LITERATURE CITED

Burger, K. M., *Fresenius' Z. Anal. Chem.* **224**, 425 (1967).
Davison, K. L., U.S. Department of Agriculture, Agricultural Research Service, Metabolism and Radiation Research Laboratory, Fargo, N.D., personal communication, 1974.
Davison, K. L., Sell, J. L., *Arch. Environ. Contam. Toxicol.* **2**, 222 (1974).
Feil, V. J., Lamoureux, C. H., Styrvoky, E., Zaylskie, R. G., Thacker, E. J., Holman, G. M., *J. Agric. Food Chem.* **21**, 1072 (1973).
Haegele, M. A., Tucker, R. T., *Bull. Environ. Contam. Toxicol.* **11**, 98 (1974).
Inoi, T., Gericke, P., Horton, W. J., *J. Org. Chem.* **27**, 4597 (1962).
Loev, B., Goodman, M. M., *Chem. Ind. (London)*, 2026 (1967).
Paulson, G. D., *Poultry Sci.* **48**, 1331 (1969).
Sell, J. L., Davison, K. L., *Fed. Proc., Fed. Amer. Soc. Exp. Biol.* **32**, 2003 (1973).

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Metabolism of 2-Chloro-4-cyclopropylamino-6-isopropylamino-*s*-triazine (Cyprazine) in the Rat

Gerald L. Larsen* and Jerome E. Bakke

Rats given single oral doses of ring-^{14}C-labeled cyprazine (I) excreted 97.6% of the dose within 72 hr (urine, 72.7%; feces, 24.9%). Less than 0.1% of the radioactivity was detected as $^{14}CO_2$ in the expired air. The rat carcasses contained 7.5% of the radioactivity at sacrifice (72 hr after dosing). Four urinary metabolites were identified by comparison of their mass spectra with those of authentic compounds. These compounds and the percentages of the urinary radioactivity that they represented were: 2-hydroxy-4,6-diamino-*s*-triazine (II, 3.0%); 2-chloro-4,6-diamino-*s*-triazine (III, 13.9%); 2-hydroxy-4-amino-6-isopropylamino-*s*-triazine (IV, 5.3%); and 2-chloro-4-amino-6-isopropylamino-*s*-triazine (VII, 2.1%). Two fecal metabolites were identified as II and IV. Four additional urinary metabolites were characterized by mass spectrometry as 2-chloro-4-amino-6-[(2-hydroxy-1-methylethyl)-amino]-*s*-triazine (V, 5.9%); 2-chloro-4-amino-6-cyclopropylamino-*s*-triazine (VI, 7.7%); [2-chloro-4-cyclopropylamino-*s*-triazinyl(6)]alanine (VIII, 9.9%); and 2-chloro-4-cyclopropylamino-6-[(2-hydroxy-1-methylethyl)amino]-*s*-triazine (IX, 1.6%).

Bohme and Barr (1967) identified rat and rabbit urinary metabolites from the 2-chloro-*s*-triazines: simazine [2-chloro-4,6-bis(ethylamino)-*s*-triazine]; atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine); and propazine [2-chloro-4,6-bis(isopropylamino)-*s*-triazine]. The metabolites of these 2-chloro-*s*-triazines were all 2-chloro analogs of their respective parent molecules. Mono- and di-N-dealkylation and oxidation of the *N*-alkyl groups to acids were major routes of metabolism for these 2-chloro-*s*-triazines.

Bakke et al. (1972) identified four and characterized two metabolites from atrazine in rat urine. As isolated, these urinary metabolites were identified as 2-hydroxy analogs of atrazine. However, these metabolites were isolated by ion-exchange chromatography at elevated temperatures, and subsequent investigations indicated that these metabolites may have been artifacts. For instance, when atrazine and 2-chloro-4,6-diamino-*s*-triazine were placed on the ion-exchange column used in their isolation procedure, both compounds were quantitatively recovered from the ion-exchange column as their 2-hydroxy analogs. Bakke et al. (1972) concluded that any 2-chloro-*s*-triazine metabolites present in the urine were quantitatively converted to 2-hydroxy analogs. However, the presence of 2-hydroxy-*s*- triazine metabolites in the urine could not be ruled out.

This report describes the metabolism of the 2-chloro-*s*-triazine cyprazine (2-chloro-4-cyclopropylamino-6-isopropylamino-*s*-triazine, I) by the rat. The urinary metabolites were isolated by using paper chromatography to minimize hydrolysis of the 2-chloro group that occurs with the use of ion-exchange chromatography.

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

EXPERIMENTAL SECTION

Chemicals. 2-Chloro-4-cyclopropylamino-6-isopropylamino-*s*-triazine-^{14}C, uniformly labeled in the ring (9.4 μCi/mg), and unlabeled cyprazine were provided by Gulf Research and Development Co., Merriam, Kan. Radiopurity of the radioactive chemical was greater than 99.0% as determined by thin-layer chromatography (TLC). Authentic standard compounds were obtained from Geigy Chemical Corporation.

Animal Treatment. All rats were dosed by stomach tube with ring-^{14}C-labeled cyprazine dissolved in 0.5 ml of ethanol. Urine and feces were collected at 24-hr intervals.

Two rats (each weighing 310–360 g) were each given 0.5 mg of cyprazine containing 0.93 μCi of ^{14}C as a single oral dose. These rats were housed in all-glass metabolism cages, and CO_2 was collected as previously reported (Bakke et al., 1967). After 3 days, the rats were sacrificed, and total carcass radioactivity was determined.

Twelve rats (each weighing 300–420 g) were each given 5.0 mg of cyprazine containing 0.51 μCi of ^{14}C as a single oral dose. These rats were housed in stainless steel cages, and urine was collected daily and pooled. Feces were also collected daily and pooled. The pooled urine and pooled fecal samples were assayed for radioactivity and used for metabolite isolation. After 3 days, the rats were sacrificed.

Instrumentation and Quantitation of Radioactivity. All column eluates were monitored with a Packard Model 320E liquid scintillation flow system. With aqueous eluates, anthracene was used as the scintillator in the flow cell, and with organic eluates, cerium-activated lithium glass beads were used as the scintillator in the flow cell. Radioactive components on paper chromatograms and

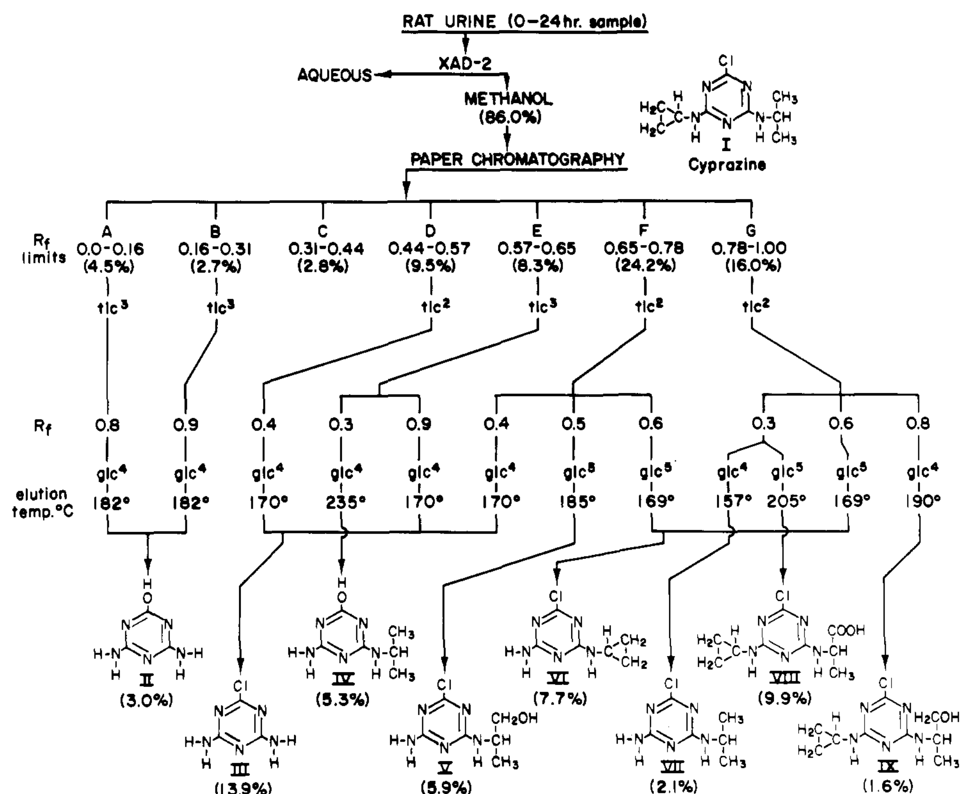

Figure 1.

thin-layer chromatograms were located with a Packard Model 7200 chromatogram scanner. Liquid scintillation in counting solution A (Bakke et al., 1967) was used to quantitate radioactivity with a Nuclear-Chicago Mark I spectrometer. Combustion of freeze-dried samples (100–200 mg) was used to quantitate carcass and fecal ^{14}C, with a Model 305 Packard Tri-Carb sample oxidizer. Mass spectra were obtained with a Varian M-66 mass spectrometer equipped with a V-5500 control console; a solid-sample inlet system was used.

Chromatographic Methods. Amberlite XAD-2 (Mallinckrodt Chemical Works) was equilibrated with water in a 1.5 × 25 cm column. The XAD-2 column was conditioned by a two-column volume wash with methanol followed by a two-column volume wash with water. This process was repeated three times. With the column equilibrated with water, the samples (10 ml) of rat urine were applied to the column under nitrogen pressure. The column was first washed with three-column volumes of water, and the radioactivity was then eluted with methanol.

The cation-exchange column (AG 50-X8 [H^+]) was converted to the ammonia form with 1 *N* NH_4OH and washed free of base with water. Acidified (pH 2.0) aqueous samples were applied to the column, and the column was washed with three-column volumes of water. The radioactivity was then eluted from the column with 1 *N* NH_4OH.

The anion-exchange column (AG 1-X8 [OH^-]) was washed with water before the samples were applied. Aqueous samples were applied under nitrogen pressure, and the column was washed with three-column volumes of water. The radioactivity was then eluted from the column with 1 *N* NH_4HCO_3.

The paper chromatograms were all developed with the organic phase from the mixture of isoamyl alcohol-concentrated NH_4OH-H_2O (40:10:50, v/v/v); Whatman No. 1 paper was used.

Thin-layer chromatography was accomplished with precoated silica gel thin-layer plates (0.5 mm thickness, Brinkman No. 5769/0020). Three solvent systems were used: solvent 1, $CHCl_3$-ethanol (5:1, v/v); solvent 2, 1-butanol-H_2O-concentrated NH_4OH (4:5:1, v/v/v); and solvent 3, methanol-concentrated NH_4OH (9:1, v/v).

Gas-liquid chromatography (GLC) was performed with a 6 ft × ⅛ in. i.d. glass column packed with 3% SE-30 on 60–80 mesh Chromosorb W in a Perkin-Elmer 801 gas chromatograph fitted with an effluent splitter (10% to the flame ionization detector). The oven temperature was programmed at either 10°/min or 6°/min from 100 to 250°; the injector temperature was maintained at 200°. The detector was located within the oven. The carrier gas was helium at a flow rate of 30 ml/min.

Metabolite Isolation. The flow diagram shown in Figure 1 lists the combinations of methods used to isolate each metabolite from rat urine. Aliquots of low specific activity (5.0-mg doses, 0- to 24-hr rat urine) were placed on the XAD-2 column. The radioactivity that eluted from the XAD-2 column with methanol was concentrated and spotted in a continuous band on paper. The developed paper chromatograms were cut into seven radioactive sections (A–G, Figure 1), and each section was extracted with methanol. The radioactivity extracted from each section was further purified by using the TLC solvent systems specified in Figure 1. The radioactive areas were scraped from the TLC plates and the radioactivity was extracted from the silica gel with methanol. These extracts were taken to dryness and derivatized by heating to 80° for 16 hr with an excess of *N*,*N*-bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylsilane (Regisil, Regis Chemical Co.).

The silanized metabolites were gas chromatographed and the elution of radioactivity was monitored by trapping each peak in a glass tube and assaying the radioactivity washed from each tube.

Feces were extracted sequentially with hexane, ethyl acetate, acetone, methanol, and water in a soxhlet apparatus, and the extracted radioactivity was determined.

The methanol extract was taken to dryness, dissolved in 5 ml of water, acidified to pH 2, and applied to a cation-exchange column. The radioactivity eluted with 1 *N* NH_4OH was freeze-dried, dissolved in 5 ml of water, and applied to the anion-exchange column. The radioactivity was eluted from the anion-exchange column with 1 *N* NH_4HCO_3 and freeze-dried. The bicarbonate salts were removed from the radioactivity by dissolving the residue in water, acidifying to pH 2, and chromatographing the radioactivity on the cation-exchange column. The bicarbonate salts were eluted from the column with three-column volumes of water. The radioactivity was eluted with 1 *N* NH_4OH, freeze-dried, and spotted on a paper chromatogram. The radioactive areas on the developed paper chromatogram were extracted from the paper with methanol, and the methanol extracts were taken to dryness, derivatized with Regisil, and gas chromatographed.

RESULTS AND DISCUSSION

The ^{14}C excretion and recovery data for rats given single oral doses of cyprazine-^{14}C are given in Table I. Ninety-eight percent of the radioactivity was excreted within 72 hr after dosing (urine, 72.7%; feces, 24.9%), most of which was excreted within the first 24 hr. Less than 0.1% of the dose was detected as $^{14}CO_2$ in the expired air. The carcass and hide contained 7.5% of the ^{14}C at sacrifice; this radioactivity was not identified.

Eighty-six percent of the urinary ^{14}C was extracted from the urine by using the XAD-2 column. This radioactivity was separated into seven fractions by paper chromatography. The R_f limits of these fractions are given in Figure 1, along with the subsequent thin-layer chromatographic data for each paper chromatography fraction and gas chromatographic elution temperature for the trimethylsilyl (Me_3Si) derivative of each metabolite separated by TLC. Structures assigned to each metabolite are also given in Figure 1, along with an estimate of the quantity of each metabolite present in the urine (based on total urinary ^{14}C). The structural assignments were based on mass spectral fragmentation patterns obtained from the Me_3Si derivatives of the metabolites. The mass spectra are given in Table II.

The mass spectral characterization of the Me_3Si derivatives of the metabolites, where Me_3Si derivatives of known compounds were not available, were based on the molecular ion ($M\cdot^+$), isotope peak intensities (especially chlorine isotope peaks), and fragmentations occurring in the alkylamino moieties of the Me_3Si derivatives.

Me_3Si derivatives of metabolites II, III, IV, and VII (Figure 1) gave mass spectra identical with the Me_3Si derivatives of authentic 2-hydroxy-4,6-diamino-*s*-triazine (ammeline), 2-chloro-4,6-diamino-*s*-triazine, 2-hydroxy-4-amino-6-isopropylamino-*s*-triazine, and 2-chloro-4-amino-6-isopropylamino-*s*-triazine, respectively.

The major urinary metabolite of cyprazine, 2-chloro-4,6-diamino-*s*-triazine (III, 13.9%), has been identified by Bohme and Barr (1967) as a urinary metabolite of other 2-chloro-*s*-triazines (simazine, atrazine, and propazine). Ammeline (II) was a major urinary metabolite of 2-methoxy-4-ethylamino-6-*sec*-butylamino-*s*-triazine, atrazine, and prometone (Bakke et al., 1971, 1972; Bakke and Price, 1973). However, some or all of the ammeline isolated as a metabolite from atrazine may have been an artifact resulting from hydrolysis of the 2-chloro analogs in the ion-exchange system used. The isolation of ammeline from the higher R_f fraction (fraction B, Figure 1) in this study most likely resulted from the hydrolysis of 2-chloro-4,6-diamino-*s*-triazine (III) during paper chromatography, because ammeline does not migrate from the origin in the paper chromatographic system used. Isolation of metabolite III from three fractions on the paper chromatogram (fractions D, E, and F; Figure 1) probably resulted from spreading during paper chromatography because of the high concentration of metabolite III present in the urine. Also, metabolite IV (2-hydroxy-4-amino-6-isopropylamino-*s*-triazine) was isolated from a fraction with a higher R_f (fraction E, Figure 1) than previously reported for IV (Bakke et al., 1972) with the paper chromatographic solvent system used. We can therefore assume that metabolite IV also resulted from hydrolysis of its less polar 2-chloro analog, metabolite VII, during paper chromatography.

Table I. Recovery of Administered Radioactivity from Rats after a Single 0.5-mg Dose of Ring-^{14}C-Labeled Cyprazine

	% of ^{14}C dose				
Hour	Urine	Feces	Carcass	Hide	CO_2
24	61.4	21.0			
48	4.3	2.3			
72	7.0	1.6	6.4	1.1	
Total	72.7	24.9	6.4	1.1	<0.1

The Me_3Si derivatives of metabolites V and IX (Figure 1) both had mass spectra with intense chlorine-containing fragment ions at $M\cdot^+ - 103$ [$103 = CH_2OSi(CH_3)_3$]. These intense $M\cdot^+ - 103$ fragment ions indicated the presence of the Me_3Si derivatives of primary alcohols (Pierce, 1968). Both chlorine-containing molecular ions ($M\cdot^+ = 347$ and $M\cdot^+ = 315$, respectively) appeared at odd mass values; these odd mass values require the presence of an odd number of nitrogen atoms in the molecular ions. If we assume the presence of the original five nitrogen atoms, at least one oxygen atom (because of the $M\cdot^+ - 103$ fragment ions), one chlorine atom (from the chlorine-containing isotope peak intensities given in Table II, *m/e* 332 for V and *m/e* 315 for IX), two silicon atoms in V, and one silicon atom in IX (from the isotope peak intensities, Table II, *m/e* 332 for V, and *m/e* 315 for IX), the most reasonable elemental composition for the $M\cdot^+ - CH_3$ from V is $C_{11}H_{23}N_5OClSi_2$ and that for the molecular ion from IX is $C_{12}H_{22}N_5OClSi$. The molecular ion from V would then have an elemental composition of $C_{12}H_{26}N_5OClSi_2$. The underivatized metabolites V and IX would then have elemental compositions of $C_6H_{10}N_5OCl$ and $C_9H_{14}N_5OCl$, respectively. These proposed elemental formulas and mass spectral fragmentation patterns are compatible with the structures given for V and IX in Figure 1 and are supported by the intense fragment ion at $M\cdot^+ - 103$ contained in the mass spectra of both metabolites V and IX; this intense fragment ion indicates oxidation of the *N*-alkyl group in both metabolites to an alcohol. From these mass spectral data, the structure V was assigned to metabolite V, and IX was assigned to metabolite IX. The presence of small quantities of the cyclopropanol isomer of IX in this fraction has not been excluded because of the presence of a fragment ion of weak intensity at *m/e* 196; this fragment ion could indicate the cleavage of the molecule between the trimethylsilyloxycyclopropyl ring and the amino nitrogen. However, this possibility seems unlikely because the cyclopropanol isomer would not fragment to yield an $M\cdot^+ - 103$ fragment ion as base peak without extensive rearrangement.

The mass spectra of the Me_3Si derivative of metabolite VI (VI, Figure 1) contained a chlorine-containing molecular ion at $M\cdot^+$ 257 and exhibited fragment ions at $M\cdot^+ - H$, $M\cdot^+ - Cl$, and $M\cdot^+ - 41$ ($41 = C_3H_5$). Because the molecular ion is at an odd mass value, the empirical formula of the molecular ion contained an odd number of nitrogen atoms. If we assume the presence of the original five nitrogen atoms and one chlorine atom (from the $M\cdot^+ + 2$ isotope ion intensities, Table II), the most reasonable elemental composition for this molecular ion is

Table II. Mass Spectra

m/e	Rel abundance	Isotope peak intensities[a] $m/e + 1$	$m/e + 2$	Empirical formula	Fragment ion description
			Metabolite V di-Me_3Si		
347 (Cl)	1				$M^{\cdot +}$
332 (Cl)	28	30 (24.4)	41 (40.4)	$C_{11}H_{23}N_5OClSi_2$	$M^{\cdot +} - CH_3$
257 (Cl)	17				$M^{\cdot +} - HOSi(CH_3)_3$
244 (Cl)	100	17 (15.9)	38 (36.4)	$C_8H_{15}N_5ClSi$	$M^{\cdot +} - CH_2OSi(CH_3)_3$
129.5 (Cl)	10				$M^{\cdot +} - [Si(CH_3)_3 + CH_3]$
			Metabolite VI mono-Me_3Si		
257 (Cl)	57[b]	19[b] (17.0)	44 (36.6)	$C_9H_{16}N_5ClSi$	$M^{\cdot +}$
256 (Cl)	25				$M^{\cdot +} - H$
242 (Cl)	100	20 (15.9)	39 (36.4)	$C_8H_{13}N_5ClSi$	$M^{\cdot +} - CH_3$
222	2				$M^{\cdot +} - Cl$
216 (Cl)	2				$M^{\cdot +}$ – cyclopropane
120.5 (Cl)	2				$M^{\cdot +} - (CH_3 + H)$
113.5 (Cl)	6				$M^{\cdot +} - 2CH_3$
			Metabolite VIII mono-Me_3Si		
329 (Cl)	37	24 (20.4)	43 (37.4)	$C_{12}H_{20}N_5O_2ClSi$	$M^{\cdot +}$
328 (Cl)	4	24 (20.4)	39 (37.4)	$C_{12}H_{19}N_5O_2ClSi$	$M^{\cdot +} - H$
314 (Cl)	18.5				$M^{\cdot +} - CH_3$
212 (Cl)	100	12 (10.7)	34 (33.0)	$C_8H_{11}N_5Cl$	$M^{\cdot +} - COOSi(CH_3)_3$
149.5 (Cl)	1				$M^{\cdot +} - 2CH_3$
117	4				$COOSi(CH_3)_3$
			Metabolite IX mono-Me_3Si		
315 (Cl)	26	22 (20.4)	38 (37.2)	$C_{12}H_{22}N_5OClSi$	$M^{\cdot +}$
314 (Cl)	2				$M^{\cdot +} - H$
300 (Cl)	10	22 (19.2)	38 (37.0)	$C_{11}H_{19}N_5OClSi$	$M^{\cdot +} - CH_3$
242 (Cl)	1				$M^{\cdot +} - Si(CH_3)_3$
212 (Cl)	100	12 (10.7)	34 (33.0)	$C_8H_{11}N_5Cl$	$M^{\cdot +} - CH_2OSi(CH_3)_3$
196 (Cl)	4				$M^{\cdot +} - C_3H_4OSi(CH_3)_3$
184 (Cl)	3				

[a] The isotope peak intensities $m/e + 1$ and $m/e + 2$ are listed as percentages of the associated peak. Values in parentheses are calculated for the empirical formulas given. [b] The values indicated were calculated taking into account the contribution of the isotope cluster from the $M^{\cdot +} - 1$ fragment ion to the $M^{\cdot +}$ and $M^{\cdot +} + 1$ fragment ion intensities.

$C_9H_{16}N_5ClSi$. The underivatized metabolite then had an elemental composition of $C_6H_8N_5Cl$. This elemental formula for the unsilanized metabolite VI had a molecular weight 42 less than that of cyprazine; this information suggested N-dealkylation by the rat of the *N*-isopropyl group. This is supported by the presence of the $M^{\cdot +} - C_3H_5$ fragment ion. The $M^{\cdot +} - 1$ fragment ion probably resulted from α cleavage of a proton from the cyclopropyl ring with a charge remaining on the cyclopropylamino nitrogen. From these data, structure VI was assigned to this metabolite.

The mass spectrum of the Me_3Si derivative of metabolite VIII contained intense chlorine-containing fragment ions at $M^{\cdot +} - 117$ [117 = $COOSi(CH_3)_3$] and $M^{\cdot +} - 15$, and a weak fragment ion at m/e 117. The intense chlorine-containing fragment ion at $M^{\cdot +} - 117$ in the mass spectra indicated the presence of a Me_3Si ester of a carboxylic acid (Pierce, 1968). The chlorine-containing molecular ion ($M^{\cdot +}$ 329) was at an odd mass value; this odd mass value requires an odd number of nitrogen atoms in the molecular ion. If we assume the presence of five nitrogen atoms, one chlorine atom (from the $M^{\cdot +} + 2$ isotope peak intensity, Table II), one silicon atom (from the $M^{\cdot +} + 1$ peak intensity, Table II), and at least two oxygen atoms (from the $M^{\cdot +} - 117$ fragment ion), the most reasonable elemental composition for this molecular ion is $C_{12}H_{20}N_5O_2ClSi$. The underivatized metabolite would then have the elemental formula $C_9H_{12}N_5O_2Cl$. This elemental composition containing two oxygens is supported by the large fragment ion at $M^{\cdot +} - 117$ [117 = $CO_2Si(CH_3)_3$]. The fragment ion at m/e 117 supports the presence of the carboxylic ester structure (Figure 1). From these data, the structure VIII was assigned to metabolite VIII (Figure 1).

The radioactivity extracted from the feces was as follows: hexane (1%), ethyl acetate (2%), acetone (2%), methanol (46%), and water (30%). The methanol extract was initially purified by ion-exchange chromatography and then separated into two components (II, R_f 0; IV, R_f 0.4) by paper chromatography. The Me_3Si derivative of the compound with the R_f 0 had a GLC elution temperature and gave a mass spectrum identical with that of the Me_3Si derivative of ammeline (II). After silanization, the compound with R_f 0.4 gave a mass spectrum identical with that of the Me_3Si derivative of 2-hydroxy-4-amino-6-isopropylamino-*s*-triazine (IV). Since ion-exchange columns were required to isolate these metabolites, it could not be determined whether the true fecal metabolites were 2-hydroxy or 2-chloro compounds. We were unable to isolate any metabolites from the water, acetone, ethyl acetate, or hexane extracts of the feces.

The urinary metabolites isolated accounted for 49% of the urinary radioactivity (Figure 1). It is assumed that low recovery resulted from hydrolysis of the 2-chloro metabolites to their corresponding 2-hydroxy analogs during paper chromatography and thereby caused the 2-hydroxy

analogs to be spread over the paper chromatogram and rendered them difficult or impossible to detect. Eighty-three percent of the urinary metabolites isolated were 2-chloro analogs of cyprazine. The hydrolysis of the 2-chloro group by the rat cannot be ruled out, but it does not appear to be a major route of metabolism in the rat. These findings are consistent with those of Bohme and Barr (1967) and suggest that the 2-hydroxy metabolites isolated as atrazine metabolites (Bakke et al., 1972) were artifacts resulting from hydrolysis of the corresponding 2-chloro analogs during ion-exchange chromatography.

Total N-dealkylation, partial N-dealkylation, and N-dealkylation with *N*-alkyl oxidation are major routes of cyprazine metabolism in the rat.

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LITERATURE CITED

Bakke, J. E., Larson, J. D., Price, C. E., *J. Agric. Food Chem.* **20**, 602 (1972).
Bakke, J. E., Price, C. E., *J. Agric. Food Chem.* **21**, 640 (1973).
Bakke, J. E., Robbins, J. D., Feil, V. J., *J. Agric. Food Chem.* **15**, 628 (1967).
Bakke, J. E., Robbins, J. D., Feil, V. J., *J. Agric. Food Chem.* **19**, 462 (1971).
Bohme, C., Barr, F., *Food Cosmet. Toxicol.* **5**, 23 (1967).
Pierce, A. E., "Silylation of Organic Compounds", Pierce Chemical Co., Rockford, Ill., 1968, pp 36–39.

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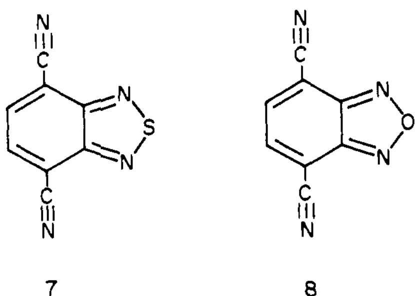
Herbicidal Activity of 2,1,3-Benzothiadiazolecarbonitriles and Related Cyanoheterocycles

Robert H. Schieferstein and Kurt Pilgram*

New carbonitriles of 2,1,3-benzothiadiazole and benzofurazan have been prepared and evaluated for herbicidal activity. 4,7-(and 4,5)-Dicyano-2,1,3-benzothiadiazole (**7** and **4**) and 4,7- (and 4,5)-dicyanobenzofurazan (**8** and **5**) were active pre- and postemergence at low rates. Substitution of one or both cyano groups by hydrogen, alkyl, chlorine, carboxy, alkoxycarbonyl, carboxamido, acylamido, and ureido reduced activity significantly. High activity was maintained in the monomethyl analog of **7**, whereas addition of two methyl groups or one amino or nitro group essentially eliminated activity. Annual ryegrass, wild oat, and corn have tolerance for **7** in relation to rates required for control of a wide range of weeds; other analogs do not appear as selective for corn as **7**.

Earlier investigations in our laboratories showed that 2,1,3-benzothiadiazole-4,7-dicarbonitrile (**7**) was active as a herbicide (Slott et al., 1968). The related benzofurazan-4,7-dicarbonitrile (**8**) also showed herbicidal activity. A



number of related 2,1,3-benzothiadiazoles and benzofurazans have now been synthesized and their properties studied. As a result of these studies, some correlations have been made of the herbicidal activity with change of position and nature of substituents in the benzothiadiazoles and benzofurazans.

MATERIALS AND METHODS

Chemical Methods. Carbonitriles of 2,1,3-benzothiadiazoles and benzofurazans were prepared by reaction of the respective bromoheterocycle with cuprous cyanide in dimethylformamide at elevated temperature. Cyanoheterocycles **3–20** and **23** were prepared by this method (Pilgram and Skiles, 1974; Pilgram and Zupan, 1974). The 2,1,3-benzothiadiazolecarbonitriles **1**, **2**, and **22** were conveniently prepared from the respective amino-2,1,3-benzothiadiazole using the Sandmeyer method (Pilgram and Skiles, 1974). Bis(thiocarboxamido)-2,1,3-benzothiadiazoles **25**, **27**, and **28**, and 4,7-bis(thiocarboxamido)benzofurazan (**26**) were prepared by the base-catalyzed addition of hydrogen sulfide to the corresponding dicarbonitrile in dimethylformamide or alcohol solution (Slott et al., 1968). Dicarboxylic acid **24**, prepared from **7** by alkaline hydrolysis, was converted, via the diacid chloride, into the diamides **29** and **30**, the dihydrazide **31**, and the dicarboxylic acid esters **32** and **33** (Pilgram, 1974). Nitration of 4-methyl-, 4-ethyl-, and 4-propyl-2,1,3-benzothiadiazole in concentrated sulfuric acid afforded the 7-nitro analogs **34**, **36**, and **38**, respectively. Compounds **34** and **36** were reduced to the amines **35** and **37**. The reduction was effected using iron filings in boiling water containing 3% of formic or acetic acid, or in aqueous sodium dithionite. Compound **39** was prepared by nitration with sodium nitrate in concentrated sulfuric acid by the method of Pesin et al. (1963). 4-Bromo-7-nitro-2,1,3-benzothiadiazole (Pilgram et al., 1970) with dimethylamine at 80° in methanol in an autoclave produced **40**, which was reduced catalytically to **41**. Chlorobenzothiadiazoles **21** and **43** were prepared by the methods of Pesin et al. (1963, 1964), whereas 4,7-dichlorobenzofurazan (**44**) was obtained following the

The Biological Sciences Research Center, Shell Development Company, Modesto, California 95352.