Metabolism of 4-Benzothienyl *N*-Methylcarbamate (Mobam) in Rats

Balance Study and Urinary Metabolite Separation

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The excretion patterns, tissue levels, and ion-exchange chromatography of the urinary metabolites of Mobam have been determined in rats. Rats (two groups, 14 per group) were dosed with Mobam-^{14}C (ring-labeled) at 2.0 and 13.0 mg. per kg. of body weight. After 24 hours, 87% (2.0 mg. per kg.) and 76% (13.0 mg. per kg.) of the ^{14}C dose were excreted as water-soluble compounds in the urine. Carcasses of rats from both groups sacrificed at 3 days contained less than 1.0% of the radioactive dose. Tissue residues were fairly evenly distributed, decreasing in the 13.0 mg. per kg. group from an average of 0.5 p.p.m. (wet tissue) after 2 days to 0.2 p.p.m. after 8 days. Anion exchange chromatography on AE cellulose separated the radioactivity in the urine into four fractions. Two metabolites in the urine, 4-benzothienyl sulfate and 4-benzothienyl glucuronide, accounted for 83 and 87% of the excreted ^{14}C from rats dosed at 2.0 and 13.0 mg. per kg., respectively. These metabolites were isolated and purified by liquid ion-exchange chromatography, paper chromatography, gel filtration, and gas-liquid chromatography.

Mobam (4-benzothienyl *N*-methylcarbamate) may become an effective pesticide for use on a wide variety of crops. Many of these crops are consumed by animals which could thus be exposed to possible residues of Mobam. This study was initiated to determine the extent of absorption from the gastrointestinal tract, the routes and rates of excretion, and the deposition in various tissues after a single oral dose of Mobam given to rats. Isolation and characterization studies of urinary metabolites were also initiated.

The metabolism of Mobam by animals has not been reported. Several studies have been conducted with ^{14}C-labeled carbamates using animals and liver-microsomal preparations to elucidate their metabolism. Most carbamates which have been studied are rapidly metabolized by animals, only small residues remaining in the animal. The metabolism of carbaryl (1-naphthyl *N*-methylcarbamate) has been studied in rats, guinea pigs, and man (Knaak *et al.*, 1965), in milk goats (Dorough and Casida, 1964), in milk cows (Dorough, 1967), and in dogs (Knaak and Sullivan, 1967). Ten ^{14}C-labeled carbamates were studied in rats by Krishna and Casida (1966). Several studies with enzyme systems have been reported (Dorough and Casida, 1964; Knaak *et al.*, 1965; Krishna and Casida, 1966; Leeling and Casida, 1966). These and other studies (Baron and Doherty, 1967; Buhler and Harpootlian, 1967; Oonnithan and Casida, 1968) indicate that carbamate compounds undergo a number of metabolic steps, including hydrolysis, hydroxylation, and conjugation, to form glucuronides and esters of sulfuric acid. Since the routes and rates of metabolism are dependent on the structure of the carbamate chemical involved, it appears necessary to study each carbamate compound in each species of animal of interest. Also, the isolation and identification of the metabolites are difficult, and to date the identification of many of those reported is tentative.

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MATERIALS AND METHODS

Chemicals. MOBAM (4-benzothienyl *N*-methylcarbamate, 4,7-^{14}C) was supplied by the Mobil Chemical Co., Metuchen, N.J. The radiolabeled product had a specific activity of 0.83 mc. per mmole (7946 d.p.m. per μg.). Purity of the radioactive chemical was greater than 99.8%, as determined by cochromatographing Mobam-^{14}C with an authentic sample of Mobam on silica gel–HF thin-layer plates developed in isopropyl ether–acetone (75 to 25) and in hexane–ethyl acetate–acetic acid (70:30:2). Mobam-^{14}C was detected with a thin-layer scanner and by viewing the chromatogram under ultraviolet light. Under these conditions, as little as 0.1% of ^{14}C impurity could be detected.

4,7-DIHYDROXYBENZOTHIOPHENE. A solution containing 1.64 grams (0.01 mole) of benzothiophene-4,7-quinone (Fieser and Kennelly, 1935) in 40 ml. of ethanol was added to a solution of 1.90 grams (0.01 mole) of stannous chloride in 25 ml. of concentrated hydrochloric acid. Then the reaction was refluxed for 30 minutes, and the ethanol was removed under reduced pressure. The residue was extracted with ether and the ether extracts were dried over magnesium sulfate. The ether was distilled off and the residue recrystallized from carbon tetrachloride to yield 0.35 gram of product melting at 165° to 168° C. The compound decomposed readily on exposure to air and was difficult to purify further. Thin-layer chromatography yielded one spot if the plate was developed immediately after spotting, but if left for a short while before development, a substantial amount of the quinone was formed. A parent ion of mass 166 was obtained as the base peak on mass spectroscopic analysis.

Analysis. Calculated for $C_8H_6O_2S$: C, 57.83; H, 3.64. Found. C, 57.40; H, 4.21.

POTASSIUM - 4 - HYDROXYBENZOTHIOPHENE SULFATE. The procedure of Feigenbaum and Neuberg (1941) was used. A solution of 9.0 grams (0.06 mole) of 4-hydroxybenzothiophene in 30 ml. of *N*-dimethylaniline was cooled to 0° C., and 8.15 grams (0.07 mole) of chlorosulfonic acid was added. The solution was then made basic with 50% potassium hydroxide, and the solid that formed was removed by filtration, washed thoroughly with ether, then dissolved in hot methanol (a small amount of solid that remained was removed by filtration). The methanol solution was concentrated and cooled, and the product filtered; it was then recrystallized from a very small amount of hot water and finally recrystallized from hot ethanol. Material obtained in this way melted at 226° to 236° C. Repeated recrystallization of the material from ethanol resulted in the isolation of potassium ethyl sulfate. The NMR spectrum showed adsorption only in the aromatic region (a complex splitting pattern between 7.3 and 7.9δ). Both chemical and enzymatic hydrolysis yielded 4-hydroxybenzothiophene.

METHYL(4-BENZOTHIENYL TRI-*O*-ACETYL-β-GLUCOPYRANOSIDE)-URONATE. A mixture of 10.32 grams (0.023 mole) of methyl-1,2,3,4-tetra-*O*-acetyl-β-D-glucuronate, 13.8 grams (0.092 mole) of 4-hydroxybenzothiophene, and 0.2 gram of *p*-toluenesulfonic acid was heated continuously on a steam bath for 2.5 hours and evacuated intermittently with an aspirator. The black residue that resulted was dissolved in benzene, and the solution was washed first with 2.5*N* sodium hydroxide, then with water. After the benzene solution had been dried with magnesium sulfate, the solvent was removed, and the residue was triturated with 2-propanol to yield 2.8 grams of product melting at 125° to 135° C. Recrystallization from ethanol-water yielded 1.8 grams (m.p. 147–49° C.).

Analysis. Calculated for $C_{21}H_{22}O_{10}S$: C, 54.07; H, 4.75; S, 6.87. Found. C, 54.13; H, 4.82; S, 6.75.

4-BENZOTHIENYL β-D-GLUCOPYRANOSIDURONIC ACID. To a solution of 0.466 gram (1×10^{-3} mole) of methyl (4-benzothienyl tri-*O*-acetyl-β-D-glucopyranoside)-uronate in 100 ml. of methanol was added 4.0 ml. of 1*N* potassium hydroxide. The reaction was allowed to stand at room temperature for 2 days. Then the methanol was removed with a flash evaporator at 40° C. The residue was placed on a 2.5 × 25.0 cm. ion-exchange column containing 25 grams of Cellex P (Bio-Rad Laboratories, Richmond, Calif.) in the H^+ form and eluted with 100 ml. of water. Most of the water was removed at 40° C. with a flash evaporator. Then 20 ml. of water was added and the solution again concentrated to remove acetic acid. Two additional portions of water were added and, in turn, removed. Finally, 30 ml. of acetonitrile was added and removed with a flash evaporator. The residue was extracted with ether and the ether removed to yield an oil which crystallized from ethyl acetate. The product (0.30 gram) melted at 84° to 94° C. and contained ethyl acetate of crystallization (as shown by NMR), which could be removed by drying at 80° C. and 0.1 mm. of Hg for 7 hours. The dried product melted at 90° to 94° C.

Analysis. Calculated for $C_{14}H_{14}O_7S$: C, 51.52; H, 4.32; S, 9.83. Found. C, 51.27; H, 4.52; S, 9.52.

An alternative, but less satisfactory, method was hydrolysis with barium hydroxide in methanol, followed by removal of barium as precipitated barium sulfate.

Apparatus. The metabolic equipment used in these experiments for collection of urine, feces, and $^{14}CO_2$ has been reported for rats (Bakke *et al.*, 1967).

Radioanalyses. A Nuclear-Chicago liquid scintillation spectrometer was used for quantitation of radioactivity. Specific methods of sample preparations for urine, fecal, and tissue analyses for ^{14}C have been described (Bakke *et al.*, 1967).

Animal Treatment. Two groups of male rats (14 per group, weighing 350 to 400 grams) were dosed with Mobam-^{14}C at 2.0 mg. per kg. (2.78 μc.) and 13.0 mg. per kg. (1.22 μc.). The chemical was dissolved in ethanol and administered by stomach tube as a single 1-ml. dose. Water and food were provided *ad libitum*. Four rats of each group were sacrificed at 2, 4, and 8 days. Urine and feces were collected daily from each rat (Table I). Two rats from each dosage level were sacrificed at 3 days for determination of total carcass residues for ^{14}C, including digestive tract and skin. Expired $^{14}CO_2$ from these animals was also determined.

Metabolite Separation. A liquid anion-exchange column was developed which removed the water-soluble radioactivity from the urine. Two milliliters of trioctylamine (Eastman Organic Chemicals, Rochester, N.Y.) was dissolved in 10 ml. of ethanol. This solution was applied to the top of a chromatography column (0.5 × 133 cm.) containing 150- to 200-mesh Porapak Q (Waters Associates, Inc., Framingham, Mass.). Nitrogen pressure was used to force the solution of trioctylamine on the column. One hundred milliliters of distilled water was pumped through the column to remove the ethanol. Rat urine diluted (1 + 9) with distilled water was pumped through the column at 0.5 ml. per minute. This column removed all of the radioactivity from the urine of four rats. The column effluent was monitored for radioactivity with a con-

Table I. Radioactivity Recovered from Rat Urine and Feces after a Single Oral Dose of Mobam-^{14}C

	Accumulated % of ^{14}C Dose			
	Urine		Feces	
Collection Interval, Days	2.0 mg./kg.	13.0 mg./kg.	2.0 mg./kg.	13.0 mg./kg.
0–1	87.4	76.2	8.4	7.3
1–2	88.7	77.8	10.6	12.0
2–3	88.9	78.2	10.8	12.6
3–4	89.2	78.6	11.0	12.9
4–5	89.3	78.8	11.1	13.1
5–6	89.3	78.9	11.1	13.1
6–7	89.4	79.1	11.1	13.1
7–8	89.4	79.2	11.1	13.2

tinuous-flow liquid scintillation detector (Packard Instrument Co., La Grange, Ill.). The column was again washed with 100 ml. of distilled water. The radioactivity was subsequently removed by pumping 100 ml. of methanol through the column. The methanol eluate was taken to dryness and transferred again to the Porapak column in a 2-ml. volume of methanol and eluted at 0.5 ml. per minute with methanol. A radioactive fraction occurred at 90 to 110 ml. Methanol was removed from the fraction and the residue dissolved in 10 ml. of chloroform. The fraction was then partitioned with 10 ml. of 1*N* NH_4OH. Radioactivity in the fraction completely partitioned into the NH_4OH layer. The NH_4OH layer was reduced to dryness, redissolved in 10 ml. of H_2O, and further purified by chromatography on ion-exchange cellulose.

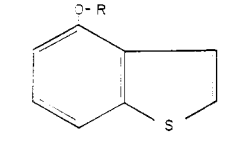
An anion-exchange cellulose (Cellex-AE, Bio-Rad Laboratories, Richmond, Calif.) was used for further fractionization of the urinary metabolites. A 1.8 × 47 cm. column was packed with 25 grams of AE-cellulose by pouring as a slurry in methanol and then washed with 100 ml. of H_2O, 30 ml. of 1*N* HCl, 100 ml. of H_2O, and 30 ml. of 1*N* NH_4OH, and washed to pH 6 with 200 ml. of distilled water. The urinary metabolites were transferred to the AE-cellulose column in 10 ml. of H_2O and eluted with ammonium formate buffer (pH 4, 0.1*M*). The elution pattern, including the final elution with 1.0*N* NH_4OH, is shown in Figure 1. Diluted urine containing 100,000 d.p.m. was chromatographed in the same manner on the AE-cellulose column to obtain the per cent of radioactivity in the various fractions.

A cation-exchange cellulose column (1.8 × 40 cm.), containing 25 grams of cellulose P (Bio-Rad Laboratories, Richmond, Calif.), was used to remove the ammonium ion from the radioactive fractions that eluted from the AE-cellulose column. The eluate containing fractions B and C was reduced to 10 ml. with a flash evaporator at 40° C. The formic acid was removed by repeated addition and distillation of three 25-ml. volumes of water. Then, 1 ml. of 1*N* NH_4OH was added to prevent hydrolysis and the fractions were taken to dryness. The residue was dissolved in methanol for further cleanup by paper chromatography.

Whatman No. 1 paper strips (19 × 55 cm.) were spotted with 0.5 mg. of Mobam-^{14}C equivalents of fractions B and C and developed in acetic acid–isoamyl alcohol–water (10:40:50). A 5-cm. strip was cut from each side of the paper chromatogram and scanned for radioactivity. The area containing the radioactivity was cut into 1-sq. cm. strips and placed in the barrel of a 10-ml. syringe with an attached needle. Three 5-ml. volumes of methanol were added to the top of the paper in the syringe for elution.

Fraction C (4-benzothienyl sulfate) was finally purified by gel filtration chromatography on a column (1 × 30 cm.) of Sephadex LH-20 (Pharmacia Fine Chemicals, Piscataway, N. J.) which was poured and washed with methanol. A 0.1-ml. volume containing 100,000 d.p.m. of fraction C was pipetted on the Sephadex LH-20 column and eluted with methanol, with 1-ml. fractions assayed for radioactivity. Fractions 24 to 31 containing ^{14}C were subjected to infrared spectrometry. Fraction B could not be purified by gel filtration because of adsorption of the radioactivity on the column, which resulted in excessive band spreading and loss.

Gas-liquid chromatography was used for final purification of fraction B (4-benzothienyl glucuronide). The glucuronide was chromatographed as the trimethyl silyl ether derivative (Horning *et al.*, 1967). One hundred micrograms of the compound reacted with 0.1 ml. of bistrimethylsilylacetamide (BSA) and 10 μl. of trimethylchlorosilane in a glass-stoppered tube for 30 minutes at 75° C. A 6-foot × ⅛-inch glass column containing 2% XE-60 coated on Chromosorb W with a helium flow of 40 ml. per minute was used for the separation on a Perkin Elmer Model 801 instrument with a flame detector and a 10-to-1 effluent splitter. Radioactivity in the effluent of the GC was determined by a method of Robbins and Bakke (1967). A temperature program rate of 10° C. per minute, starting at 100° C. and ending at 250° C., was used. Retention times and elution temperatures were: 4-benzothienyl glucuronide trimethylsilyl ether, 230° C.; 4-OH-benzothiophene, 190° C.; 4-7-OH-benzothiophene, 165° C.; and benzothiophene-4,7-quinone, 168° C.



Mobam, R= $\overset{O}{\overset{\|}{C}}NHCH_3$

Metabolite B, R= β - glucuronide

Metabolite C, R= SO_3H

Capillary tubes were used for trapping the compounds for infrared spectrometry. Flushing TMS derivatives of glucose and glucuronic acid while in capillary tubes with anhydrous HCl gas at 10 ml. per minute for 3 minutes resulted in infrared spectra identical to those of glucose and glucuronic acid. This procedure was followed in obtaining infrared spectra of the 4-benzothienyl glucuronide metabolite by using the micro KBr technique (1.5-mm. disk with a 4× beam condenser) with 20 μg. of the compound.

The diluted urine was also partitioned with chloroform (1 to 1) and each layer assayed for radioactivity. Thin-layer chromatography and GLC, as previously described, were used for isolating 4-hydroxybenzothiophene from the chloroform. Similar R_f values were obtained in the TLC solvent systems for 4-hydroxybenzothiophene, 4,7-dihydroxybenzothiophene, and benzothiophene-4,7-quinone. After TLC chromatography of chloroform-soluble radioactivity, the ^{14}C area was removed from the plate, eluted with methanol, and subjected to GLC to obtain infrared spectra.

Enzymatic hydrolysis of 4-benzothienyl sulfate and 4-benzothienyl glucuronide was done according to Colowick and Kaplan (1955). Forty micrograms of the

acid ester was incubated at 39° C. with β-glucuronidase aryl sulfatase (pH 6.2) for 1 hour. Forty micrograms of the glucuronide metabolite (after paper chromatography) was incubated with 1000 Fishman units from three sources of β-glucuronidase (bovine liver and helix pomatia, Calbiochem, Los Angeles, Calif.; bovine liver, Nutritional Biochemicals, Cleveland, Ohio) at pH 4.5 for 18 hours. The reactions were stopped and the solution was extracted with diethyl ether. The aqueous and organic phases were counted for ^{14}C and the ether-soluble components chromatographed by TLC and GLC.

RESULTS AND DISCUSSION

Excretion of radioactivity was most rapid during the first 24 hours (Table I). At this time, 87 and 76% of the ^{14}C had been excreted in the urine, and 8 and 7% in the feces for dosage levels of 2.0 and 13.0 mg. per kg., respectively. After 48 hours, only trace amounts of ^{14}C were found in the urine and feces. These data showed that Mobam was rapidly absorbed from the gastrointestinal tract and excreted in the urine primarily in the first 24 hours after receiving the chemical.

Tissue residues are given in Table II. Within each level of dosage, the ^{14}C residues decreased with time. After 48 hours, the highest levels of ^{14}C residues were in the liver and lung tissues, which contained 0.14 and 0.17 p.p.m. (2.0 mg. per kg.) and 1.23 and 0.68 p.p.m. (13.0 mg. per kg.). Also, a factor of approximately 6 exists between the parts per million residues of Mobam-^{14}C equivalents with respect to the same tissues from rats given the 2.0- and 13.0-mg. per kg. doses. At these levels of ^{14}C, the limit of detectability of the combustion analyses was 0.01 p.p.m. for tissues from rats given 2.0 mg. per kg. and 0.04 p.p.m. at the 13.0-mg. per kg. dose. No tissue selectivity of the ^{14}C residues was apparent, since a rather even distribution of ^{14}C residues existed, and the residues decreased with time. No $^{14}CO_2$ was found in the expired CO_2.

Two rats from each level of treatment were killed at 3 days for total carcass residues. These provided a check against the accuracy of the excretion data (Table I). Carcasses of rats receiving the 2.0-mg. per kg. dose contained 0.82% of the dose, while rats treated at 13.0 mg. per kg. contained 0.92% of the dose. This indicated that 99% of the dose from each treatment had been excreted by the rats within 3 days. The apparent differences in the radioactivity recovered in the urinary and fecal excretions between the two treatments are probably due to experimental error. Such errors can occur in data collected from rat metabolism units operated under the most careful conditions. In either case, it is obvious that the ring portion of Mobam is readily excreted by rats.

Separation of the water-soluble metabolites in the urine by ion-exchange chromatography on AE-cellulose is shown by Figure 1, and the percentages of ^{14}C in these fractions are given in Table III. This procedure would fractionate small quantities of urinary ^{14}C components (100,000 d.p.m.) for quantitation; however, when larger amounts of urine were put on the column,

Table II. Radioactive Residues Detected in Rat Tissues after a Single Oral Dose of Mobam-^{14}C

	P.P.M. of Mobam-^{14}C Equivalents					
	2 Days[a]		4 Days[a]		8 Days[a]	
Tissue	2.0 mg./kg.	13.0 mg./kg.	2.0 mg./kg.	13.0 mg./kg.	2.0 mg./kg.	13.0 mg./kg.
Brain	0.02	0.10	0.01	0.10	0.01	0.06
Heart	0.06	0.42	0.05	0.28	0.05	0.30
Lung	0.17	1.23	0.13	1.07	0.13	0.79
Kidney	0.13	0.64	0.05	0.43	0.04	0.37
Digestive tract	0.72	0.95	0.06	0.65	0.01	0.08
Muscle	0.01	0.06	0.01	0.06	0.005	0.03
Liver	0.14	0.68	0.07	0.60	0.07	0.45
Omental fat	0.03	0.26	0.03	0.15	0.01	0.05

[a] Time of sacrifice.

Table III. Per Cent Distribution of Mobam-^{14}C Urine Metabolites[a]

	Dosage Level	
Fraction	2 mg./kg.	13 mg./kg.
A	7.8	5.9
B[b]	20.5	8.2
C[c]	63.1	79.2
D	8.4	6.6

[a] Separation of 0–24-hour composite sample on AE cellulose.
[b] 4-Benzothienyl glucuronide.
[c] Benzothienyl sulfate.

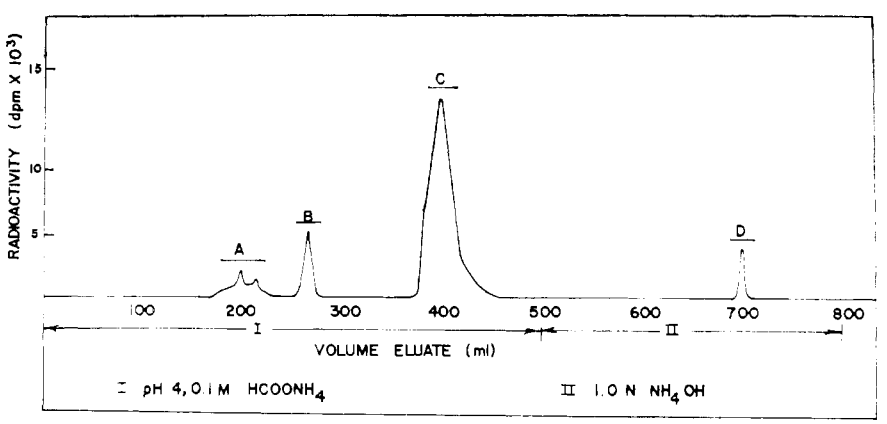


Figure 1. Cellulose ion-exchange chromatography of urinary metabolites of Mobam-^{14}C

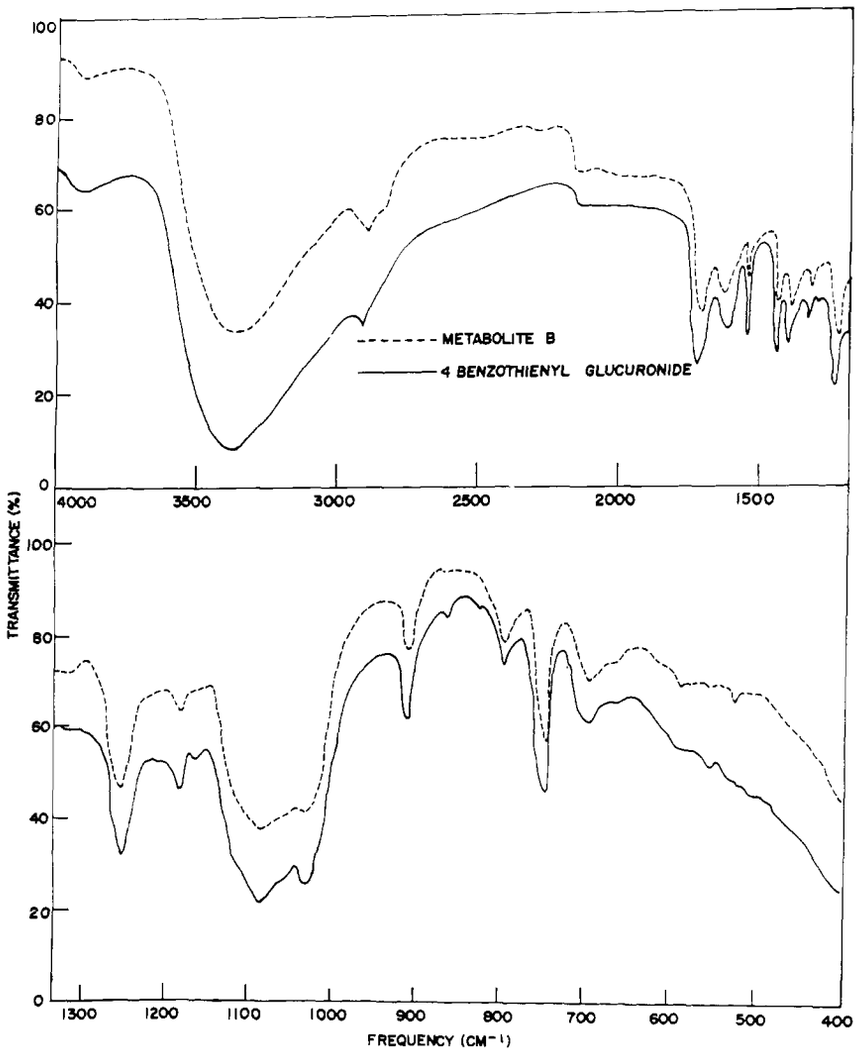


Figure 2. Infrared spectrum of 4-benzothienyl glucuronide

the fractions did not separate satisfactorily because of a large excess of other ions present in the untreated urine. DEAE cellulose of several preparations was found unsatisfactory because of swelling which resulted in extremely slow flow rates when untreated urine was chromatographed.

Attempts to chromatograph the radioactivity of untreated urine on anion-exchange resins resulted in irreversible adsorption of the ^{14}C by nonionic forces. The radioactivity could not be eluted by either acids or bases. Nonionic adsorption on anion-exchange resins has been reported with conjugates of sterols (Bush, 1961). By using the liquid anion-exchange column (Porapak Q coated with trioctylamine), this property was used to obtain a preliminary cleanup of the urinary metabolites. Since the trioctylamine-metabolite complex could be removed with methanol and metabolites partitioned into NH_4OH from a chloroform solution, a significant cleanup was obtained. The chloroform-soluble radioactivity which did not partition was isolated by TLC and GLC and found to be 4-hydroxybenzothiophene, which was also directly chloroform-extractable from the untreated urine (1 to 2% of the total ^{14}C).

The NH_4OH-soluble fraction (10 to 15 $\times$ 10^6 d.p.m.) from the liquid anion-exchange column satisfactorily chromatographed on the AE-cellulose column, and the ^{14}C fractions eluted similarly to those from untreated urine (Figure 1).

Fractions B and C in the acid form were readily hydrolyzed if taken to dryness from an acid solution. This occurred in the first attempts at isolation and the resulting 4-hydroxybenzothiophene was identified. In preliminary studies, these fractions were from 40 to 90% hydrolyzed, as measured by diethyl ether extraction of ^{14}C-labeled 4-hydroxybenzothiophene. However, when untreated urine was heated with 5*N* HCl (1 to 1) at 75° C. for 5 hours, less than 10% of the total radioactivity was ether soluble. Thus, these conjugates, in the presence of a buffered solution such as urine, appeared fairly resistant to acid hydrolysis.

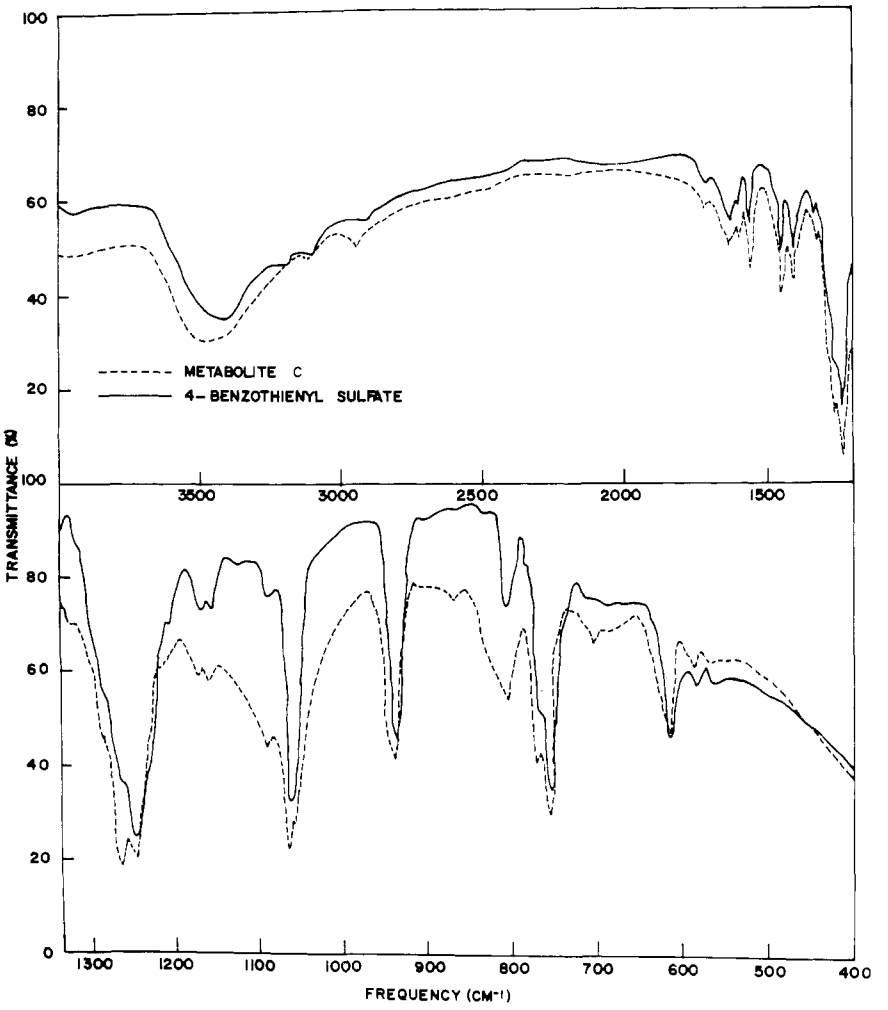


Figure 3. Infrared spectrum of 4-benzothienyl sulfate

Comparative infrared spectra of the metabolites and synthesized compounds are shown in Figure 2 (4-benzothienyl glucuronide) and Figure 3 (4-benzothienyl sulfate). Excellent spectra of 4-benzothienyl glucuronide were obtained from GLC purification and subsequent removal of the silyl groups by HCl. Infrared spectra of 4-benzothienyl sulfate, as isolated from the LH-20 column, were used for identification.

Fraction B (4-benzothienyl glucuronide) treated with glucuronidase was only 5.2% hydrolyzed after 18 hours. Similar results were obtained with the synthesized 4-benzothienyl glucuronide incubated with three sources of glucuronidase. Complete hydrolysis occurred after 1 hour when fraction C (4-benzothienyl sulfate) was incubated with β-glucuronidase aryl sulfatase.

From these data, Mobam appears to be rapidly hydrolyzed to 4-hydroxybenzothiophene by the rat to form principally conjugates of glucuronic acid and sulfuric acid. The percentages of these conjugates varied with the level of dosage (Table III). Rats receiving the 2-mg. per kg. treatment excreted 20% of the ^{14}C as the glucuronide, as compared to 8% for those dosed at 13 mg. per kg. Excretion of 4-benzothienyl sulfate was 63 and 79% of the ^{14}C radioactivity excreted in the urine for the 2- and 13-mg. per kg. treatments, respectively. Fractions A (7.8 and 5.9%) and D (8.4 and 6.6%), for the 2- and 13-mg. per kg. treatments, were less affected by level of dosage. Fraction A contained at least three compounds and fraction D one component, as evaluated by chromatography on AE cellulose. At present, our purification methods have not been adequate for further identification.

All of the urinary metabolites were acidic. Previous studies on carbamate metabolism have indicated ring hydroxylation and *N*-glucuronide formation in addition to *O*-glucuronide and sulfuric acid conjugation. These compounds may account for the unknown metabolites in this study, and attempts are being made to elucidate the structures of the unknown metabolites.

Methodology for the isolation and identification of

glucuronides and sulfuric acid esters has been developed and should be useful when such conjugates are present in small amounts in biological materials.

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