Metabolism of Benzo[b]thien-4-yl Methylcarbamate (Mobam) in Dairy Goats and a Lactating Cow

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The excretion patterns, tissue and milk levels, chromatographic separations, and identification of major metabolites of Mobam-^{14}C have been determined in milking goats and a dairy cow. Within 24 hours, the goats (dosed at 4.6 and 12.5 mg. per kg.) had excreted 96 and 95% of the ^{14}C dose as water-soluble compounds in the urine, and the cow (dosed at 1.9 mg. per kg.) had excreted 87% of the radioactive dose in the urine. Mobam-^{14}C equivalents found in the milk of goats were 3.1 p.p.m. at 8 hours (4.6 mg. per kg.) and 20.5 and 5.5 p.p.m. in the 8- and 16-hour milk samples at the 12.5 mg. per kg. dose. Milk-^{14}C residues from the dairy cow (1.9 mg. per kg.) were 1.3, 0.7, and 0.1 p.p.m. for samples taken at 8-, 16-, and 24-hour collections. Two metabolites in urine (4-benzothienyl sulfate and 4-benzothienyl sulfate-1-oxide) accounted for 88 to 91% of the ^{14}C excreted in the urine. In milk, 96 to 98% of the radioactivity consisted of 4-benzothienyl sulfate-1-oxide.

Mobam (benzo[*b*]thien-4-yl methylcarbamate) is one of a series of heterocyclic carbamates derived from benzothiophene. These compounds have been studied by Kilsheimer *et al.* (1969) to determine their effectiveness as pesticides.

A previous study with rats (Robbins *et al.*, 1969a) indicated that Mobam was rapidly hydrolyzed to remove the carbamate group, and the radioactivity was excreted principally in the urine as 4-benzothienyl sulfate and 4-benzothienyl glucuronide.

The present study was designed to determine the extent of absorption from the gastrointestinal tract and the routes and rates of excretion after a single oral dose of Mobam was given to lactating goats and a dairy cow. The major metabolites found in the urine and milk were isolated and identified.

MATERIALS AND METHODS

Chemicals. Mobam (benzo[*b*]thien-4-yl methylcarbamate; 4,7-^{14}C) with a specific activity of 0.45 mCi. per mmole and unlabeled Mobam were supplied by Mobil Chemical Co., Metuchen, N. J. Mobam-^{14}C, labeled in the carbonyl position, (1 mCi. per mmole) was synthesized by New England Nuclear Corp., Boston, Mass. Methyl-^{14}C-labeled Mobam (34.5 mCi. per mmole) was supplied by G. L. Lamoureux, Crops Research Division, of this laboratory. Purity of the radiolabeled Mobam was greater than 99%, as determined by thin-layer chromatography (TLC) or by gas–liquid chromatography as the *N*-trifluoroacetate ester. Potential metabolites, 4,7-dihydroxybenzothiophene, 4-benzothienyl sulfate, and 4-benzothienyl glucuronide were available from previous study (Robbins *et al.*, 1969a).

Apparatus, Radioanalysis, and Tissue Preparation. Metabolic equipment for collecting excreta and $^{14}CO_2$ from goats has been described (Robbins and Bakke, 1967). The cow was confined to a raised metabolism stall for collecting urine and feces. Urine was obtained by a catheter placed in the urinary bladder prior to treatment. Radioanalysis and techniques of tissue preparation have been reported (Bakke *et al.*, 1967).

Animal Treatment. Animals were dosed by dissolving Mobam in ethanol, pipetting the solution into gelatin capsules, and giving it as a single oral dose with a balling gun. A dairy cow (number 16) weighing 530 kg. was given 1.016 grams (1.9 mg./kg.; 462.4 μCi.) of ring-labeled ^{14}C-Mobam. Goat 57 received 4.6 mg./kg. (249 mg.; 121.9 μCi.), and goat 55 received 12.5 mg./kg. (789 mg.; 121.9 μCi.) of ring-labeled ^{14}C-Mobam. Goat 57 and goat 55 weighed 54 and 63 kg., respectively. Another milk goat weighing 56 kg. was dosed with 258 mg. (31.6 μCi.) of carbonyl-^{14}C-labeled Mobam. A ewe weighing 47 kg. was given 500 mg. (22.4 μCi.) of methyl-^{14}C-labeled Mobam. Collection of $^{14}CO_2$ was made on goat 55 and the goat and ewe receiving the carbonyl and methyl radiolabeled Mobam. Specific times of urine, feces, and milk collections, and slaughter for tissue samples are given in Table I.

Metabolite Separation and Characterization. Many of the chromatographic procedures used in this study were developed for isolation of the metabolites of Mobam in rat urine (Robbins *et al.*, 1969a). However, ruminant urine is basic and contains greater amounts of cations, which require changes in some of the chromatographic procedures. Procedures previously reported will not be described in detail.

Figure 1 presents the general outline for the cleanup and isolation. A liquid anion-exchange column for extracting the metabolites from urine was prepared by coating 50 grams of 200- to 325-mesh Porapak Q (Waters Associates, Inc., Framingham, Mass.) with 10 ml. of trioctylamine (Eastman Organic Chemicals, Rochester, N. Y.). A solution containing 10 ml. of trioctylamine dissolved in 75 ml. of ethanol was slowly added to 50 grams of Porapak Q while stirring the solid support with a glass rod to ensure an even coating, and the ethanol was removed on a flash evaporator. The coated Porapak Q was poured into a 2.5 × 60 cm. chromatographic column and a 1-cm. plug of cotton placed on top of the support. Two hundred milliliters of water was pumped at 2 to 4 ml. per minute through the column, followed by 30 ml. of 1*N* formic acid. Two hundred to 400 ml. of goat or cow urine was acidified to pH 5 with formic acid and placed on the column, followed by 200 ml. of distilled water. The column was then eluted with 200 ml. of 1*N* NH_4OH and washed to pH 6 with water. Two hundred milliliters of methanol was used to elute the remaining radioactivity as the trioctylamine salt. Methanol was removed by flash evaporation and the residue dissolved in 20 ml. of chloroform. This solution was partitioned three times with 20-ml. portions of 1*N*

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Table I. Radioactivity of Urine, Feces, and Milk of Goats and Cow after a Single Oral Dose of Mobam-^{14}C (Ring-Labeled)[a,b]

Collection Interval, Hr.	Accumulated % of ^{14}C Dose in Urine and Feces: Urine			Feces			Milk Residues, P.P.M. of Mobam-^{14}C Equivalents		
	Goat 55	Goat 57	Cow 16	Goat 55	Goat 57	Cow 16	Goat 55	Goat 57	Cow 16
0–8	83.8	81.3	71.4				20.5	3.1	1.4
8–16	93.8	94.4	84.2				5.5	1.1	0.7
16–24	95.1	96.4	86.9	6.7	4.8	11.9	1.0	0.3	0.1
24–32		96.7	87.5				...	0.1	...[c]
32–40		96.9	87.7				...	...[c]	...[c]
40–48		97.1	87.8		8.2	13.9	...	...[c]	...[c]
48–56			87.9				...	...	...[d]
56–64			87.9				...	...	...[d]
64–72			87.9			14.4	...	...	...[d]

[a] Goat 55 (12.5 mg./kg.) sacrificed at 24 hr.; goat 57 (4.6 mg./kg.) sacrificed at 48 hr.; cow 16 (1.9 mg./kg.) sacrificed at 72 hr. [b] Fecal collections taken at 0–24, 24–48, and 48–72 hours. [c] Less than 0.05 p.p.m. [d] No detectable radioactivity.

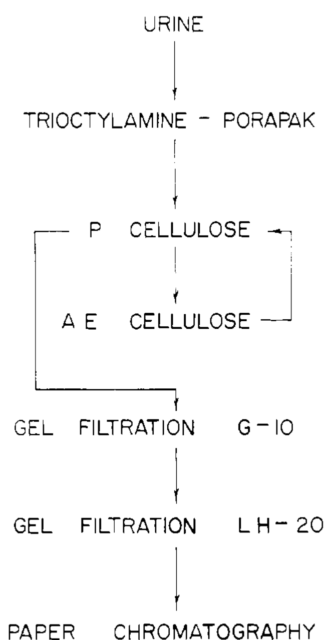

Figure 1. Outline of isolation of water-soluble metabolites of Mobam-^{14}C from urine

NH_4OH. The NH_4OH solution was taken to dryness and the residue dissolved in 20 ml. of water.

The above fractions were further purified on cellulose ion-exchange columns. A cation exchange cellulose column (2.2 × 40 cm.) packed with 25 grams of Cellex-P (Bio-Rad Laboratories, Richmond, Calif.) was converted to the H^+ form with HCl. An anion-exchange cellulose column (2.2 × 48 cm.) containing 25 grams of Cellex-AE (Bio-Rad Laboratories, Richmond, Calif.) was converted to the OH^- form with $1N$ NH_4OH.

Each fraction from the trioctylamine-Porapak (NH_4OH and methanol eluates) was put on the Cellex-P column and eluted with water to remove cations and amphoteric compounds. Then each fraction from the Cellex-P column, without reduction in volume, was chromatographed on the Cellex-AE column.

Figure 2 shows the elution sequence from the Cellex-AE column eluted with H_2O, $0.5M$ formic acid, $0.1M$ ammonium formate (pH 4), and $1N$ NH_4OH.

For quantitation of the metabolites, urine (10 to 20 ml.) containing 400,000 d.p.m. from the 0- to 8-hour samples was acidified to pH 5 with formic acid and run through the Cellex-P column. The eluate containing the radioactivity was then chromatographed on the Cellex-AE column.

Fraction 3 (4-benzothienyl sulfate-1-oxide) and fraction

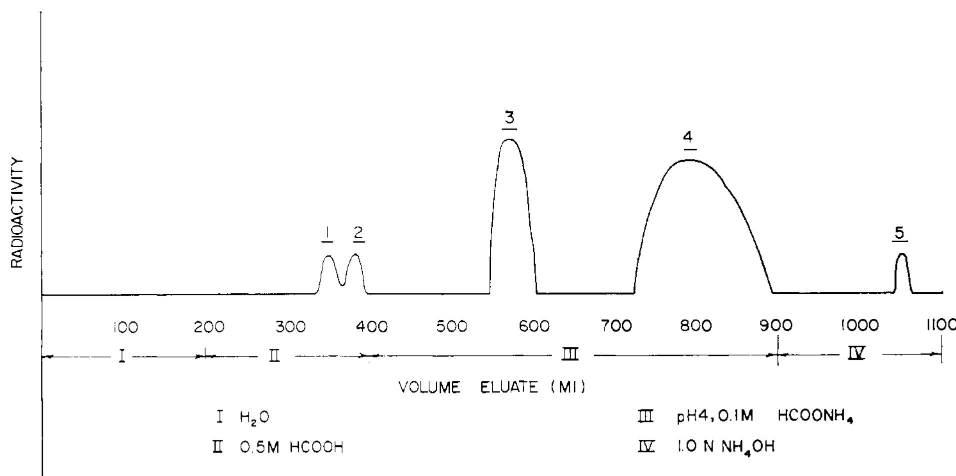

Figure 2. AE-cellulose ion-exchange chromatography of urinary metabolites of Mobam-^{14}C

Table II. Radioactive Residues Detected in Cow and Goat Tissues after a Single Oral Dose of Mobam-^{14}C (Ring-Labeled)[a]

Tissue and Organs	Tissue Residues, P.P.M. of Mobam-^{14}C Equivalents		
	Goat 55, 12.5 mg./kg.	Goat 57, 4.6 mg./kg.	Cow 16, 1.92 mg./kg.
Kidney fat	...[b]	...[b]	...[c]
Omental fat	...[b]	...[b]	...[c]
Blood	0.11	...[b]	...[c]
Lung	0.27	...[c]	...[c]
Liver	1.30	0.16	...[c]
Spleen	0.17	...[c]	...[c]
Heart	0.11	...[c]	...[c]
Kidney	0.61	0.06	...[c]
Muscle	0.18	...[c]	...[b]
Brain	0.42	0.11	0.10

[a] Goat 57 sacrificed at 48 hr.; goat 55 sacrificed at 24 hr.; cow 16 sacrificed at 72 hr. [b] No detectable radioactivity. [c] Less than 0.05 p.p.m.

4 (4-benzothienyl sulfate), after elution from the Cellex-AE column, were chromatographed on the Cellex-P column to remove the ammonium ion. Flash evaporation at 50° C. was used to codistill the formic acid with water from the samples. Three 20-ml. volumes of water were added and distilled to completely remove the formic acid. The volume of liquid in the flask was not reduced below 20 ml. during the distillation of the formic acid. Next 1 ml. of 1N NH_4OH was added, and the metabolites were taken to dryness as ammonium salts.

Further purification of fractions 3 and 4 was accomplished by gel filtration on a column (2.2 × 40 cm.) of Sephadex G-10 (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) and then on a 0.9 × 40 cm. column of Sephadex LH-20 developed in methanol.

When a high degree of purity was desired for fractions 3 and 4, they were also chromatographed on Whatman No. 1 paper strips (19 × 55 cm.) developed in acetic acid–butanol–water (10:40:50). R_f values were 0.43 for fraction 3 (4-benzothienyl sulfate-1-oxide) and 0.65 for fraction 4 (4-benzothienyl sulfate).

Thirty-gram samples of freeze-dried milk, collected 8 hours after dosing, were extracted three times with 100 ml. of methanol by stirring the solvent and milk, centrifuging the sample, and carefully removing the supernate. The methanol extract was taken to dryness and the residue dissolved in 100 ml. of water. This was partitioned with 100 ml. of hexane and then against 100 ml. of chloroform. The water layer was placed on the Cellex-P column and the eluate chromatographed on the Cellex-AE column by the procedure used for urinary metabolites. Fraction 3 (4-benzothienyl sulfate-1-oxide), after chromatography on the Cellex-AE column, was adsorbed on the trioctylamine–Porapak Q column, removed by methanol elution, and further purified by gel filtration on a Sephadex G-10 column. At this point, this fraction was sufficiently pure to obtain infrared spectra, which were identical to this fraction in urine.

The hexane-soluble radioactivity from milk partitioned quantitatively into 100 ml. of acetonitrile. This fraction was reduced to 0.4 ml. and chromatographed in the paper chromatography system as above and by TLC (silica gel HF, developed in xylene–ethylacetate–formic acid–water (1:35:2:2)).

Reactions of Metabolites 3 and 4. Metabolite 4 (4-benzothienyl sulfate) was hydrolyzed by heating 0.5 mg. with 1 ml. of 3N HCl or 3N HBr for 1 hour at 75° C. Methylene chloride soluble radioactivity was acetylated with 0.1 ml. of acetic anhydride and 10 μl. of pyridine. Metabolite 3 was subjected to the same conditions, and the products were separated by GLC under the following conditions: 6 ft. × $^1/_8$-in. i.d. glass column containing 3% SE-30 coated on Chromasorb Q. A temperature program rate of 10° C. per minute, starting at 100° C., was used. Radioactive fractions were trapped in glass capillary tubes and subjected to infrared and mass spectrometry.

Metabolite 3 (0.5 mg. of the purified conjugate) was eluted with water from a cation-exchange (0.5 × 6 cm.) column packed with AG 50W-X8 (Bio-Rad Laboratories, Richmond, Calif.) in the H^+ form. The elutate was taken to dryness, the methylene chloride soluble portion extracted from the flask, and an infrared spectrum obtained.

Metabolite 3 was studied to see if a Diels-Alder reaction would occur. One-half milligram of this metabolite was reacted with 0.4 ml. of isoprene and 0.2 ml. of dimethylformamide in a sealed tube at 50° C. for 48 hr. Another tube containing only dimethylformamide and metabolite 3 was treated similarly. Vacuum distillation was used to remove the reagents, and the residue was dissolved in methylene chloride. Infrared and mass spectra were obtained of the methylene chloride soluble products of each tube. The reaction products were slowly heated from 20° to 180° C. in the sample probe of the mass spectrometer (M-66, Varian Associates, Palo Alto, Calif.) and mass spectra obtained at 10° C. intervals during the heating period.

RESULTS AND DISCUSSION

Excretion of the radioactivity administered as ring-labeled Mobam was highest during the first 24 hours in all cases. The goats excreted 96 and 95% of the ^{14}C in the urine for dosage levels of 4.6 and 12.5 mg. per kg. (Table I). Within the first 8 hours, more than 70% of the radioactivity had been excreted by each of the treated animals. When fecal excretion was considered, over 100% of the ^{14}C was recovered. This high recovery was due to error associated with the experimental techniques. However, urinary excretion was the major route for disposal of the ring portion of Mobam.

Mobam-^{14}C residues appearing in fresh milk from the goats and the cow are given in Table I. Milk from goat 57 (4.6 mg. per kg.) had a maximum metabolite content in the 8-hour sample (3.1 p.p.m.), and milk from goat 55 (12.5 mg. per kg.) contained 20.5 p.p.m. and 5.5 p.p.m. in the 8- and 16-hour milk samples, respectively. Milk residues from the dairy cow were 1.40, 0.70, and 0.10 p.p.m. for milk samples taken at 0–8, 8–16, and 16–24 hour periods. Goat 55 excreted 1.9% of the ^{14}C in the milk during the first 24 hours. Milk collected for 48 hours from goat 57 contained 1.2% and milk collected for 24 hours from cow 16 had 1.0% of the administered radioactivity. Milk residues compared on a dosage basis (mg. per kg. of body weight) were related to the amount of Mobam that each animal received.

Tissue residues are given in Table II. The highest tissue residue of the three animals was 1.3 p.p.m. in the liver of goat 55 (12.5 mg. per kg.) slaughtered after 24 hours. Brain tissue of cow 16 contained a measurable amount (0.10 p.p.m.) of ^{14}C residue. All other tissues taken from cow 16 contained less than 0.05 p.p.m. of ^{14}C residues. Residues of ^{14}C were distributed among the various tissues other than fat. This would be expected for a compound that was undergoing rapid metabolism to water-soluble conjugates.

Combustion methods used for milk and tissues in this study could detect 0.1 p.p.m. or higher ^{14}C residues with a 95% accuracy. Residues lower than 0.05 p.p.m. are not presented.

Studies with the carbonyl and methyl ^{14}C-labeled Mobam

indicated that a significant amount of the carbamate ester was hydrolyzed and metabolized, in that 75% of the carbonyl-^{14}C and 38% of the methyl-^{14}C were exhaled as $^{14}CO_2$ within 24 hours. Urinary excretions of ^{14}C from carbonyl and methyl radiolabeled Mobam were 13.6 and 13.2%, respectively.

A study with rumen bacterial suspensions (Williams and Robbins, 1969) demonstrated that ring-labeled ^{14}C-Mobam was completely degraded to 4-hydroxybenzothiophene. No $^{14}CO_2$ was found in expired CO_2 from goat 55, which was dosed with the ring-labeled ^{14}C-Mobam.

Separations and identifications of the water-soluble metabolites in the animal urine were made by modification of a series of techniques previously developed to study the metabolism of Mobam in rats. Initial extraction of the water-soluble metabolites was made by non-ionic adsorption on a column containing Porapak Q coated with trioctylamine. This column was converted to the formate form and the urine acidified with formic acid to prevent a basic front from developing on the column which would remove fraction 3. Since the metabolites would not partition from urine into chloroform, diethyl ether, or ethyl acetate, this procedure was extremely important as a first step in the cleanup (Figure 1). This technique has been used as a step for purification of dieldrin metabolites in sheep urine (Hedde, 1969) and propazine metabolites in the urine of goats (Robbins, *et al.*, 1969b).

Separation of water-soluble metabolites found in urine and milk was accomplished by chromatography on AE cellulose. Data from this procedure are given in Table III. Five radioactive fractions were eluted and quantitated from urine and milk. Preliminary chromatographic studies on AE cellulose with urine which was not free of cations resulted in a part of fractions 3 and 4 being eluted with fractions 1 and 2. This was caused by anions being removed from the solution and a basic front developing. Prior removal of the cations by chromatography of urine and milk samples on P cellulose resulted in reproducible separations of the radioactive fractions by the AE cellulose column. Fractions 3 and 4 represented 88, 91, and 91% of the radioactivity in the urine of animals 55, 57, and 16. Metabolite 3 was the largest percentage (61%) of the ^{14}C in the urine of goat 55 (12.5 mg. per kg.), while in the urine of cow 16 (1.9 mg. per kg.) fraction 4 was the major metabolite (65%).

In milk, fraction 3 accounted for 96 to 98% of the water-soluble radioactivity. Thus, a selectivity for excretion of this metabolite in milk existed regardless of dosage or the relative amount of this fraction excreted in the urine. However, the amounts of ^{14}C present in the milk decreased with decreasing dosage (Table I). Three to 5% of the ^{14}C in the 0–8 hour milk samples partitioned into hexane and then into acetonitrile. When the acetonitrile extract was chromatographed on paper, the major peak was estimated to be 80 to 90% of the ^{14}C by area obtained from a radioactive scanner. This radioactivity co-chromatographed with fraction 3 from the urine. No 4-hydroxybenzothiophene was detected when the organic-soluble ^{14}C was chromatographed by TLC. Therefore, the major metabolite in milk resulting from Mobam metabolism was fraction 3 (Table III).

Table III. Separation and Quantitation of Radiolabeled Metabolites in Urine and Milk

Metabolites[c] (0–8 Hour Sample)	Per Cent of Water Soluble Radioactivity[a,b]					
	Milk			Urine		
	Goat 55	Goat 57	Cow 16	Goat 55	Goat 57	Cow 16
1	0.5	0.4	0.1	3.5	2.4	2.9
2	0.1	0.1	0.1	3.5	4.2	3.1
3	96.0	95.9	97.5	60.7	39.9	26.4
4	2.1	1.9	0.9	28.1	51.3	65.0
5	1.3	1.6	1.4	4.0	2.3	2.6

[a] As determined by chromatography on AE cellulose. [b] Goat 55, 12.5 mg./kg.; goat 57, 4.6 mg./kg.; cow 16, 1.9 mg./kg. [c] Metabolites 1, 2, and 5 were not identified; metabolite 3 was 4-benzothienyl sulfate-1-oxide; metabolite 4 was 4-benzothienyl sulfate.

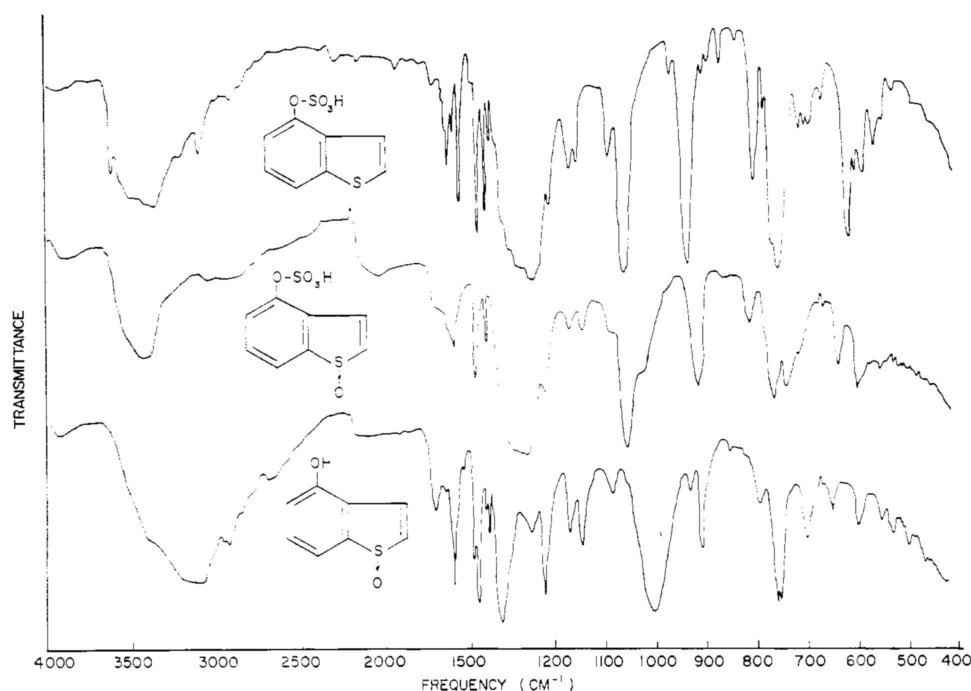

Figure 3. Infrared spectra of 4-benzothienyl sulfate, 4-benzothienyl sulfate-1-oxide, and 4-hydroxybenzothiophene-1-oxide

Structural identification was made of fractions 3 and 4 isolated from urine. Infrared spectra were obtained of these fractions (Figure 3). Fraction 4 was identical to authentic 4-benzothienyl sulfate. Fraction 3 was assigned to be a conjugate of sulfuric acid on the basis of bands in the infrared spectrum at 1250 and 1050 cm^{-1}. Fraction 4 (4-benzothienyl sulfate) exhibited a moderately strong band at 940 cm^{-1} which shifted in fraction 3 to 910 cm^{-1}. In addition, 4-benzothienyl sulfate showed two overlapping bands at 760 cm^{-1}, while fraction 3 had distinct bands at 730 and 765 cm^{-1} (Figure 3).

Hydrolysis of fractions 3 and 4 was made with HCl and HBr. The methylene chloride soluble residues were acetylated with acetic anhydride and separated by GLC. Hydrolysis of fraction 4 yielded only 4-hydroxybenzothiophene (GLC at 160° C.). Fraction 3, treated with HCl, gave two radioactive products (GLC at 168° and 188° C.). After hydrolysis with HBr, fraction 3 showed three products by GLC (168°, 196°, and 220° C.). Mass spectra, obtained from the trapped radioactive peaks, indicated that fraction 3 was partly hydrolyzed to 4-hydroxybenzothiophene and a monosubstituted product of 4-hydroxybenzothiophene containing chlorine after treatment with HCl. Hydrolysis in HBr produced 4-hydroxybenzothiophene and two bromine monosubstituted products of 4-hydroxybenzothiophene. At this point the presence of a sulfoxide could not be determined, since the oxygen, if present, was removed during the reaction. But, an inference could be made that fraction 3 had reactivity at positions 2 and 3 of the thiophene portion of the molecule, which indicated a sulfoxide was present.

To stabilize the postulated sulfoxide, a Diels-Alder addition of isoprene was attempted. Thiophene 1-dioxide has been shown to be a reactive dienophile which gave an adduct with 1,2-dimethylenecyclohexane (Bailey and Cummins, 1954). Metabolite 3 was reacted with isoprene in dimethylformamide. Another portion of metabolite 3 was heated in dimethylformamide only. Infrared spectra obtained from the methylene chloride soluble residue from the conjugate heated in dimethylformamide were identical to infrared spectra obtained after drying the metabolite in the acid form (from the AG 50W-X8 column in the H^+ form). This compound, 4-hydroxybenzothiophene-1-oxide, (Figure 3) was also quantitatively produced by heating the conjugate in dimethylformamide at 110° C. for 1 hour. A characteristic infrared absorption was present for a sulfoxide (1000 cm^{-1}). Thin-layer chromatography (silica gel HF, developed in xylene–ethyl acetate–formic acid–water, 1:35:2:2) gave an R_f of 0.62. Infrared spectra of the methylene chloride soluble portion from the isoprene reaction showed no band characteristic of sulfate esters, with loss of the band at 905 cm^{-1} and a single band at 780 cm^{-1}. These data indicated an adduct was formed.

Further proof of the structure of metabolite 3 was obtained from mass spectra of the Diels-Alder adduct. Mass spectra of the control (conjugate heated with DMF) obtained at 50° C. showed only a *m/e* 150 ion which was characteristic of 4-hydroxybenzothiophene. No parent ion (*m/e* 166) was observed for 4-hydroxybenzothiophene-1-oxide because of the rapid loss of oxygen. However, when the adduct was subjected to mass spectrometry at a sample probe temperature of 180° C., a molecular ion of *m/e* 234 (6% abundant) was present. The base peak was *m/e* 214 (M − 20, loss of 0 and 4 hydrogen atoms). A mass 150 ion (26% abundant) indicated that a retro Diels-Alder reaction had occurred. Fortunately, the contamination had disappeared after heating the sample probe to 110° C., which provided the true mass spectra of the adduct obtained at 180° C. (4-hydroxybenzothiophene-1-oxide rapidly disappeared at 50° to 60° C.).

Metabolite 3 was characterized as 4-benzothienyl sulfate-1-oxide from the physical data. Attempts to synthesize this compound and 4-hydroxybenzothiophene-1-oxide were unsuccessful. Several attempts to oxidize 4-hydroxybenzothiophene to 4-hydroxybenzothiophene-1-oxide resulted in the formation of 4-OH-benzothiophene-1,1-dioxide. The same result was observed by Kaufman (1969) when simple derivatives of 4-hydroxybenzothiophene were oxidized.

Metabolites in milk and urine, other than metabolites 3 and 4, were not identified. These metabolites may have resulted from oxidation of the benzothiophene ring. With the rather small amounts present and the inability of the present cleanup procedures to isolate these compounds in a high degree of purity, structural identification was not accomplished. From their behavior in the chromatographic systems, the unknown metabolites are acids which are highly water soluble, indicating them to be conjugates. One of the major metabolites of Mobam metabolism in rats, 4-benzothienyl glucuronide, was not found in this study.

The present study not only elucidated the metabolism of Mobam in ruminants but also extended the methodology for extraction and purification of conjugates from urine. All or part of these techniques are being used in this laboratory for isolating water-soluble compounds from urine. Some of these procedures should be useful in any study concerned with the metabolism of organic compounds by animals.

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