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Metabolism of Monensin in the Steer and Rat

A. Donoho,* J. Manthey, J. Occolowitz, and L. Zornes

The metabolism of [^{14}C]monensin was studied in both cattle and rats. Monensin was metabolized to many different compounds by both species. The metabolite pattern was qualitatively similar but quantitatively different in the two species. Six fecal metabolites were tentatively identified. Five resulted from O-demethylation and/or hydroxylation and one resulted from demethylation and decarboxylation. The radioactive residue in steer liver was characterized and the metabolite pattern was qualitatively similar to that of feces. The most abundant metabolite accounted for only 6% of the liver radioactivity.

Herberg et al. (1978) have described the excretion and tissue distribution pattern when [^{14}C]monensin was fed to cattle. The radioactivity was excreted rapidly and quantitatively in the feces. Liver was the only edible tissue which contained a residue of radioactivity greater than 0.021 ppm in cattle sacrificed 12 h after either a 2- or 5-day [^{14}C]monensin dosing period. The mass spectrometry of four monensin metabolites from the rat was described by Donoho and Occolowitz (1975).

The studies described in this report were undertaken to characterize the radioactivity in feces and liver from [^{14}C]monensin-treated cattle and to compare the metabolic patterns in cattle and rats. The sodium salt of [^{14}C]-monensin was used throughout the studies. For convenience, the term monensin is used to indicate monensin sodium except where stated otherwise.

EXPERIMENTAL SECTION

Radiolabeled Compound. [^{14}C]Monensin was prepared by fermentation as described by Day et al. (1973), using as substrates propionate-*1*-^{14}*C* in combination with either propionate-*2*-^{14}*C* or butyrate-*1*-^{14}*C*. The propionate/propionate combination labeled the monensin in 14 positions including the carboxyl carbon and two carbons in each ring except ring C (see Day et al., 1973, for details). The propionate/butyrate combination labeled monensin in eight positions, including the carboxyl carbon and each of the five rings.

Experimental Samples. The cattle feces sample used for the comparative metabolite study was from a steer dosed orally for 5 days with 330 mg/day of [^{14}C]monensin, 0.25 μCi/mg, synthesized using the propionate/butyrate combination of substrates (steer 6 from the studies of Herberg et al., 1978). All other samples were derived from animals fed [^{14}C]monensin synthesized using the propionate/propionate combination.

Liver was obtained from a steer which was dosed orally for 2 days with 300 mg/day of [^{14}C]monensin, 0.283 μCi/mg, and sacrificed 12 h after the last dose (steer 4 from the studies of Herberg et al., 1978). Feces for isolation and identification of metabolites were obtained from steer 4 or one dosed in a similar manner.

Feces from Wistar-derived albino rats dosed orally ([^{14}C]monensin, sp act. 0.576 μCi/mg, 16 mg/kg) for 2 days were used for comparative studies.

Comparative Metabolite Study. Two grams of rat feces were macerated with added water to give a smooth paste and extracted with 200 mL of methanol by blending for 15 min. The sample was filtered, the extraction was repeated, and the combined filtrates were evaporated to 200 mL. The sample was diluted with 100 mL of 10% aqueous sodium chloride solution (aqueous NaCl) and extracted with $CHCl_3$–CCl_4 (1:1, 3 × 100 mL, then 3:1, 2 × 100 mL). The combined $CHCl_3$–CCl_4 fractions were evaporated, and the sample was reconstituted in 100 mL of methanol–aqueous NaCl (8:2). This sample was extracted with hexane (2 × 100 mL), and the combined hexanes were extracted with 100 mL of methanol–aqueous NaCl (8:2). The combined aqueous methanol fraction was diluted with 80 mL of aqueous NaCl and extracted with $CHCl_3$–CCl_4 (3:1, 3 × 200 mL). The extract was purified by gradient elution column chromatography on a 1 cm i.d. × 60 cm stainless steel column packed with Quantum LP-1 silica gel, 10–20 μ (Quantum Industries, Fairfield, N.J.). The sample was evaporated onto 8 mL of Woelm dry column silica gel, No. 202 (ICN Pharmaceuticals, Cleveland, Ohio), and placed in a 10-cm stainless steel extension on top of the column. The column was eluted at a flow rate of 9.9 mL/min using a Waters Model 6000 liquid chromatography pump. A nonlinear gradient was developed by pumping solvent from a 190-mL stirred reservoir filled with one solvent into which a more polar solvent was introduced as the elution progressed. The pump was situated between the reservoir and the column to avoid pressurization of the glass reservoir. The solvent sequence for elution is described in the Results and Discussion section. Fractions of 20 mL each were collected and aliquots were assayed for radioactivity by liquid scintillation counting (LSC). Specific fractions were pooled and aliquots of the pools were separated by thin-layer chromatography (TLC) on silica gel plates with a preadsorbent zone for sample application (Quantum LQ plates). Plates were developed in equilibrated chambers.

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Table I. Primary Ions from EI Mass Spectra of Monensin Sodium and Its Metabolites

fragmentation	monensin sodium[a]	m/e M-1	M-2	M-3	M-4	M-5
M^+	692	678	694	694	694	708
a	661	647	663	663	663	677
b	617	617	633	633	633	633
c	575	561	561	561	577	591
d	547	547	563	563	563	563
e	491	477	477	477	493	507
f	463	463	479	479	479	479
g	405	405	421	421	421	421
h	379	365	365	365	365	379
k	321	321	337	337	337	337
l	449	435	435[b]	435[b]	435[b]	449

[a] Peak assignments for all metabolites except M-5 were supported by elemental compositions determined by accurate mass measurement. [b] For these metabolites, *m/e* 435 was found to consist of ions with the compositions $C_{22}H_{36}O_7Na$ and $C_{23}H_{40}O_6Na$. The former arises from fragmentation l while the latter involves fragmentation across the B ring (fragmentation g + CH_2).

Solvent system 1 consisted of ethyl acetate–diethyl ether-3A alcohol–water–diethylamine, 100:100:3:3.5:4. Solvent system 2 consisted of toluene–ethyl acetate–methanol, 90:100:10. Solvent system 3 consisted of ethyl acetate–methanol–water, 70:30:3. Metabolites were visualized by autoradiography or by reaction with acid–vanillin (1 mL of concentrated H_2SO_4 and 3 g of vanillin in 100 mL of 3A alcohol) according to the procedure described by Golab et al. (1973).

A 100-g sample of feces from steer 6 was processed as described above except that twice the volumes of solvents were used in the extraction and partition steps.

Extraction and Purification of Steer Liver. A 500-g sample of steer liver was extracted by blending with methanol (2 mL/g), followed by in vacuo filtration after addition of Hyflo Super-Cel as a filter aid. This extraction was performed four times, and the combined extracts were diluted with one-half volume of aqueous NaCl and the metabolites were extracted into $CHCl_3$. The $CHCl_3$ extract was evaporated and partitioned between hexane and aqueous methanol essentially as described above. The metabolites were recovered from the diluted aqueous methanol by extraction with $CHCl_3$. The $CHCl_3$ extract was evaporated and purified by chromatography on an 8 mm i.d. × 1 m glass column packed with Woelm dry column silica gel. The column was eluted by gradient elution as described above using the solvent sequence described in the Results and Discussion section.

Isolation and Identification of Fecal Metabolites. Rat and steer feces samples of various sizes were extracted and partitioned generally as described above and metabolites were separated by gradient elution chromatography on columns packed with Woelm dry column silica gel. For the preparative scale separations, column size was varied to accommodate feces samples of 50–500 g.

Some partially purified metabolite fractions were further purified by reversed-phase high-pressure liquid chromatography (LC) on a 4.6 mm i.d. × 1 m stainless steel column packed with Bondapak, C18/Porasil B (Waters Associates, Framingham, Mass.), using methanol–water mixtures as the mobile phase.

During fractionation, radioactivity was monitored by LSC using the same techniques described by Herberg et al. (1978). Solid samples were counted after combustion to $^{14}CO_2$ and liquid samples were counted directly.

Mass spectra were obtained with a Varian-MAT Model 731 mass spectrometer. Electron impact spectra were obtained by direct introduction of the sample into the ion source. The electron energy was 70 eV and the ion source temperature was 250 °C. Accurate mass measurements

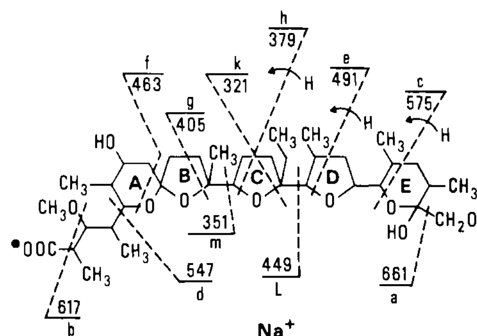

Figure 1. Structure of monensin sodium showing the major fragments which arise from electron impact mass spectrometry. The sodium ion is retained in all of the fragments shown.

were made from photoplates or by peak matching at an electrical resolution of 15 000. Field desorption spectra were obtained from carbon dendrite emitters with the Model 731 mass spectrometer.

RESULTS AND DISCUSSION

Identification of Fecal Metabolites. Extensive preliminary studies were conducted to find appropriate procedures for extraction and separation of monensin and its metabolites. The application of these procedures in evaluation of the number and quantity of metabolites is described in the next section.

During the metabolism studies it became apparent that monensin was converted to a large number of metabolites by both the steer and rat. Six fecal metabolites, designated as M-1 to M-6, were isolated. Tentative identification of these six metabolites was established by comparison of their mass spectra to the spectrum of monensin. Monensin sodium and monensin acid give different fragmentation patterns upon electron impact mass spectrometry, and all of the major ions from monensin sodium contain a sodium atom (Chamberlin and Agtarap, 1970). The structure of monensin sodium and the major ions produced by electron impact mass spectrometry are shown in Figure 1. The tentative structures of the six fecal metabolites are shown in Figure 2. Five of the metabolites had fragmentation patterns similar to that of monensin sodium. The major ions of monensin sodium and the corresponding ions from these five metabolites are shown in Table I. The mass spectrum of M-1 is given in Figure 3. The sixth metabolite had a fragmentation pattern similar to that of monensin acid and that mass spectrum is shown in Figure 4.

Metabolite M-1 was isolated from both rat and steer feces. The appropriate fraction from the silica gel column

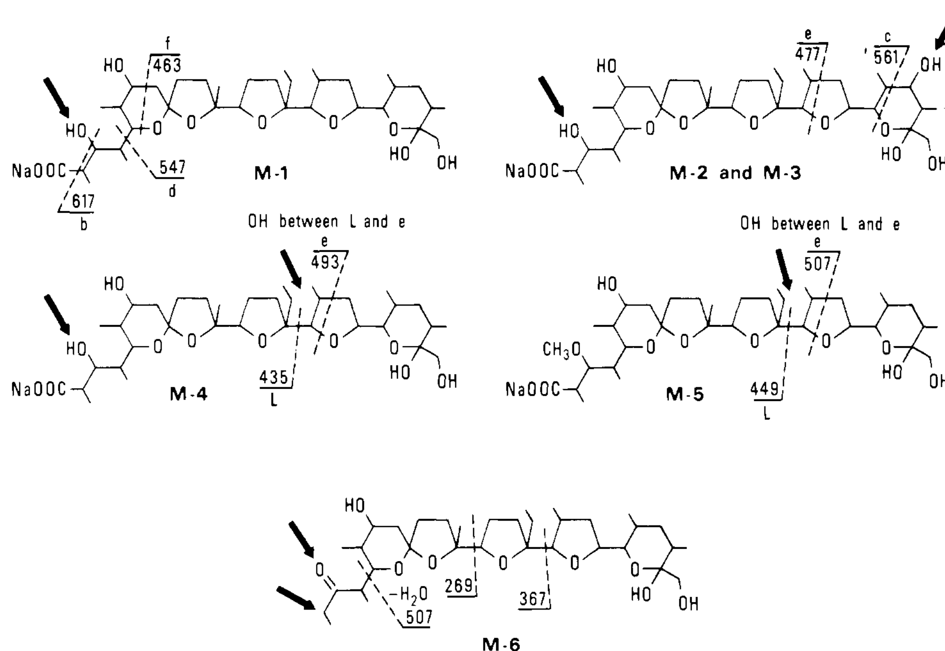

Figure 2. Structures of monensin metabolites showing critical fragmentations for structure elucidation. Arrows indicate areas of metabolism.

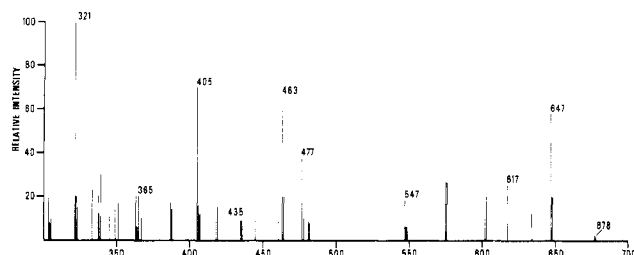

Figure 3. Electron impact mass spectrum of M-1.

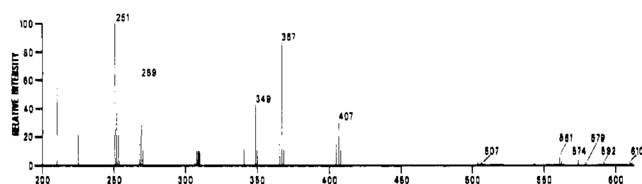

Figure 4. Electron impact mass spectrum of M-6.

was collected and further purified by LC (Bondapak C18, methanol–water, 7:3). High- and low-resolution mass spectra indicated that M-1 was O-demethylated monensin. The indicated molecular weight was 678, equivalent to monensin minus CH_2. The fragmentation pattern, shown in Figure 2, indicated that the loss of methyl occurred to the left of fragmentation f since both monensin sodium and M-1 gave m/e 463. Monensin sodium gave ions of m/e 617 and 547 as a result of losing $CH_3O + CO_2$, then loss of C_5H_{10}. M-1 had identical ions corresponding to loss of $OH + CO_2$, then loss of C_5H_{10}. This identified the missing methyl group as the methoxy methyl.

Additional evidence was obtained by acetylation of the primary and secondary -OH groups and methylation of the carboxyl group of M-1 as described by Chamberlin and Agtarap (1970) for monensin. The mass spectrum of this derivative indicated a molecular weight of 796. The corresponding monensin derivative had a molecular weight of 768. Thus, the M-1 derivative differed by 28, equivalent to replacement of $-OCH_3$ by acetate in the derivative or by -OH in the underivatized molecule.

Metabolites M-2 and M-3 were isolated from rat feces. A fraction containing the metabolites was recovered from the silica gel column, and M-2 and M-3 were separated by LC (Bondapak C18, methanol–water, 6:4). M-2 constituted about two-thirds of the total fraction and M-3 about one-third. These two compounds had identical mobilities by TLC in solvent system 1 and were poorly separated in solvent system 2. Both gave a yellow-to-orange vanillin color. Also, both gave low-resolution electron impact mass spectra which were identical in all major points. The high-resolution spectrum of the sodium salt, together with its field desorption spectrum, indicated that M-2 had a molecular weight of 694 and a composition of $C_{35}H_{59}O_{12}Na$. This is equivalent to O-demethylated monensin plus one oxygen.

M-2 and M-3 had fragmentation patterns similar to M-1 except that many of the fragments contained an extra oxygen. Ions at m/e 477 and 561 from fragmentations e and c, respectively, occurred in both M-1 and M-2. This indicated that the additional oxygen in M-2 (and M-3) was to the right of fragmentation c (Figure 2). Determination of the exact position is not possible from the mass spectra. However, the close similarity of the M-2 and M-3 spectra, together with the similarity of their chromatographic characteristics, suggest that they are an epimer pair rather than positional isomers. Thus, they were tentatively identified as shown in Figure 2.

Metabolite M-4 was isolated from rat feces. It had a TLC mobility slightly greater than M-2 in solvent system 1 and gave a yellow-orange vanillin color. The mass spectrum showed the molecular weight to be 694, i.e., the same as M-2 and M-3. The fragmentation pattern indicated M-4 to be an O-demethylated compound with an additional oxygen in the middle of the molecule. The critical fragments for location of the oxygen were m/e 435 and 493 which placed the O to the right of fragmentation l and to the left of fragmentation e (Figure 2).

Metabolite M-5 was a minor component which was separated by LC and TLC during the isolation of M-1 from the steer feces. This metabolite gave a pink vanillin color similar to that of monensin. The mass spectrum indicated the molecular weight to be 708 (i.e., monensin sodium + oxygen). The critical fragments for location of the oxygen were m/e 449 and 507 which placed it to the right of fragmentation l and left of fragmentation e (Figure 2). The

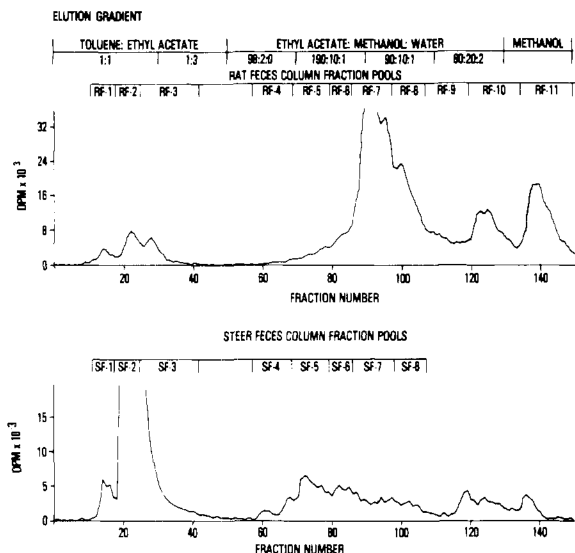

Figure 5. Comparative silica gel (Quantum LP-1) column chromatography of feces extracts from [^{14}C]monensin-dosed rat (above) and steer (below). Elution was initiated with toluene and proceeded with the gradient shown.

exact location is not known. It is possible that M-4 and M-5 have the same structure except that M-4 is O-demethylated.

Metabolite M-6 was the major component of the silica gel column fraction eluting before monensin. This metabolite was observed first in steer liver and was subsequently isolated from steer feces. It constituted 2% or less of the total radioactivity in the steer feces. This compound had a TLC mobility similar to monensin in solvent system 1 and a TLC mobility less than monensin in solvent system 2. The vanillin color was purple and the reaction was rather insensitive relative to monensin. The mass spectrum of M-6, which gave no m/e 23 (Na^+), indicated that it was not the sodium salt as were the other metabolites. By field desorption and electron impact mass spectrometry, the indicated molecular weight was 610. The high-resolution mass spectrum gave a fragmentation pattern somewhat similar to monensin acid. The critical points in the mass spectrum are the molecular weight of 610 (monensin acid minus CH_3COOH) and the fragment m/e 507. This fragment which had a composition of $C_{29}H_{47}O_7$ (observed mass 507.332, calculated 507.333) is derived from the loss of the side chain off ring A in monensin together with the elimination of a molecule of water. Since this ion occurs in M-6 also, the loss of CH_3COOH is probably in the side chain. The proposed structure of this metabolite is shown in Figure 2.

Numerous other fractions from both rat and steer feces extracts were processed in an attempt to isolate additional metabolites. All of these fractions contained several metabolites, none of which were isolated in sufficient quantity for identification.

Comparative Metabolite Study. Steers given oral doses of [^{14}C]monensin excreted the radioactivity quantitatively in the feces. During the preliminary phase, balance studies were conducted in rats and less than 1% of an oral [^{14}C]monensin dose was excreted in the urine. The remainder was in the feces. Therefore, feces were the only excreta samples examined for metabolites.

A 2-g rat feces sample containing approximately 1.5 × 10^6 dpm and a 100-g feces sample from steer 6 containing approximately 8 × 10^5 dpm were processed as described in the Experimental Section. Distribution of radioactivity during the extraction and partition steps is shown in Table II. The bulk of the radioactivity was extracted from the feces and carried through into the $CHCl_3$–CCl_4 extracts (89 and 76% for the steer and rat, respectively). The radioactive metabolites in these extracts were fractionated by silica gel column chromatography, and the resulting chromatograms are shown in Figure 5. Selected fractions were pooled together for further analysis. Details of the column elution gradient and the pooling of fractions are shown in Figure 5.

Table II. Percent of Radioactivity in Various Fractions during Extraction and Partition of Feces Samples

	steer	rat
feces after extraction	4	11
hexane extract	3	1
aqueous methanol (after $CHCl_3/CCl_4$)	4	12
$CHCl_3/CCl_4$ extract	89	76

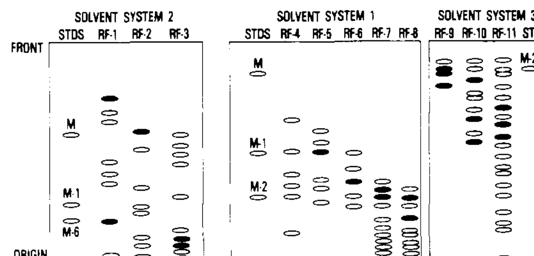

Figure 6. Diagrammatic representation of TLC autoradiograms from rat feces column fraction pools. Selected metabolites and monensin (M) were applied as standards. Dark zones (●) and light zones (○) of radioactivity are indicated.

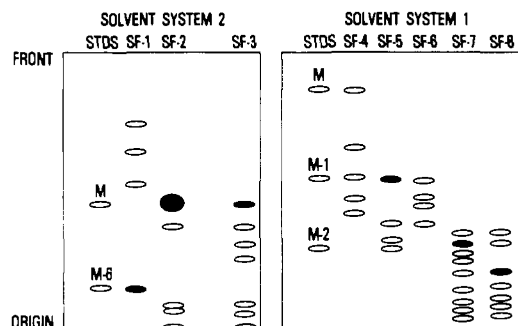

Figure 7. Diagrammatic representation of TLC autoradiograms from steer feces column fraction pools. Selected metabolites and monensin (M) were applied as standards. Dark zones (●) and light zones (○) of radioactivity are indicated.

Metabolites in the column pools were further separated by TLC, and autoradiograms of the plates were prepared. The results of this analysis are shown in Figure 6 and 7 for the rat and steer samples, respectively. Fraction pools 1–8 for both the rat and steer were each applied to two sets of TLC plates along with selected metabolite standards. One set was developed in solvent system 1 and the other set in solvent system 2. For fraction pools 1–3 the best resolution was obtained with solvent system 2. For fraction pools 4–8 the best resolution was obtained with solvent system 1. These autoradiograms are shown in Figure 6 and 7. The radioactivity in the polar column fractions remained at or near the origin of TLC plates developed in solvent systems 1 and 2. Therefore, fractions RF-9–RF-11 were separated using solvent system 3. Definitive autoradiograms of the corresponding fractions from the steer feces sample were not obtained because of large quantities of feces coextractives which resulted in overloading of the TLC plates.

The proportion of total fecal radioactivity in each fraction pool was determined, and estimates of quantities

Table III. Radioactivity (as Percent of Total) in Various Column and TLC Fractions from Extracts of Rat and Steer Feces

rat				steer			
column		TLC		column		TLC	
fraction	%	metabolite	%	fraction	%	metabolite	%
RF-1	1.2	M-6	1	SF-1	2.9	M-6	2
RF-2	2.5	monensin	2	SF-2	48.0	monensin	45
RF-3	2.3			SF-3	9.5	monensin	6
RF-4	0.7			SF-4	2.3		
RF-5	2.0	M-1	>1	SF-5	6.2	M-1	4
RF-6	2.9	M-1	<1	SF-6	3.1	M-1	1
RF-7	26.4	M-2, M-3	20	SF-7	4.0	M-2	2
		M-4	1			M-4	<1
RF-8	9.9	M-2, M-3	1	SF-8	2.3		
		M-4	<1				
RF-9	5.3						
RF-10	7.5						
RF-11	11.4						

of the more abundant metabolites were made by LSC of segments scraped from the TLC plates. These results are presented in Table III. Recovery of radioactivity from the silica gel columns was greater than 97% as determined by combustion analysis of the spent column packing.

The most prominent difference between the rat and steer feces was the relative abundance of monensin. Fractions SF-2 and SF-3 contained 57% of the steer feces radioactivity and by TLC at least 90% of this was monensin. Thus, the monensin level in feces from steer 6 was estimated to be approximately 50% of the total radioactivity. In contrast, the monensin peak from rat feces, RF-2, contained approximately 2% of the radioactivity.

In a separate experiment, a similar fractionation was conducted on a feces sample from steer 4. Approximately 40% of the radioactivity in that sample was monensin.

The nonmonensin radioactivity in the feces of both the rat and steer was widely distributed among many metabolites. Even allowing that metabolites in adjacent column fractions with the same TLC mobility were identical, there was evidence for approximately 50 metabolites in the rat feces. The pattern was even more complex than appears from Figures 6 and 7. For example, the metabolite with the same mobility as M-1 in solvent system 1 in fractions SF-4 and RF-4 was not M-1 since it was separated from M-1 in solvent system 2. The metabolite zone in RF-7 with the mobility of M-2 contained both M-2 and M-3 as described previously. Most of the metabolites which were visualized by autoradiography appeared as faint zones which individually did not exceed 1% of the total fecal radioactivity.

The most abundant metabolite in the steer feces sample was M-1 which was present primarily in SF-5 and to a lesser extent in SF-6, giving a combined estimate of 5% (total fecal radioactivity). Similar analysis indicated a level of 2% for M-2 which appeared as the dark zone in SF-7 (Figure 7) and a level of 1% or less for M-4 which was just above M-2 in SF-7. Presumably, the M-2 zone contained both M-2 and M-3. However, this was not verified for the steer.

Metabolite M-6 was present in SF-1 and the estimated level was 2%. The exact location of M-5 on these chromatograms was uncertain because there was insufficient metabolite isolated for use as a TLC standard. However, the chromatographic characteristics observed during isolation suggested that M-5 was the metabolite in SF-4 which had the same TLC mobility as M-1 (Figure 7).

A similar evaluation was made for the rat feces metabolites. The most abundant metabolite zone was M-2 and M-3 (approximately 21%) which occurred in fractions RF-7 and RF-8. In separate studies described previously, this zone from rat feces contained approximately two-thirds M-2 and one-third M-3. Metabolites M-1 (RF-5 and RF-6), M-4 (RF-7 and RF-8), and M-6 (RF-1) gave estimates of 2, 2, and 1% of the total fecal radioactivity, respectively.

The dark zone below M-2 in RF-8 contained approximately 4% of the radioactivity. An attempt was made to identify this metabolite. However, upon further fractionation, the zone was found to consist of two or more compounds and none were identified. No other single TLC zone contained more than 3% of the fecal radioactivity. Fraction RF-9 contained radioactive metabolites which had the same mobility as M-2 in solvent system 3, but TLC of RF-9 in solvent system 1 demonstrated that there was no appreciable M-2 in that fraction.

Estimates of metabolite levels were based on the assumption that no ^{14}C was lost from the monensin through metabolism. This assumption is valid for all of the metabolites which were tentatively identified with the exception of M-6. Loss of the carboxyl carbon in producing M-6 would amount to a loss of 1 in 8 or 1 in 14 labeled carbon atoms, depending upon the substrates used for synthesis. This loss would not materially affect the above estimates of M-6.

Comparison of the data from the steer and rat indicated that the qualitative metabolite pattern was similar, but that there were quantitative differences. The steer appeared to metabolize 50–60% of the [^{14}C]monensin while the rat metabolized more than 95%. Both species produced a large number of metabolites. None of these were present at a concentration greater than 5% with the exception of the isomers M-2 and M-3 in the rat which, together, accounted for 21% of the fecal radioactivity.

Biliary excretion appeared to be the primary excretory pathway for monensin metabolites. Bile from steer 4 contained radioactivity equivalent to approximately 14 ppm monensin. Fractionation of this radioactivity indicated that less than 3% was monensin. No direct measurements on the absorption of monensin were made; however, the data from bile suggest that the monensin which was absorbed was metabolized and that the quantitative difference in monensin metabolism between the steer and rat may be due to a difference in monensin absorption.

To determine if there was extensive degradation of monensin in the alimentary canal, monensin was incubated for 2 days in cattle feces (37 °C), for 5 h in sheep abomasum fluid (38 °C), or overnight in steer rumen fluid (38 °C). At least 90% of the monensin was recovered in all cases. These results suggest that monensin was not ex-

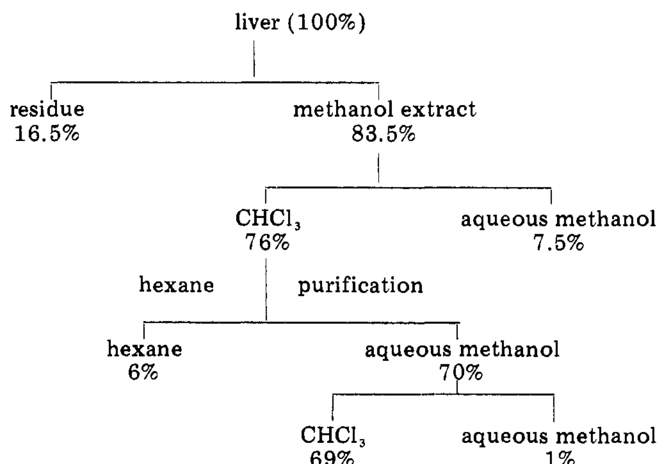

Figure 8. Distribution of radioactivity extracted from steer liver.

tensively degraded in the alimentary canal of the ruminant and support the conclusion that the fecal metabolites were the result of metabolism by the steer.

All of the monensin metabolites which were isolated gave a positive vanillin reaction. Metabolites M-1 through M-4, which were O-demethylated, gave a yellow-orange color, metabolite M-5 gave the typical red-pink monensin color, and M-6 gave a purple color. The metabolites were nonvolatile and were relatively stable during laboratory manipulation.

Fractionation of Radioactivity in Steer Liver. After preliminary studies to establish fractionation procedures, 500 g of liver from steer 4 were processed as described in the Experimental Section. The distribution of radioactivity during the extraction and partitioning steps is shown in Figure 8. The $CHCl_3$ extract containing 69% of the total liver radioactivity was evaporated and chromatographed on a silica gel column using gradient elution. The results of this separation are shown in Figure 9. A 5% aliquot from each fraction was counted and the remainder was pooled as shown. The following studies were conducted on the pooled fractions to characterize the primary metabolites:

SL-1 contained 13% of the liver radioactivity and was similar in elution characteristics to the frontal fraction from steer feces from which M-6 was isolated. Therefore, this fraction was rechromatographed on a silica gel column without gradient elution and then chromatographed by LC (Bondapak C18, methanol–water, 8:2). These separations revealed only one prominent peak of radioactivity. This compound had the same characteristics as M-6 from steer feces on the silica gel column, the LC column, and by TLC in two different solvent systems. It also gave the same purple color upon reaction with vanillin. Quantitatively, this fraction constituted about 45% of SL-1 and would, therefore, amount to 6% or less of the total radioactivity in the liver. There was not a sufficient quantity of this compound from liver for mass spectral analysis.

Aliquots of SL-2 through SL-5 were chromatographed on a TLC plate and visualized by autoradiography. SL-6

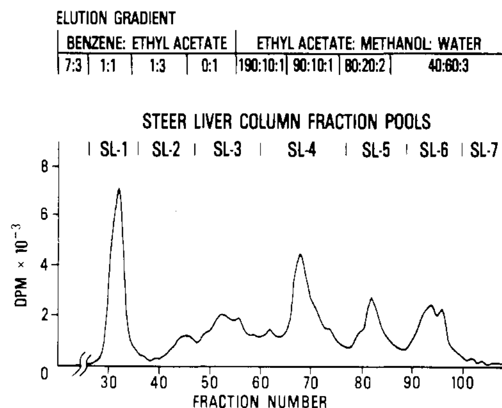

Figure 9. Gradient elution silica gel (Woelm No. 202) column chromatography of liver extract from [^{14}C]monensin-dosed steer.

and SL-7 contained too many coextractives for suitable TLC.

SL-2 contained a single zone with the mobility of monensin. This zone was scraped from the plate and counted to determine the radioactivity. The monensin level was estimated to be 2–3% of the total liver radioactivity. The presence of monensin was confirmed by microbiological assay.

SL-3 contained radioactivity but produced no discrete zone of consequence after separation by TLC. SL-4 contained several metabolites. The most abundant was M-1, which constituted only 4–5% of the total radioactivity in the liver. SL-5 contained several metabolites, one of which was M-2. M-2 was estimated to account for 1% of the total radioactivity.

The fractionation studies on liver may be summarized as follows: the total drug-derived radioactivity in steer liver as a result of oral dosing with [^{14}C]monensin was 0.59 ppm and consisted of many metabolites. Metabolites M-6, M-1, monensin, and metabolite M-2 were the most abundant compounds in the liver, and the concentration of each was estimated at 6, 5, 3, and 1% of the total radioactivity, respectively.

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