occur, as appears to be the case. It suggests that perhaps 2,4-D-Glu might be a good substrate for this hydroxylation reaction, resulting eventually in a lowering of its concentration due to this reaction as well as accumulation of the other conjugates such as 2,4-D-Asp.

Additional experiments are being performed to test this proposed mechanism of metabolism of 2,4-D. It is hoped that information gained from metabolism in callus tissue will be similar to the metabolism in the whole plant.

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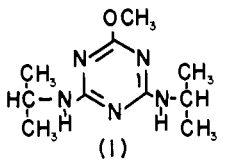
Rat Urinary Metabolites from 2-Methoxy-4,6-bis(isopropylamino)-*s*-triazine (Prometone)

Jerome E. Bakke* and Connie E. Price

Nine urinary metabolites from ^{14}C-ring-labeled prometone (I) were either identified or characterized by mass spectrometry of their trimethylsilyl derivatives. The major urinary metabolites were ammeline (31.5%) and 2-methoxy-4,6-diamino-*s*-triazine (10–14%). The other metabolites were dealkylation and oxidation products of prometone.

Bohme and Barr (1967) identified three rat urinary metabolites from prometone [2,4-bis(isopropylamino)-6-methoxy-*s*-triazine, I]. These metabolites were the mono- and di-*N*-dealkylated analogs of I and *N*-[2-methoxy-4-amino-*s*-triazinyl-(6)]alanine. Bakke *et al.* (1967) reported the excretion pattern and tissue residues resulting from a single oral dose of ^{14}C-ring-labeled prometone given to rats, and demonstrated the presence of at least 11 urinary metabolites from I by ion-exchange chromatography.



(I)

The present study reports the identification or mass spectral characterization of these rat urinary metabolites.

EXPERIMENTAL SECTION

The preparation of ^{14}C-ring-labeled prometone, animal treatment, radioanalysis, sample preparation, and ion-exchange chromatography of the urinary metabolites have been previously reported (Bakke *et al.*, 1967). The methods used to isolate and characterize the metabolites were identical with those reported for atrazine and 2-hydroxyatrazine urinary metabolites (Bakke *et al.*, 1972).

Rat urine (0–48 hr collections) was freeze dried and the radioactivity was extracted from the dry solids with methanol. Aliquots of this methanol extract were dried, taken up in 3 ml of water, adjusted to pH 3 with 6 *N* HCl, and applied to a 0.6 × 100 cm column of beadform, strong cation exchange resin (Chromabeads, Type A, Technicon, Inc., Column A). The column was jacketed with water circulating at 65°. The column preparation, equilibration, and regeneration, as well as buffer preparation (pH 2.875, 3.80, and 5.00 sodium citrate buffers) and buffer sequence in the variable-gradient device, were those given in the instruction manual for the automatic amino acid analyzer (Technicon Chromatography Corporation, 1962) and were similar to the system reported by Piez and Morris (1960), with the following exceptions. The column was monitored by a continuous-flow liquid scintillation detector; the detergent and bacteriostat were not included in the buffers; and after the end of the normal buffer cycle (675 ml), the elution was continued with a two-chamber gradient consisting of 75 ml of the pH 5 buffer in the first chamber and 75 ml of 0.2 *N* NaOH containing 0.6 mol/liter of NaCl in the second chamber.

The column flow rate was 0.5 ml/min, and 10-ml fractions were collected. The fractions containing activity were quantitated by liquid scintillation in counting solution A (Bakke *et al.*, 1967).

The radioactive fractions collected from column A were separately freeze dried and the residues were taken up in 5 ml of water, acidified to pH 3 with 6 *N* HCl, and applied to a 1- × 20-cm column of AG-50X8 cation-exchange resin in the ammonium form (column B). The citrate present from the column A buffer was eluted with water and the radioactivity was then eluted with 1 *N* NH_4OH. The recoveries from column B ranged from 71 to 95%. The citrate-free fractions were freeze-dried, and the residue from each fraction was dissolved in methanol and chromatographed on Whatman No. 1 paper. The chromatograms were developed in isoamyl alcohol–acetic acid–water (40:10:50, v/v/v). The radioactive fractions were extracted from the paper with methanol, the extracts were concentrated, and each was separately applied to a 0.9- × 100-cm column of methanol-equilibrated Sephadex LH-

United States Department of Agriculture, Agricultural Research Service, Metabolism and Radiation Research Laboratory, Fargo, North Dakota 58102.

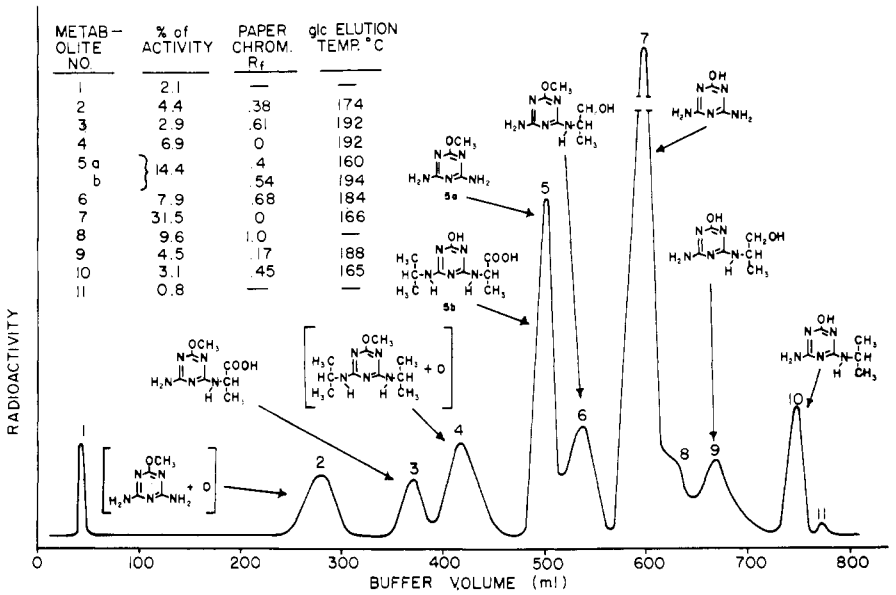


Figure 1. Ion-exchange chromatographic separation of the rat urinary metabolites from prometone with identified and proposed structures, quantitation of urinary metabolites and chromatographic data. The glc elution temperatures are for the trimethylsilyl derivatives. Structures 5a, 7, and 10 were identified by comparisons with known compounds; the rest were characterized by mass spectrometry.

20. The radioactive fractions eluted from the LH-20 column with methanol were taken to dryness, and each was silanized by heating with 50 μl of bis-trimethylsilyl trifluoroacetamide containing 1% trimethylchlorosilane (Regisil). The silanized metabolites were gas chromatographed on a 6-ft, ⅛-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/min. The temperature was programmed from 100 to 250° at 10°/min. The detector was located within the column oven, and its temperature varied with the oven temperature. The injector was maintained at 220°. Ten percent of the effluent from the column went to the flame detector.

The elution of radioactivity from the gas chromatograph was monitored by trapping each peak in a glass tube, and each tube was assayed for radioactivity.

Infrared spectra were obtained from samples in micro-KBr pellets (10 mg of 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.

RESULTS AND DISCUSSION

The results of the ion-exchange separation of the rat urinary metabolites resulting from a single oral dose of prometone-^{14}C are shown in Figure 1. The quantitation of each fraction based on the radioactivity applied to the column and the structures assigned to the identified and characterized metabolites are also shown in Figure 1. The paper chromatographic R_f values for each fraction and the temperatures at which the trimethylsilyl derivative (TMS) of each fraction eluted from the gas chromatographic column are also given in Figure 1. The mass spectra of the TMS derivatives of the isolated metabolites are given in Tables I and II.

The mass spectral characterizations of the TMS metabolites for which known compounds were not available were based upon the molecular ions ($M\cdot^+$), isotope peak intensities (where measurable), and fragmentations in the alkylamino portions of the TMS derivatives. The mass spectra of the TMS metabolites, in most cases, contained two sets of molecular ions with associated fragment ions. These resulted from the hydrolysis of a TMS group after trapping from the gas chromatograph but prior to ionization in the mass spectrometer, as has been observed for the TMS metabolites from other triazines (Bakke *et al.*, 1971, 1972).

Where sufficient sample was available, infrared spectra were obtained, but the data were of value only in demonstrating the presence of the triazine ring system.

The TMS derivatives of metabolites 5a, 7, and 10 gave mass spectra identical with the TMS derivatives of 2-methoxy-4,6-diamino-*s*-triazine, 2-hydroxy-4,6-diamino-*s*-triazine (ammeline), and 2-hydroxy-4-amino-6-isopropylamino-*s*-triazine, respectively. Two of these (5a and 7) have been identified as the major rat, cow, and goat urinary metabolites of a similar triazine, 2-methoxy-4-ethylamino-6-*sec*-butylamino-*s*-triazine (Bakke *et al.*, 1971; Larson *et al.*, 1971). Metabolites 5a and the 2-methoxy analog of 10 were identified by Bohme and Barr (1967) as rat urinary metabolites from prometone.

A second minor metabolite, approximately 2–3% of the urinary radioactivity, was separated from metabolite 5 (5b) by paper chromatography. The mass spectrum (5b, Table I) of its TMS derivative contained molecular ions and fragment ions compatible with both the mono- and di-TMS derivatives of structure 5b in Figure 1. The presence of a TMS ester of a carboxylic acid was indicated by the presence of intense fragment ions for the losses of 117 amu [$COOSi(CH_3)_3$ = 117] from both of the molecular ions ($M\cdot^+$ = 385 and 313) present in the spectrum. The isotope peak intensities for the $M\cdot^+$ at 385 and its associated $M\cdot^+ - 15$ fragment ion were compatible with the elemental compositions given in Table I. These elemental compositions each contained two atoms of silicon. The isotope peak intensity for the fragment ion at *m/e* 268 (385–117) was compatible with the elemental composition for the $M\cdot^+ - COOSi(CH_3)_3$ ion fragment.

N-[2-Methoxy-4-amino-*s*-triazinyl-(6)]alanine, the carboxylic acid isolated by Bohme and Barr (1967), was isolated from metabolite 3 (Figure 1). The mass spectrum (3, Table I) contained spectra for both the mono- and di-TMS derivatives. The major fragment ion in both spectra resulted from the loss of 117 amu [$COOSi(CH_3)_3$] from each molecular ion. The isotope peak intensities for the $M\cdot^+ - 15$ and $M\cdot^+ - 117$ fragment ions were compatible with those calculated for the elemental compositions given in Table I.

Fractions 2, 4, 6, and 9 were characterized by mass

Table I. Mass Spectra of the Trimethylsilyl Derivatives from Rat Urinary Metabolites from Prometone

m/e[a]	Relative abundance	M + 1 intensity[b]	Fragment ion description
		Metabolite 3 (Figure 1)	
		Di-TMS	
357	3		$M\cdot^+$
342	14	27 ($C_{12}H_{24}N_5O_3Si_2$; 25.6)	$M\cdot^+ - CH_3$
252	0.6		
240	100	18 ($C_9H_{18}N_5OSi$; 17.0)	$M\cdot^+ - COOSi(CH_3)_3$
224*	5		
163.5	0.5		$M\cdot^+ - 2CH_3$
112.5	0.3		$M\cdot^+ - [CH_3 + COOSi(CH_3)_3]$
		Mono-TMS	
285	2		$M\cdot^+$
270	9.5	18.5 ($C_9H_{16}N_5O_3Si$; 17.1)	$M\cdot^+ - CH_3$
168	100	10 ($C_6H_{10}N_5O$; 8.6)	$M\cdot^+ - COOSi(CH_3)_3$
127.5	0.1		$M\cdot^+ - 2CH_3$
		Metabolite 4 (Figure 1)	
313	5.2	22 ($C_{13}H_{27}N_5O_2Si$; 21.5)	$M\cdot^+$
298	7.5		$M\cdot^+ - CH_3$
254	7.0		
240	100		$M\cdot^+ - Si(CH_3)_3$
224*	11		$M\cdot^+ - OSi(CH_3)_3$
198	6.5		
168	14		
112.5	8		$M\cdot^+ - [Si(CH_3)_3 + CH_3]$
		Metabolite 5b (Figure 1)	
		Di-TMS	
385	3.5	30 ($C_{15}H_{31}N_5O_3Si_2$; 28.9)	$M\cdot^+$
370	15.5	28 ($C_{14}H_{28}N_5O_3Si_2$; 27.8)	$M\cdot^+ - CH_3$
341	<0.5		$M\cdot^+ - CO_2$
326	<0.5		$370 - CO_2$
268	100	20 ($C_{11}H_{22}N_5OSi$; 19.2)	$M\cdot^+ - COOSi(CH_3)_3$
		Mono-TMS	
313	11		$M\cdot^+$
298	13.5		$M\cdot^+ - CH_3$
196	100		$M\cdot^+ - COOSi(CH_3)_3$
		Metabolite 6 (Figure 1)	
343	1		$M\cdot^+$
328	24	25 ($C_{12}H_{26}N_5O_2Si_2$; 25.5)	$M\cdot^+ - CH_3$
312	1		$M\cdot^+ - OCH_3$
286	0.4		
270	0.6		
267	2.4		
253	7		
252	1.9		
240	100	17 ($C_9H_{15}N_5OSi$; 17.0)	$M\cdot^+ - CH_2OSi(CH_3)_3$
238*	3.4		
224*	4		
214	4		
198	3.4		
127.5	2.4		$M\cdot^+ - 2CH_3$
		Metabolite 9 (Figure 1)	
401	0.8		$M\cdot^+$
386	12.8	34 ($C_{14}H_{32}N_5O_2Si_3$; 32.8)	$M\cdot^+ - CH_3$
311	4.2		
310	1.7		
298	100	24 ($C_{11}H_{24}N_5OSi_2$; 24.3)	$M\cdot^+ - CH_2OSi(CH_3)_3$
296	4		
256	2		
240	1		

Table I *(Continued)*

m/e[a]	Relative abundance	M + 1 intensity[b]	Fragment ion description
		Metabolite 10 (Figure 1)	
		Di-TMS	
313	72	28 ($C_{12}H_{25}N_5OSi_2$; 25.6)	$M\cdot^+$
312	8		$M\cdot^+ - H$
298	100	28 ($C_{11}H_{22}N_5OSi_2$; 24.5)	$M\cdot^+ - CH_3$
271	26.5		$M\cdot^+ - CH_3CHCH_2$
256	47		$M\cdot^+ - CH_3CH(NH)CH_2$
141.5	0.5		$M\cdot^+ - 2CH_3$
		Mono-TMS	
241	58	17 ($C_9H_{17}N_5OSi$; 17.1)	$M\cdot^+$
240	9		$M\cdot^+ - H$
226	100	16 ($C_8H_{14}N_5OSi$; 16.0)	$M\cdot^+ - CH_3$
199	45		$M\cdot^+ - CH_3CHCH_2$
184	26		$M\cdot^+ - CH_3CH(NH)CH_2$
105.5	11		$M\cdot^+ - 2CH_3$

[a] An asterisk denotes a metastable transition. Ions at half mass values are doubly charged species. [b] $M\cdot^+ + 1$ isotope peak intensities are listed relative to the associate peak being 100%. Values in parentheses are calculated for the empirical formulas given.

Table II. Mass Spectra of the Trimethylsilyl Derivatives of Rat Urinary Metabolites from Prometone

	Metabolites 2 and 5a (Figure 1)					Metabolites 2 and 5a (Figure 1)			
m/e[a]	Metabolite 2	Metabolite 2a	Metabolite 5a	M + 1 intensity[b] and fragment ion description	m/e[a]	Metabolite 2	Metabolite 2a	Metabolite 5a	M + 1 intensity[b] and fragment ion description
373	12			$M\cdot^+$ ($M + 1 = 35\%$; calcd for $C_{13}H_{31}N_5O_2Si_3$, 31.8%)	181	3.7			
					171.5	3			373 − $2CH_3$
358	100			$M\cdot^+ - CH_3$ ($M + 1 = 35\%$; calcd for $C_{12}H_{28}N_5O_2Si_3$, 30.7%)	171	12	57	57	
					157	9.5			
					156	2.5	15	14	
342	6.5			$M\cdot^+ - CH_3O$	155	1.5			
328	0.5				141	2			$M\cdot^+$ for 5a
326	0.5				135.5	<0.5			301 − $2CH_3$
301	4				130	1.7			
286	5.5				127.5	<0.5	9	9	285 − $2CH_3$
285	3	45	45	$M\cdot^+$ for di-TMS-5a	115	2			
270	6	100	100		105	1.5			
254	1.5	2	4		100	5	24	22	
244	1.5				99	9.5	53	63	
240		3	1		89	15	15	16	
228*		5	4		86	1			
214	1				85	2			
213	1.5	4	5	$M\cdot^+$ for mono-TMS-5a	75	2.5	31	7	
198	5	12	24		73	22	100	54	
197	2.5				69	5.5			
187	8.5				59	10	14	14	

[a] An asterisk denotes a metastable transition. Ions at half mass values are doubly charged species. [b] $M\cdot^+ + 1$ isotope peak intensities are listed relative to the associate peak being 100%. Values in parentheses are calculated for the empirical formulas given.

spectrometry of their TMS derivatives to contain hydroxy functional groups other than or in addition to a 2-hydroxyl group on the *s*-triazine ring.

The mass spectra for the TMS derivatives of metabolites 6 and 9 (6 and 9, Table I) contained $M\cdot^+$ and $M\cdot^+ - 103$ [$103 = CH_2OSi(CH_3)_3$] fragment ions consistent with the TMS ethers of 2-methoxy- and 2-hydroxy-4-amino-6-(1-hydroxy)-isopropylamino-*s*-triazine, respectively. The isotope peak intensities for the $M\cdot^+ - 15$ and $M\cdot^+ - 103$ fragment ions for both metabolites were in agreement with those calculated for the elemental compositions given in Table I. Metabolites 6 and 9 were therefore assigned structures 6 and 9, respectively, in Figure 1.

The mass spectra from the TMS derivatives of metabolites 2 (2, Table II) and 4 (4, Table I) do not exhibit fragment ions which enable one to locate the positions of the proposed hydroxyl groups in either of the metabolites. Yields of the TMS derivatives of both of these metabolites from the gas chromatograph were approximately 10% of the activity injected into the gas chromatograph. However, some observations are of interest.

The mass spectrum of the TMS derivative of metabolite 2 (2, Table II) contained mass spectra for a di-TMS and a tri-TMS derivative, along with the mass spectra for the

mono- and di-TMS derivatives of 2-methoxy-4,6-diamino-*s*-triazine (structure 5a, Figure 1; mass spectrum 5a, Table II).

During gas chromatography of the TMS derivative of metabolite 2, radioactivity bled from the column from 160° (the elution temperature for di-TMS-5a) until the peak appeared at 174°. The mass spectrum (2a, Table II) of this bleed prior to the peak at 174° was identical to that of di-TMS-5a (2-methoxy-4,6-diamino-*s*-triazine), indicating that the tri-TMS derivative of 2 is converted to di-TMS-5a during gas chromatography. It has not been excluded that this bleed is the di-TMS derivative of 2-hydroxy-4-amino-6-methylamino-*s*-triazine.

The tri-TMS derivative of metabolite 2 (2, Table II) had a molecular ion at *m/e* 373, with the most intense fragment ion at *m/e* 358 ($M\cdot^+ - 15$) and a fragment ion at ($M\cdot^+ - 31$). These ions had isotope clusters typical for silicon-containing ions, and their relative intensities indicated the presence of three silicon atoms. Subtracting three TMS groups would give the underivatized metabolite a mol wt of 157. The fragment ion at $M\cdot^+ - 31$ indicated the presence of a methoxyl moiety. The $M\cdot^+$ at an odd mass requires the presence of an odd number of nitrogens.

The evidence that the apparent mol wt of 2 (157) is 16 greater than that of 5a suggests that a metabolite is obtained from metabolite 2, whose structure is some hydroxylated form of 2-methoxy-4,6-diamino-*s*-triazine (structure 5a, Figure 1) or 2-hydroxy-4-amino-6-methylamino-*s*-triazine. A hydroxymethylamino group, a possibility from the latter structure, was not indicated, since most of the fragment ions in the mass spectrum of the TMS derivative of 2-chloro-4-hydroxymethylamino-6-isopropylamino-*s*-triazine (Bakke *et al.*, 1972) were attributed to fragmentations and/or rearrangements in the trimethylsilyloxymethylamino group, with the base peak in the spectrum being $[NHCH_2OSi(CH_3)_3]^+$. The corresponding fragment ions were not present in the mass spectrum of metabolite 2. A 2-TMS-methylenedioxy group was not indicated, since the free hemiacetal would not be stable under the ion-exchange conditions (approximately 2 hr at 65° and pH 3). This leaves the possibilities of either a hydroxyl on one of the ring nitrogens or a hydroxylamine structure.

The mass spectrum from the TMS derivative of metabolite 4 (4, Table I) had a molecular ion at *m/e* 313. Although this mass would suggest the di-TMS derivative of fraction 10, the fragmentation pattern and isotope peak intensity were not compatible with that structure. The M + 1 isotope peak intensity for the $M\cdot^+$ at 313 indicated the presence of one TMS moiety. This would give the underivatized metabolite a mol wt of 241, which is 16 amu greater than that for prometone, and indicates the metabolite is some oxygenated form of prometone. The location of the oxygen could not be determined from the fragment ions. It could not be present as a TMS ether of a primary alcohol on one of the isopropyl groups, for this structure gives rise to a large $M\cdot^+ - 103$ fragment ion. The base ion fragment at $M\cdot^+ - 73$ $[M\cdot^+ - Si(CH_3)_3]$ and the metastable ion at $M\cdot^+ - 89$ $[M\cdot^+ - OSi(CH_3)_3]$ lead one to speculate the existence of a $NOSi(CH_3)_3$ structure on either a ring or an alkyl nitrogen.

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Metabolic Studies with Chloropropylate Acaricide in the Dairy Cow

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Excretion and metabolism of the acaricide chloropropylate (isopropyl 4,4′-dichlorobenzilate) was studied in a dairy cow. At a level of 5 ppm in the feed for 4 days, 0.11 and 5.93% of the compound was excreted intact in the milk and feces, respectively. Major elimination occurred in the urine with excretion of 28.13 and 55.23% of the total chloropropylate dose, respectively, as 4,4′-dichlorobenzilic acid and conjugates of the latter compound. Excretion of 4,4′-dichlorobenzilic acid in feces represented 5.44% of the dose. Chloropropylate was stable in rumen fluid but it decomposed in the presence of the 10,000 × *g* supernatant fraction of beef liver.

Chloropropylate (isopropyl 4,4′-dichlorobenzilate) is an effective acaricide for control of various mites on apples and pears. The use of pesticides on fruit always raises the possibility of cattle forage contamination through drift or residues in fruit pomace, which is sometimes used in dairy rations. Investigations in this laboratory have shown that virtually all of the chloropropylate in apples remains in the pomace after juice expression (Gutenmann and Lisk, 1972). No published work has appeared on the fate of chloropropylate in the bovine. Bourke *et al.* (1970) studied the fate of chloropropylate in rats. Using the carbon-14 (acetate)-labeled compound administered *via* stomach tube and measuring total radioactivity, elimination was shown to occur in the feces (64.5%) and urine (5.1%), with tissue storage in the liver (8.9%) and gastrointestinal tract (8.4%) and minor amounts in other organs and expired carbon dioxide. In the work reported, a study of the passage of chloropropylate from apples into pomace and the fate of the pure compound in a lactating cow has been made.

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