND DISCUSSION

The accountability of the dose recovered in the feces and urine for the group of rats sacrificed 1–7 days postdose is shown in Table I. Only 0.42% of the dose was recovered in the urine for all 7 days postdose. In the feces, 94% of the dose was recovered with most within the first 3 days postdose.

The total residue concentration in the tissues 7 days postdose is shown in Table II. For all tissues analyzed, the total residue was less than 100 ppb, except for fat which was 106 ppb. The GI tract contained the next highest residue level, but this was probably due to the contents within. Therefore, at 7 days after a dose of 1.4 mg/kg 8,9-*Z*-B_{1a} the total residue levels in all tissues analyzed were low (i.e., less than 100 ppb).

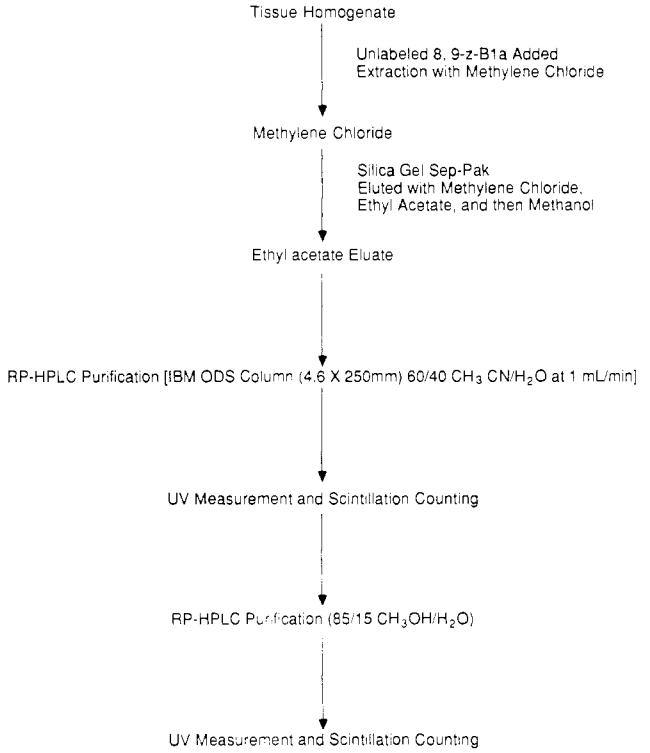

Figure 2. Fractionation and purification procedure for unchanged 8,9-*Z*-B_{1a} in rat tissues.

Table I. Recovery of Radioactivity in Rat Urine and Feces Expressed as a Percent of the Administered Dose

days postdose	feces	urine
1	58.8	0.13
2	15.2	0.12
3	9.0	0.07
4	4.6	0.03
5	3.0	0.03
6	2.2	0.02
7	1.2	0.02
total	94.0	0.42

The total residue levels in the liver, kidney, muscle, and fat tissues at various times postdose are shown in Table III. At all time periods postdose, the fat contained the highest levels. At 1 day postdose, the residue levels ranged from 278 to 1426 ppb. At 7 days postdose, these residue levels decreased to 17–106 ppb. The total residue levels in these tissues were observed in the following order: muscle < kidney < liver < fat. The depletion of the total residue in these tissues followed apparent first-order kinetics. The half-lives were calculated by a linear regression analysis and are shown in Table III. For all tissues, the depletion half-life of the total residue was very similar and between 1.45 and 1.64 days. Therefore, the depletion of the total residue occurs relatively rapidly in liver, kidney, muscle, and fat tissue, and persistence of the residue does not occur when 8,9-*Z*-B_{1a} is administered to rats.

The level of the unchanged 8,9-*Z*-B_{1a} in various tissue samples, as measured by RIDA, is shown in Table IV. For the liver tissue, 91% of the total residue was accounted for as 8,9-*Z*-B_{1a} at 1 day and 50% at 7 days postdose. The depletion half-life of 8,9-*Z*-B_{1a} in liver tissue was 1.23 days as determined by linear regression analysis. At 4 days postdose, the levels of 8,9-*Z*-B_{1a} in liver, kidney,

Table II. Concentration of Total Residue in Various Tissues 7 Days after Administration of the [^{3}H]-8,9-Z Isomer of Avermectin B_{1a}

tissue	residue, ppb	tissue	residue, ppb
blood	3.8	GI tract	59.7
spleen	18.6	bone	12.2
gonads	9.7	muscle	16.9
heart	26.5	fat	106.3
brain	0.6	liver	55.6
lung	27.9	kidney	48.0

Table III. Residue Levels and Depletion Half-Lives in Tissues at Time Periods after [^{3}H]-8,9-Z-B_{1a} Administration

days postdose	[^{3}H]-8,9-Z-B_{1a} equiv, ppm			
	liver	kidney	fat	muscle
1	0.912	0.596	1.426	0.278
2	0.640	0.440	1.381	0.227
4	0.298	0.218	0.647	0.098
7	0.056	0.048	0.106	0.017
half-life, days	1.48	1.64	1.54	1.45

Table IV. Characterization of the Tissue Residue by RP-HPLC Radioactivity Chromatography and Levels of 8,9-Z-B_{1a} by RIDA Analysis (%)

			radioactivity profiles		
				metabolite	
tissue	days postdose	RIDA: 8,9-Z-B_{1a}	8,9-Z-B_{1a}	A	B
liver	1	91	81.6	0.2	16.1
	2	79	72.9	0.2	24.0
	4	67	63.7	<0.2	30.7
	7	50	51.7	<0.2	40.2
kidney	4	71	68.6	<0.2	27.2
muscle	4	68	69.7	<0.2	26.4
fat	4	95	94.1	<0.2	3.6

and muscle tissue were similar (67–71%) while the level in fat was higher (95%).

The liver, kidney, muscle, and fat tissue residue was characterized by HPLC radioactivity chromatograms. A representative chromatogram of the liver tissue 4 days postdose is shown in Figure 3. The HPLC system was the first system shown in Figure 2. The 8,9-Z-B_{1a} was removed for subsequent RIDA analysis and is therefore not shown in the radiochromatogram. The peaks of radioactivity corresponding to metabolites A and B are indicated. From these chromatograms, the levels of these two metabolites and 8,9-Z-B_{1a} as a percent of the total residue were determined and are shown in Table IV. Radioactivity in several small peaks and column wash accounted for a few percent of the total radioactivity. As shown in Table IV, the level of 8,9-Z-B_{1a} when measured as a percent of the eluting radioactivity was similar to the level when determined by RIDA. The level of metabolite B in the liver tissue was 16.1% at 1 day and 40.2% at 7 days postdose. At 4 days postdose, the level of metabolite B was similar for liver, kidney, and muscle tissue (26–31%) but only 3.6% for fat tissue. The presence of metabolite A was observed for most tissues analyzed; however, its levels were ≤0.2% of the total extractable tissue residue. Metabolite A was of interest since it may correspond to a metabolite that was identified in tissues from rats administered avermectin B_{1a}. From these data, the residue was qualitatively similar in all tissues analyzed.

To obtain the identity of metabolites A and B, the metabolites were isolated from liver tissue (group 1) and cochromatographed with known standards. These standards were generated and purified from rat liver microsomal incubations of [^{3}H]-8,9-Z-B_{1a} and identified by NMR and MS. These standards had the same retention times as the corresponding metabolites on several HPLC systems. Metabolite B isolated from liver tissue was mixed with tritiated 3″-desmethyl-8,9-Z-B_{1a} (3″-DM-8,9-Z-B_{1a}) and chromatographed with the first HPLC system shown in Figure 2. Since both metabolite B and the 3″-DM-8,9-Z-B_{1a} were ^{3}H-labeled, the criteria for cochromatography were the sum of the added radioactivity and the peak shape. The resulting radioactivity chromatogram is shown in Figure 4. All the radioactivity corresponding to metabolite B and the 3″-DM-8,9-Z-B_{1a} eluted as a single peak. This peak was collected from this HPLC system and injected on the second HPLC system shown in Figure 2. The second cochromatography is shown in Figure 5. Again, all the radioactivity corresponding to metabolite B and 3″-DM-8,9-Z-B_{1a} eluted as a single, well-defined peak. Therefore, the major metabolite in the liver, kidney, muscle, and fat tissues from rats administered 8,9-Z-B_{1a} was identified to be 3″-DM-8,9-Z-B_{1a}.

The cochromatography of metabolite A present in liver tissue extract and the 24-OH-8,9-Z-B_{1a} standard was conducted in a manner different from that done for metabolite B. A polar metabolite fraction from the liver tissue extract (group 1) was isolated by a previously reported method involving two solvent partitioning steps (Miwa et al., 1982). A radioactivity profile of this polar metabolite fraction from the liver tissue extract is shown in Figure 6. For this HPLC system the column was the same as before only with a mobile phase of CH_3OH/CH_3CN/H_2O (32.5:32.5:35) at a flow rate of 1 mL/min. Metabolite A elutes in fractions 20–22 with the presence of other metabolites observed in this isolated polar fraction (e.g., 11, 13, 16, 26, 34). A known amount of 24-OHMe-8,9-Z-B_{1a} standard was added to this polar metabolite fraction and chromatographed on the same RP-HPLC system. The radioactivity corresponding to the 24-OHMe-8,9-Z-B_{1a} standard eluted with metabolite A (fractions 20–22) as a single, well-defined peak (Figure

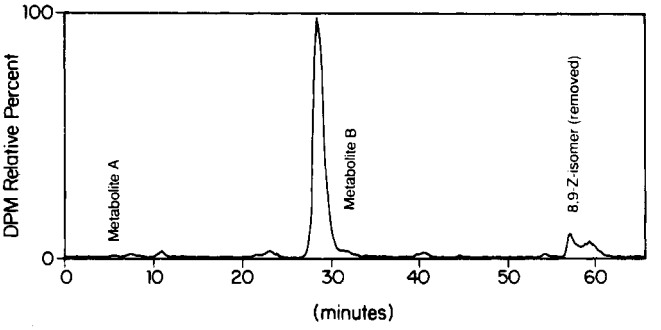

Figure 3. RP-HPLC radioactivity chromatogram of liver tissue extract 4 days after dosing. RP-HPLC conditions: IBM ODS column (0.46 × 25 cm) with a mobile phase of 60:40 CH_3CN/H_2O at a flow of 1 mL/min.

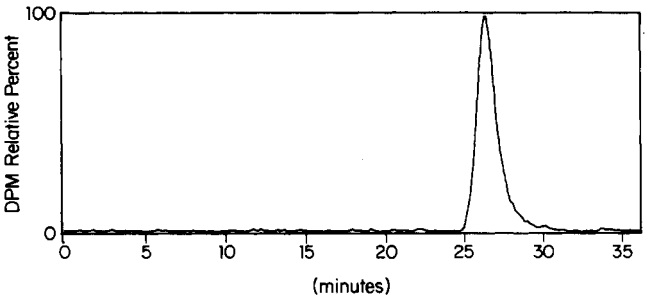

Figure 4. RP-HPLC radioactivity chromatogram of metabolite B isolated from liver tissue and 3″-DM-8,9-Z-B_{1a} standard. Same HPLC conditions as Figure 3.

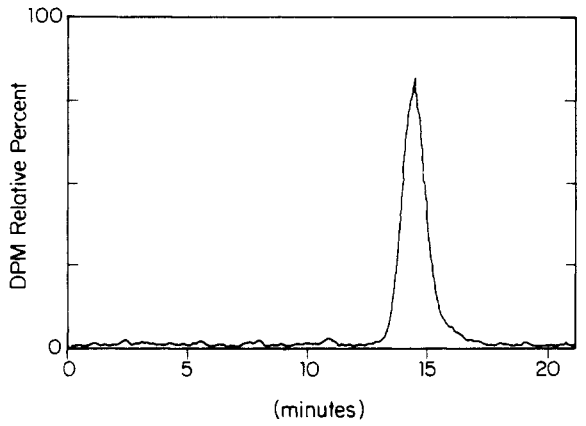

Figure 5. RP-HPLC radioactivity chromatogram of metabolite B from liver tissue and 3″-DM-8,9-*Z*-B_{1a} standard combined and isolated from peak in Figure 4. Same column as Figure 3 with 85:15 CH_3OH/H_2O at a flow rate of 1 mL/min.

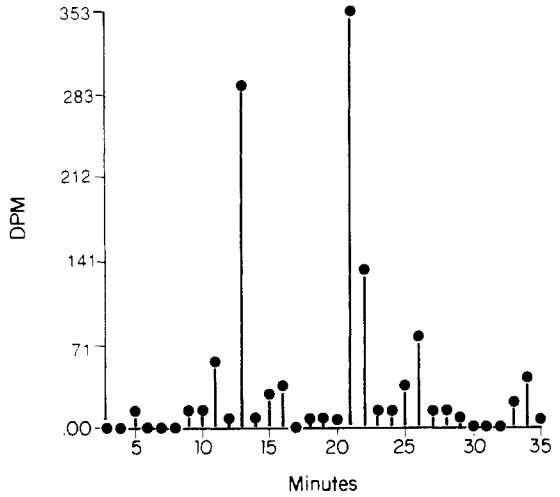

Figure 6. RP-HPLC radioactivity profile of the polar metabolite fraction from liver tissue extract 1 day after dosing. Same column as Figure 3 with 32.5:35:32.5 $CH_3OH/H_2O/CH_3CN$ at a flow rate of 1 mL/min.

7A). The ^3H from the 24-OHMe-8,9-*Z*-B_{1a} standard added and metabolite A present was accounted for in this peak. Futhermore, the absorbance peak from the 24-OHMe-8,9-*Z*-B_{1a} standard corresponded to the radioactivity peak from the standard and metabolite A. This composite peak (20–22) from the first cochromatography was collected and injected on a second RP-HPLC system, and the radioactivity and absorbance cochromatogram are shown in Figure 7B. Again, the same column but a different mobile phase of CH_3CN/H_2O (50:50) at a flow rate of 1 mL/min was used. As shown, the radioactivity from the standard and metabolite A eluted identically in a single peak. The absorbance from the standard again corresponded to the radioactivity peak. Therefore, the identity of metabolite A in liver tissue was confirmed by two cochromatographies to be 24-OHMe-8,9-*Z*-B_{1a}.

From these results, the major metabolite, 3″-DM-8,9-*Z*-B_{1a}, was identified in liver, kidney, muscle, and fat tissues while the minor metabolite, 24-OHMe-8,9-*Z*-B_{1a}, was identified in liver tissue and observed in most of the other tissues. These two metabolites and unchanged 8,9-*Z*-B_{1a} accounted for 92–98% of the total tissue residue. These two corresponding metabolites (3″-DM-B_{1a} and 24-OHMe-B_{1a}) were also identified in these tissues from rats dosed with avermectin B_{1a} and in rat liver microsomal incubations of avermectin B_{1a} (Maynard et al., 1985a). In addition, the 24-OHMe-B_{1a} and 3″-DM-B_{1a} were identified

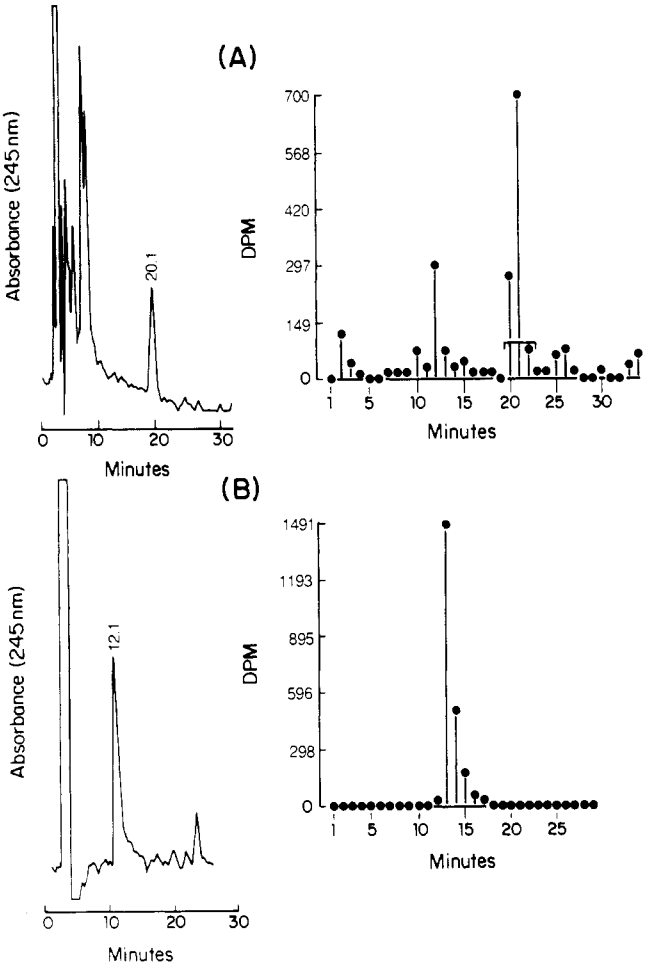

Figure 7. RP-HPLC radioactivity profile and chromatograms: (A) polar metabolite fraction from liver tissue and 24-OHMe-8,9-*Z*-B_{1a} standard; (B) collection and injection of combined peak (20–22) from (A) on a second HPLC system. RP-HPLC conditions: (A) same as described in Figure 6; (B) same column as Figure 3 with 50:50 CH_3CN/H_2O at a flow rate of 1 mL/min.

in tissues from goats administered avermectin B_{1a} (Maynard et al., 1985b, 1989).

In conclusion, the fate of 8,9-*Z*-B_{1a} in rats was determined. The initial tissue residue concentrations were 1 ppm or less, with tissue residues depleting to 100 ppb or less within 7 days postdose. Of the dose 94% was excreted in the feces, with 2% or less recovered in the urine or tissues. The total residue in the liver, kidney, muscle, and fat tissues depleted with a half-life of 1.5 days, indicating that these low levels of residue of 8,9-*Z*-B_{1a} do not persist. The characterization of the residue was qualitatively similar among these tissues and between male and female rats administered 8,9-*Z*-B_{1a}. Identification of 92–98% of the tissue residue was accounted for as 8,9-*Z*-B_{1a}, 24-OHMe-8,9-*Z*-B_{1a}, and 3″-DM-8,9-*Z*-B_{1a}. The metabolism of 8,9-*Z*-B_{1a} in rats was similar to the metabolism of avermectin B_{1a} (Maynard et al., 1985a) and ivermectin B_{1a} (22,23-dihydroavermectin B_{1a}) (Chiu et al., 1986) in rats.

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Registry No. 8,9-(*Z*)-Avermectin B_{1a}, 113665-89-7; 24-(hydroxymethyl)-8,9-(*Z*)-avermectin B_{1a}, 12327-29-6; 3″-demethyl-8,9-(*Z*)-avermectin B_{1a}, 123237-30-9.

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Lactating goats were administered [^{3}H]avermectin B_{1a} by 10 daily oral doses at one of three dose levels (0.005, 0.05, 1.0 mg/kg). Excreta and milk were collected daily and the total radiolabeled residues measured. After the fourth dose, maximal residue levels were observed in the excreta and milk. One day after the last dose, various goat tissues were obtained and the total radiolabeled residue was measured. Tissue residue levels were dose dependent and were less than 100 ppb for goats treated at the high dose (1 mg/day). Most of the dose (89–99%) was recovered in the feces, with <1% recovered in the urine or milk. The levels of avermectin B_{1a} in the edible tissues were mostly between 70 and 95% of the total residue for all goats. The major metabolite in the edible tissues and milk was identified as 24-(hydroxymethyl)avermectin B_{1a}. A minor metabolite, 3″-desmethylavermectin B_{1a}, was also identified in the edible tissues. Both metabolites were previously identified in the corresponding tissues of rats administered avermectin B_{1a}. The parent and these two metabolites accounted for 85–99% of the total residue in the edible tissues, milk, and feces of the goats. The extent of tritium exchange was determined to be <0.1% of the dose.

Avermectins are a new class of biological agents that contain a macrocyclic lactone and are produced by *Streptomyces avermitilis* (Burg et al., 1979). The structures have been elucidated (Albers-Schonberg et al., 1981), and some of the biological activities have been reported (Campbell et al., 1983). Abamectin is a commercial product of the avermectins that is being developed by Merck & Co. Inc. as an acaricide/insecticide. The major active ingredient in abamectin is avermectin B_{1a} (Figure 1). This report describes the findings from goat metabolism studies with avermectin B_{1a}. Some of these data have been previously presented (Maynard et al., 1985a).

In these studies, lactating Nubian goats (40–51 kg) were orally dosed daily for 10 days with 3H-labeled avermectin B_{1a} at three dose levels. The dosage rates of avermectin B_{1a} administered to lactating goats were 0.005, 0.05, and 1.0 mg/goat per day. The low dose was calculated on the basis of an anticipated total daily feed intake of range grass treated with abamectin fire ant bait (Affirm) at 50 mg/acre. The intermediate dose was calculated on the basis of an anticipated total daily feed intake of dried citrus pulps from fruit treated with abamectin miticide (Agrimec) (Maynard et al., 1989a). The high dose represents an exaggerated rate to better study the metabolism in lactating ruminants.

The objective of this study was to determine the fate of the total [^{3}H]avermectin B_{1a} residue in goat tissues, excreta, and milk. Furthermore, the level of avermectin B_{1a} and the identification of metabolites were deter-

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