Metabolism of 2-Methoxy-4-ethylamino-6-*sec*-butylamino-*s*-triazine by the Dairy Cow and the Goat

Jerome E. Bakke,* Joe D. Robbins, and Vernon J. Feil

A lactating cow was given a single oral dose (1 mg/kg) of ^{14}C-ring-labeled 2-methoxy-4-ethylamino-6-*sec*-butylamino-*s*-triazine. Eighty-nine percent of the dose was recovered 120 hr after dosing. Percentages recovered were: urine 76.1%, feces 10.6%, and milk 2.4%. The urine contained at least 19 radioactive components. Seven urinary components, representing 77% of the urinary radioactivity at the period of maximum excretion, were characterized by mass spectrometry. Two fecal metabolites were characterized by paper chromatography. The parent herbicide was not detected in either the urine or the feces. Fat tissues contained no detectable radioactivity. Liver and spleen were the only assayed tissues with significantly detectable radioactivity. Residues in the milk reached a maximum of 0.36 ppm during the 32- to 40-hr collection period.

The metabolism of the herbicides of the triazine series by the rat and ruminant have been under investigation in our laboratory (Bakke *et al.*, 1967; Robbins *et al.*, 1968). The compound 2-methoxy-4-ethylamino-6-*sec*-butyl-amino-*s*-triazine (GS-14254) is proposed as a preemergence herbicide for use on alfalfa and sugar cane. The rat LD_{50} is 2680 mg per kg; the cow and goat toxicity is not known. Its metabolism by the dairy cow is the subject of this report.

MATERIALS AND METHODS

Radio-Labeled Compounds. Uniformly ring-labeled 2-methoxy-4-ethylamino-6-*sec*-butylamino-*s*-triazine (GS-14254) was supplied by Geigy Chemical Co. The ^{14}C-labeled herbicide contained less than 1% of an unknown radioactive impurity.

Methoxy-^{14}C-labeled GS-14254 was synthesized by the following procedure. Two vials, each containing 1.0 mCi of ^{14}C-methanol, were cooled on dry ice before opening. The contents of each vial were transferred with two 0.5 ml washings of dimethyl sulfoxide (DMSO) into a 2-dram vial equipped with a Teflon lined cap and a small magnetic stirring bar. To the ^{14}C-methanol solution were added 32 μl (0.78 mmole) of methanol, 229 mg (1.0 mmole) of 2-chloro-4-ethyl-amino-6-*sec*-butylamino-*s*-triazine, 0.20 ml (1.0 mmole) of 5.0 N NaOH, and a trace of sodium iodide. The solution was stirred with a magnetic stirrer at room temperature. After 2 days, an additional 41 μl (1.0 mmole) of methanol was added and the stirring continued an additional 2 days. The volatile solvents were removed *in vacuo* with a water aspirator. The solution was extracted with chloroform, and the organic layer was washed with water and dried with anhydrous magnesium sulfate. The solvent was removed to yield an oil; the oil was taken up in methanol, water added, and the solution was seeded with authentic 2-methoxy-4-ethylamino-6-*sec*-butylamino-*s*-triazine. On cooling, 163 mg of crystalline product was collected. After drying in a desiccator, the yield was 134 mg (1.35 mCi ^{14}C), having an infrared spectrum identical with that of an authentic sample.

Animal Treatment. A 484-kg lactating dairy cow was acclimatized to the metabolism crate over a period of 7 days. Three days prior to dosing, the cow was catheterized, placed in the crate, and control collections of milk, urine, and feces

U.S. Department of Agriculture, Animal Science Research Division, ARS, Metabolism and Radiation Research Laboratory, Fargo, N.D. 58102

were obtained. The cow was given 4×10^6 units of penicillin after catheterizing.

After 3 days of control collections, the cow was given, by gelatin capsule, 482.9 mg (0.997 mg per kg) of ring-labeled GS-14254 containing 966.7 μCi of ^{14}C. Milk and urine were collected at 8 hr intervals for 72 hr, followed by 24 hr composites of 12 hr collections for the 72 to 120 hr period. Feces were collected in 24 hr intervals for the full 120 hr. Blood samples were taken at 24 and 120 hr. The animal was sacrificed 120 hr after dosing.

A 52-kg female goat was catheterized and given 57.6 mg (1.1 mg per kg) of methoxy-labeled GS-14254 containing 48.8 μCi of ^{14}C. Expired $^{14}CO_2$ was collected for 24 hr (Robbins and Bakke, 1967) and urine was collected for 48 hr. After 48 hr the goat was given 828 mg of ring-labeled GS-14254 (containing 124.5 μCi of ^{14}C) for the isolation of metabolites.

Analytical Methods. Urinary radioactivity was determined by diluting the urine 1 to 10 with distilled water and counting 1 ml in dioxane counting solution A (Bakke *et al.*, 1967). Tissue, feces, and milk residues were determined by Parr bomb combustion of homogenized, freeze-dried aliquots as previously reported (Bakke *et al.*, 1967).

Separation of the urinary metabolites on the amino-acid analyzer (column I) and quantitation of the radioactive components were accomplished as previously reported (Bakke *et al.*, 1967). The characterized metabolites were quantitated in the urine by individually chromatographing the purified samples on column I, and comparing their elution volumes with those in the total urine.

Isolation of Cow Urinary Metabolites. Cow urine (800 ml containing 30×10^6 dpm) from the 8 to 6 hr collection was saturated with $(NH_4)_2SO_4$ and extracted first with 400 ml of diethyl ether (37.6% of the activity) and then with 400 ml of tetrahydrofuran (36.6% of the activity).

The diethyl ether fraction was dried, dissolved in 2 ml of water, acidified to pH 3 with 6 N HCl, and applied to a column of Aminex A-5 resin (Bio-Rad Laboratories, Richmond, Calif.) that had been equilibrated with 0.35 N sodium citrate buffer at pH 5.28 (column II). The column was 0.9 cm in diameter and contained 15 g of resin. The radioactivity separated into fractions 1 through 7, with the buffer pumped at 0.5 ml per min. An eighth fraction was obtained by elution with 0.2 N NaOH.

The citrate was removed from the Aminex A-5 fractions using columns of Chelex 100 (Bio-Rad Laboratories, Richmond,

Calif.) in the Cu^{+2} form, as described by Goldstein (1967) for the separation of nucleic acid components. Fractions 6, 7, and 8 passed through the column with water; fraction 5 eluted from the column with 1 N NH_4OH. All fractions were freeze-dried.

Fractions 5 and 6 were paper chromatographed on Whatman No. 1 using the organic phase from isoamyl alcohol-acetic acid-water (40:10:50, v/v/v). Fraction 7 was applied to a silica gel thin-layer plate and the plate developed with chloroform ethanol (27:3, v/v). In each case, the radioactive fraction on the chromatogram was extracted from the chromatogram with methanol.

Fractions 5, 6, 7, and 8 were next separately applied to a 100 × 0.9 cm column of Sephadex LH-20 packed in methanol. The radioactive fractions eluted with methanol were dried under vacuum. Silyl derivatives for gas-liquid chromatography of fractions 5, 6, and 7 were prepared by heating the dry samples with 50 μl of bis-(trimethylsilyl)-trifluoroacetamide containing 1% trimethylchlorosilane (Regisil). Fraction 8 was gas chromatographed without derivatization.

The tetrahydrofuran-soluble radioactivity was dissolved in methanol and separated into six fractions using a 100 × 4.5 cm column of Sephadex LH-20 poured in methanol. The third fraction was dried, acidified to pH 3 with 6 N HCl, and applied to a 5-g column of Cellex-P [H⁺] (Bio-Rad Laboratories). The column was washed with 50 ml of water and the radioactivity eluted with 1 N HCl. The eluate was freeze-dried and paper chromatographed in the isoamyl alcohol solvent used for the ether-soluble metabolites. The major radioactive fraction (R_f 0.1) was eluted from the paper with methanol and applied to a 10-g column of Cellex-GE [OH⁻] (Bio-Rad Laboratories). The column was washed with 70 ml of water and the radioactivity eluted with 1 N NH_4OH. The sample was freeze-dried and silanized by heating with 50 μl of Regisil prior to gas-liquid chromatography.

Isolation of Metabolites from Goat Urine. Goat urine (220 ml, 52 × 10⁶ dpm) was extracted three times with 100 ml of n-butanol. The aqueous phase was freeze-dried, taken up in water (500,000 dpm/ml), acidified with 4 N formic acid, and 0.2 ml of trioctylamine added per 500,000 dpm. The trioctylamine complexes were extracted into tetrahydrofuran and separated into two radioactive fractions on a 100 × 0.9 cm column of LH-20 in methanol. The second fraction was silanized with Regisil and gas chromatographed.

Goat urine (220 ml, 52 × 10⁶ dpm) was applied to a 20 × 2.5 cm column of Amberlite CG-400 in the chloride form. The radioactivity that eluted with water (51.5%) was applied to a 20 × 2.5 cm column of Bio-Rad AG-1 in the hydroxide form. The radioactivity that eluted with water (42% of the urine activity) was freeze-dried, dissoved in water, acidified to pH 3 with 6 N HCl, and separated into four fractions on column I.

The citrate was removed from these four fractions by acidifying each to pH 3 with 6 N HCl and applying each separately to a 10 × 0.9 cm column of Bio-Rad AG-50 × 8 in the ammonium form. The citrate was eluted with water and the radioactivity with 1 N NH_4OH. The radioactive fractions were freeze-dried, silanized with Regisil, and separately gas chromatographed.

All gas chromatography utilized a 6 ft, ¹/₈ in. i.d. glass column of 3% SE-30 on 60/80 mesh Chromosorb W in a Perkin-Elmer 801 gas chromatograph fitted with an effluent splitter. The carrier gas was helium at 30 ml per min. The temperature was programmed at 10° per min from 150° to 225° C. The injector was maintained at 230° C. The detector was located within the column oven and its temperature

Table I. Radioactivity in the Urine, Feces, and Milk from a Cow after a Single Oral Dose of GS-14254-¹⁴C

Collection Interval Hr	% of ¹⁴C Dose in Urine, Feces, and Milk			Milk Residues ppm of GS-14254 Equivalents
	Urine	Feces	Milk	
0–8	4.4		0.15	0.15
8–16	11.8		0.21	0.23
16–24	11.3	2.9	0.22	0.22
24–32	7.2		0.22	0.23
32–40	17.2		0.43	0.36
40–48	9.6	5.1	0.25	0.22
48–56	4.2		0.19	0.18
56–64	2.9		0.15	0.15
64–72	2.5	1.5	0.15	0.13
72–96	3.7	0.8	0.28	0.08
96–120	1.3	0.3	0.16	0.05
Total	76.1	10.6	2.4	

varied with the oven temperature. Ten percent of the effluent from the column went to the flame detector.

All infrared spectra were obtained from samples in micro-KBr pellets (10 mg KBr; 2 mm diameter pellets) using a Perkin-Elmer 337 infrared spectrometer equipped with a 4X beam condenser. Mass spectra were obtained on samples trapped from the gas chromatograph using the solid sample inlet system of the Varian M-66 mass spectrometer equipped with a V-5500 control console.

Chromatography of Fecal Metabolites. One hundred grams of freeze-dried 24 to 48 hr cow feces was extracted with methanol and the concentrated extract applied to a florisil column (50 × 2 cm) packed in chloroform. The radioactivity was eluted with a stepwise elution of ethanol in chloroform. The radioactivity eluted with a 30:70 (v/v) mixture of ethanol in chloroform. The fractions containing activity were paper chromatographed on Whatman No. 1 with the isoamyl alcohol-acetic acid-water system.

RESULTS AND DISCUSSION

Approximately 89% of the administered ring-labeled GS-14254 was recovered in the urine (76.1%), feces (10.6%), and milk (2.4%) from the cow 120 hr after dosing (Table I). Two periods of maximum excretion of radioactivity in the urine were observed which were not related to urine volume. The first was a broad maximum during the 8 to 24 hr collections, and the second was a sharp maximum during the 32 to 40 hr collection. The second maximum corresponded with the period of highest milk secretion (0.43% of the dose) and concentration (0.36 ppm GS-14254 equivalents).

The milk and tissue residues in ppm of GS-14254 equivalents are given in Tables I and II, respectively. Residues of of 0.05 ppm were considered the detectability limits (twice background on 40 min counts). The spleen and liver were the only tissues with significantly detectable residues. The milk residues reached a maximum of 0.36 ppm, decreasing to near the detectability limits after 5 days.

The goat rapidly excreted the radioactivity from the methoxy-¹⁴C-labeled GS-14254 in the urine (73.8% after 24 hr, 83% after 48 hr). The respiratory CO_2 contained 3.3% of the administered dose after 48 hr. The feces and tissues were not assayed.

Ion-exchange chromatography (column I) of the cow urine collected during the two periods of maximum excretion of radioactivity separated 14 (8 to 16 hr sample) and 19 (32 to 40 hr sample) urinary metabolites of ring-labeled GS-14254 (see Figure 1). The major differences between the metabolite
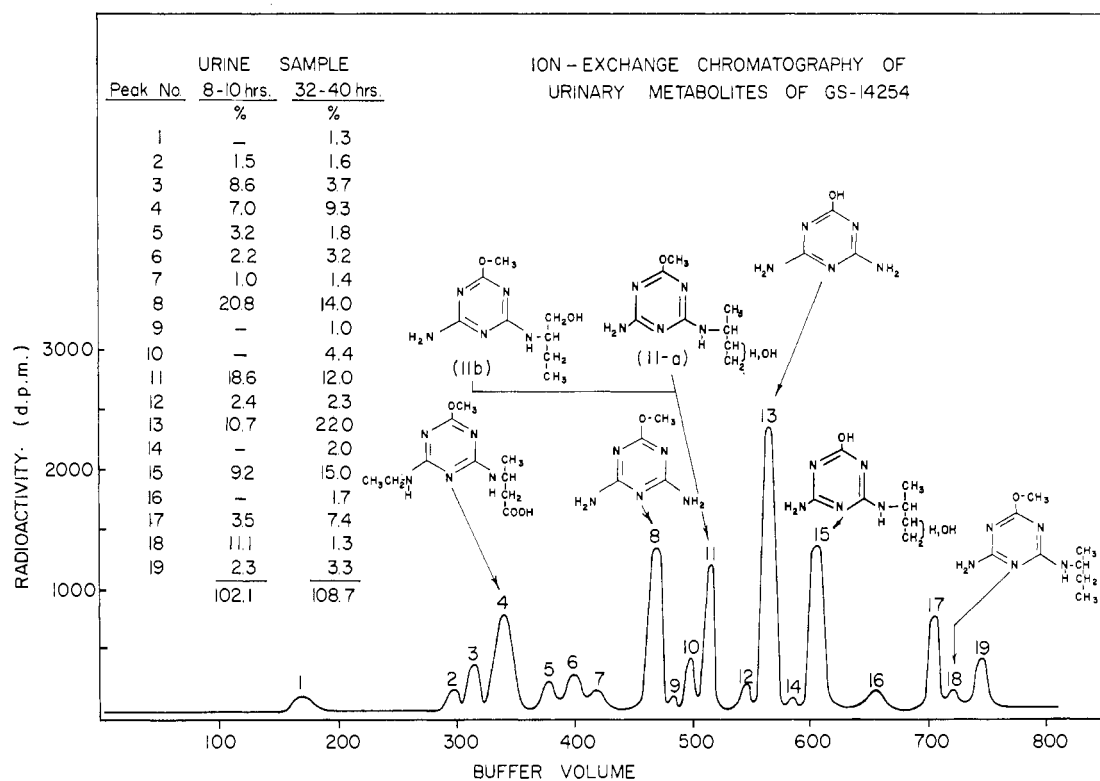
Figure 1. Ion-exchange chromatography of the metabolites from ring-labeled GS-14254-^{14}C in cow urine

Peak No.	URINE SAMPLE 8-10 hrs. %	32-40 hrs. %
1	—	1.3
2	1.5	1.6
3	8.6	3.7
4	7.0	9.3
5	3.2	1.8
6	2.2	3.2
7	1.0	1.4
8	20.8	14.0
9	—	1.0
10	—	4.4
11	18.6	12.0
12	2.4	2.3
13	10.7	22.0
14	—	2.0
15	9.2	15.0
16	—	1.7
17	3.5	7.4
18	11.1	1.3
19	2.3	3.3
	102.1	108.7

concentrations in the two urine samples, other than the appearance of five new minor metabolites in the 32 to 40 hr urine, were the decreases in metabolites 3, 8, 11, and 18 with increases in metabolites 13, 15, and 17.

Goat urine (8 to 24 hr sample) containing the ring-^{14}C-labeled metabolites gave the same elution pattern of radioactivity from the ion-exchange column as the 32 to 40 hr sample of cow urine. Ion-exchange chromatography of goat urine containing the methoxy-^{14}C-labeled metabolites demonstrated that ^{14}C-activity was associated with ring-labeled fractions 4, 7, 8, 11, 12, 18, and 19 in Figure 1. Approximately 50% of the methoxy-^{14}C radioactivity in the goat urine eluted as one peak near the buffer front (40–50 ml) unassociated with any ring-^{14}C- labeled fraction from either cow or goat urine.

The paper, thin-layer, gas-liquid, and amino-acid analyzer chromatographic (column I) data for the ring-labeled metabolites isolated from cow and goat urines and cow feces are given in Table III. Table III also correlates the several isolated fractions (first column, Table III) with the peak numbers

Table II. Radioactive Residues Detected in Tissue Samples of a Cow at Sacrifice (120 hr) after a Single Oral Dose of GS-14254-^{14}C

Tissue	Residues, ppm of GS-14254 Equivalents
Brain	trace
Leg muscle	trace
Spleen	0.49
Adrenal gland	0.07
Perirenal fat	ND[a]
Omental fat	ND
Tailhead fat	ND
Kidney	0.07
Liver	0.24
Heart	trace
Blood	ND

[a] None detected.

(numbers in parentheses in column 4, Table III) and the assigned structures given in Figure 1.

The ether extract of cow urine separated into eight radioactive fractions on column II. The first four fractions represented less than 2% of the radioactivity applied to the column and were not characterized. Fractions 5, 6, 7, and 8 contained 11.6, 32.9, 4.2, and 5.8%, respectively, of the radioactivity applied to column II. Fraction 5 contained peak 8 in Figure 1. Fractions 6 and 7 were both contained in peak 11 in Figure 1 and will be referred to as metabolites 11a and 11b, respectively. Fraction 8 was contained in peak 18 in Figure 1. Chromatography of either the ether extracts or the butanol extracts of goat urine on column II separated all the above components, except the 11b was not present.

The major metabolite in the tetrahydrofuran extract (fraction 3 off LH-20) of the ether-extracted cow urine contained peak 15 in Figure 1. The remaining fractions contained the same components as were found in the ether extract.

The trioctylamine complexes from the butanol-extracted goat urine contained two radioactive components. The larger second fraction chromatographed on column I as peak 4 in Figure 1.

The four radioactive fractions isolated from goat urine using column I had elution volumes identical with peaks 8, 11, 13, and 15 (Figure 1) from cow urine.

The removal of the citrate buffer from the radioactive components eluted from columns I and II was accomplished by two methods. The Chelex 100 [Cu^{2+}] resin quantitatively bound the citrate as the copper chelate. This chelate could not be eluted from the column with either water or 1 N NH$_4$-OH. The metabolites either passed through the column with water or were eluted with ammonia. The other method for removal of the citrate buffer utilized a sulfonic acid resin in the ammonium form. The acidified metabolites bound to the AG-50 [NH$_4$$^+$] resin, and the citrate eluted from the column with water. The metabolites were subsequently recovered from the column by elution with 1 N NH$_4$OH.

Table III. Paper Chromatography R_f Values, Gas Chromatographic Elution Temperatures, and Column I Elution Volumes for the Characterized Metabolites

Fraction	R_f Values[a]	Glc Elution Temperatures[b] °C	Elution Volume from Column I[c] ml
Cow urine ether extract[d]			
5	0.54	180	450–470 (8)
6	0.77	208	510–520 (11a)
7	0.77 (0.33)[e]	208	510–520 (11b)
8	...	184[f]	710–730 (18)
Cow urine tetrahydrofuran extract[g]			
3	0.1	198	590–620 (15)
Goat urine fractions from column I			
1	0.5	180	450–470 (8)
2	0.77	208	500–520 (11)
3	0.1	186	550–580 (13)
4	0.1	198	590–620 (15)
Goat urine trioctylamine extract[h]			
2	...	215	320–350 (4)
Cow feces methanol extract[i]			
1	0.54	...	... (8)
	0.77	...	... (11)

[a] Solvent: isoamyl alcohol-acetic acid-water 40:10:50 (v/v/v). [b] Silanized fractions were gas chromatographed on a 6 ft, 3% SE-30 on Chromosorb W column temperature programmed from 100° to 250° C at 10° per min. [c] Numbers in parentheses designate peak numbers and proposed structures in Figure 1. [d] Fractions from column II (see text). [e] R_f on silica gel tlc developed with chloroform-ethanol, 27:3 (v/v). [f] Not silanized. [g] Major fraction off LH-20 column (see text). [h] See text for preparation. [i] Sample eluted from florisil (see text).

Table IV. Mass Spectra

Peak 4, Figure 1 (monosilyl)

m/e	Relative Abundance	M + 1 Intensity[a]
327	24	19 ($C_{13}H_{17}N_5O_3Si$; 17.9)
312	9	
210	100	
196	88	
169	30	
168	15	
131	2	
117	6	

Peak 8, Figure 1 (disilyl)

285	50	24 ($C_{10}H_{23}N_5OSi_2$; 23.5)
270	100	25 ($C_9H_{20}N_5OSi_2$; 22.4)
171	42	
127.5	7	

Peak 11, Figure 1 (disilyl)

	11a	11b	11a	11b
357	1	0.7		
342	9	10	27	25 ($C_{13}H_{28}N_5O_2Si_2$; 26.6)
328	...	2		
254	2[b]	100		20 ($C_{10}H_{20}N_5OSi$; 17.9)
240	100	14	19.5	($C_9H_{18}N_5OSi$; 16.8)
198	15	6		
117	6	4		
103	4[b]	7		

Peak 13, Figure 1

	trisilylammiline	
343	100	30 ($C_{12}H_{29}N_5OSi_3$; 30.5)
328	80	30 ($C_{11}H_{26}N_5OSi_3$; 29.4)
	disilylammiline	
271	100	21 ($C_9H_{21}N_5OSi_2$; 22.0)
256	85	25 ($C_8H_{18}N_5OSi_2$; 20.9)
	monosilylammiline	
199	100	14 ($C_6H_{13}N_5OSi$; 13.4)
184	87	14 ($C_5H_{10}N_5OSi$; 12.3)

Peak 15, Figure 1

	trisilyl	
415	1	33 ($C_{16}H_{37}N_5O_2Si_3$; 34.9)
400	14	34 ($C_{15}H_{34}N_5O_2Si_3$; 33.8)
312	5[b]	
298	100	26 ($C_{11}H_{24}N_5OSi_2$; 23.0)
	disilyl	
343	4	34 ($C_{13}H_{29}N_5O_2Si_2$; 26.5)
328	12	25 ($C_{12}H_{26}N_5O_2Si_2$; 25.4)
240	9[b]	
226	100	20 ($C_8H_{18}N_5OSi$; 14.6)
	common to both	
117	9	
103	+[b]	

Peak 18, Figure 1

197	10
182	15
168	100
141	9

[a] M + 1 isotope peak intensities are listed relative to the associated peak being 100%. Values in parentheses are calculated for the empirical formulas given. [b] These peaks not present in the corresponding goat urine fractions.

The mass spectra for the isolated metabolites are given in Table IV. Except for metabolite 18, all mass spectra were obtained on the trimethylsilyl derivatives.

Metabolites 8 and 13 gave mass and infrared spectra identical with the trimethylsilyl derivatives of 2-methoxy-4,6-diamino-s-triazine and ammiline (2-hydroxy-4,6-diamino-s-triazine), respectively. Metabolite 13, as did known silanized ammiline, gave three sets of parent ion (M^+) and $M^+ -15$ fragments corresponding to the mono-, di-, and trisilyl derivatives. These are presumed to result from hydrolysis of the sample after elution from the gas chromatograph, since the relative intensities of the three sets changed with residence time in the spectrometer, while the relative intensities within the sets remained constant. The three derivatives distilled into the mass spectrometer from the solid sample probe in order of decreasing number of silyl groups. Metabolite 18 gave mass and infrared spectra identical with authentic 2-methoxy-4-amino-6-sec-butylamino-s-triazine.

The mass spectral characterizations of the metabolites for which known compounds were not available were based upon molecular ions (M^+), isotope peak intensities ($M^+ +1$), and fragmentations in the sec-butyl portions of the silanized metabolites. The fragmentation is summarized in Table V.

The two isomeric alcohols, 11a and 11b, isolated from cow urine on column II were differentiated by the fragmentation patterns of their silyl derivatives. The base fragment for 11a was the loss of 117 amu [C_2—C_3 cleavage, M^+, either CH_2-CH_2O—$Si(CH_3)_3$ or CH_3—CHO—$Si(CH_3)_3$] with a minor loss (2%) of 103 amu [M^+ —CH_2O—$Si(CH_3)_3$] and no loss of 29 amu (M^+ —CH_2CH_3). The base fragment from 11b was the loss of 103 amu (C_1—C_2 cleavage) with a 2% loss of 29 amu (C_2-C_3 cleavage) and a 14% loss of 117 amu. The large loss of 103 from 11b indicated the presence of the silyl ether of a primary alcohol. The alcohol could have been on either C_1

or C_4 of the butyl moiety; however, the loss of 29 amu (M^+ —CH_2CH_3) favors the alcohol being located on C_1. The m/e +1 isotope peak intensities, where measurable, for both 11a and 11b were in good agreement with values calculated for the associated fragment ions (see Table IV).

The loss of 117 amu (m/e 240) and the presence of an m/e 117 fragment in the mass spectra of 11b were inconsistent with the proposed structure. The presence of these fragments can be explained by cross-contamination of 11a and 11b, since the only apparent different in their physical properties in the isolation procedures utilized was their chromatography

	Metabolite	Bond Cleaved		
		C_1—C_2	C_2—C_3	C_3—C_4
C (1)	11a and 15	$M^{+} - 15$	$M^{+} - 117$	$(M^{+} - 103)^{a}$
R—NH—C (2)	11b	$M^{+} - 103$	$M^{+} - 29$	$M^{+} - 15$
C (3)	4	$M^{+} - 15$	$M^{+} - 131$	$M^{+} - 117$
C (4)				

a Not present in goat urine metabolites 11 and 15.

on column II. Metabolite 11b eluted as a distinct peak after 11a without the recorder trace returning to baseline. This is also supported by the evidence that 11b was not present in the organic extracts of goat urine chromatographed on column II, and that the mass spectrum of metabolite 11 isolated from goat urine gave the same fragmentation pattern as 11a from cow urine, except that no $M^{+} - 103$ fragment at m/e 254 was present. This indicated the cross-contamination of cow urine metabolites 11a and 11b.

The mass spectrum of silanized metabolite 15 from cow urine gave the same fragmentation of the sec-butyl moiety as metabolite 11a, and, as with silanized ammiline, gave sets of parent ions (m/e 415 and 343) and fragments corresponding to di- and trisilyl derivatives. The fragments within the two sets maintained constant relative intensities within each set down to m/e 226, indicating that the disilyl derivative was a product of hydrolysis prior to ionization rather than a fragmentation mode of the trisilyl derivative. The measurable $M^{+} + 1$ isotope peak intensities were in good agreement with those calculated for the molecular ions and the $M^{+} - 15$ and $M^{+} - 117$ fragments.

The mass spectrum of silanized metabolite 15 isolated from goat urine gave the same fragmentation as cow urine metabolite 15 except that, as with goat urine metabolite 11, the $M^{+} - 103$ fragments (m/e 312 and m/e 240) and the m/e 103 fragment were missing. This would indicate that cow urine metabolite 15 contained two components which could correspond to the demethoxylated analogs of metabolites 11a and 11b. Assuming that the relative intensities of the $M^{+} - 117$ and $M^{+} - 103$ fragments of metabolite 15 from cow urine are a measure of the relative amounts of the two isomers, the demethoxylated analog of 11a would be the major component.

From the mass spectral data, the location of the hydroxyl group in the major components of both metabolites 11 and 15 could be on either C_3 or C_4 of the sec-butyl moieties. The secondary alcohol structure is favored, since the mass spectra of the silanized metabolites 11 and 15 from goat urine gave m/e 117 fragments and no $M^{+} - 103$ or m/e 103 fragments. If a primary alcohol were present, a favored fragmentation mode of the silyl ether would have been cleavage of the C_3—C_4 bond (α cleavage; Pierce, 1968) when the ionization took place on the ether oxygen. This would have produced an m/e 103 fragment.

The micro KBr infrared spectra of metabolites 11 and 15 were of value only in demonstrating the presence of the triazine ring systems.

The carboxyl-containing metabolite, metabolite 4, was the only one isolated which contained the ethylamino group. Its characterization was based upon the molecular ion of the

silyl ester at m/e 327, with an isotope peak in good agreement with the calculated value and fragments at m/e 210 ($M^{+} - 117$) and 196 ($M^{+} - 131$) which would result, respectively, from C_3—C_4 and C_2—C_3 bond cleavages in the butyl moiety. The presence of fragments at m/e 131 and 117 lends confirmation to this fragmentation. The infrared spectrum was of little value, indicating only the presence of the s-triazine ring. The infrared spectrum of known 3-aminobutyric acid gave bands at 1640, 1560, and 1535 cm^{-1}. These would be masked by the strong triazine ring bands in that region.

From the mass spectral evidence, the structures were assigned as shown in Figure 1.

Rat urinary metabolites of GS-14254 eluted from column I in a pattern qualitatively similar to those of the cow and goat urine metabolites (Larson et al., 1970). Metabolites 8, 11, 13, and 15 accounted for 91% of the urinary radioactivity (8, 20.8%; 11, 19.8%; 13, 34.2%; and 15, 16.2%). Metabolite 13 (ammiline) was the major metabolite.

Larson et al. (1970) identified the rat urinary metabolites of ^{14}C-ring-labeled 2-hydroxy-GS-14254 (I), ^{14}C-ring-labeled 2-hydroxy-4-amino-6-ethylamino-s-triazine (II), and ^{14}C-ring-labeled 2-hydroxy-4-amino-6-sec-butylamino-s-triazine (III). The major urinary metabolites from II and III were identified as the unchanged compounds (89 and 78% of the radioactivity in the 0 to 24 hr urine, respectively). The major rat urinary metabolite (88% of the radioactivity in the 0 to 24 hr urine) from I was characterized as compound I with a hydroxyl on either the 3- or 4-carbon of the sec-butyl moiety. Unchanged compound I and debutylated compound I (compound II) were also present in the urine from rats dosed with I (6.8 and 5.6% of the dose, respectively). No ammiline was found as a metabolite from these three 2-hydroxytriazines, and no N-deethylation of I or II occurred. This evidence suggested that the mammalian metabolism of GS-14254 is greatly influenced by the type of substitution at the 2-position of the triazine ring, since ammiline was a major urinary metabolite from GS-14254 in cow, goat, and rat urine, and GS-14254 was extensively deethylated by the cow, goat, and rat.

Compounds I, II, and III had elution volumes from column I identical with fraction 17 in Figure 1, indicating that this fraction from cow and goat urine contained a mixture of the 2-hydroxy analog of GS-14254 and the two possible mono-N-dealkylated 2-hydroxy analogs of GS-14254. The hydroxylated rat metabolite of compound I eluted between fractions 16 and 17 in Figure 1.

The radioactivity in the cow feces cochromatographed on paper with urinary metabolites 8 and 11. No parent compound could be found.

ACKNOWLEDGMENT

LITERATURE CITED

Bakke, J. E., Robbins, J. D., Feil, V. J., J. AGR. FOOD CHEM. 15, 628 (1967).
Goldstein, G., Anal. Biochem. 20, 477 (1967).
Larson, J. D., Bakke, J. E., Feil, V. J., Proc. N.D. Acad. Sci. 24, in press (1970).
Pierce, A. E., "Silylation of Organic Compounds," Pierce Chemical Company, Rockford, Ill., 1968, p 35.
Robbins, J. D., Bakke, J. E., J. Anim. Sci. 26, 424 (1967).
Robbins, J. D., Bakke, J. E., Feil, V. J., J. AGR. FOOD CHEM. 16, 698 (1968).