(Jenkins et al., 1970, 1975; Schmeltz et al., 1979) have questioned the extrapolation from classical pyrolysis data to cigarette smoking.

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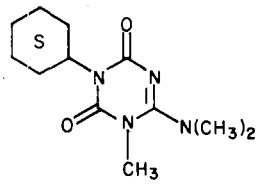
Determination of Hexazinone and Metabolite Residues Using Nitrogen-Selective Gas Chromatography

Richard F. Holt

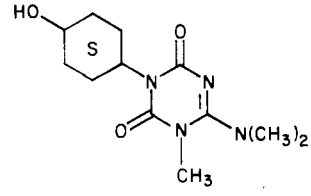
Residues of the weed killer 3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1*H*,3*H*)-dione (hexazinone) and two primary metabolites (A and B) in plants, animal tissues, and soil are determined by initial extraction with chloroform, cleanup by liquid/liquid partitioning techniques, and measurement by nitrogen-selective gas chromatography after reaction of the metabolites with trifluoroacetic anhydride. Additionally, by modification of the extraction scheme, two minor metabolites (D and E) can also be detected. A fifth metabolite (C) can be determined through a separate isolation procedure. Relative to a 25-g sample, method sensitivity is 0.04 ppm for the parent compound and metabolites A and B, 0.1 ppm for metabolites D and E, and 0.2 ppm for metabolite C.

Hexazinone is the approved common name for 3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4-(1*H*,3*H*)-dione. This material, which was formerly known as DPX-3674, is the active compound in Du Pont's Velpar weed killer.

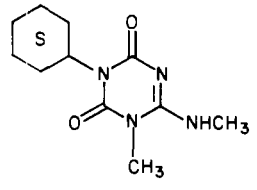
Sensitive analytical procedures are described for the determination of the parent compound and five metabolites: 3-(4-hydroxycyclohexyl)-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1*H*,3*H*)-dione (metabolite A); 3-cyclohexyl-6-(methylamino)-1-methyl-1,3,5-triazine-2,4-(1*H*,3*H*)-dione (metabolite B); 3-(4-hydroxycyclohexyl)-6-(methylamino)-1-methyl-1,3,5-triazine-2,4(1*H*,3*H*)-dione (metabolite C); 3-cyclohexyl-1-methyl-1,3,5-triazine-2,4,6-(1*H*,3*H*,5*H*)-trione (metabolite D); 3-(4-hydroxycyclohexyl)-1-methyl-1,3,5-triazine-2,4,6(1*H*,3*H*,5*H*)-trione (metabolite E). These metabolites were detected in [^{14}C]hexazinone studies with alfalfa and sugarcane. Metabolites A and B were detected in [^{14}C]hexazinone soil studies (Rhodes, 1980a). Metabolites A and C were detected in the urine and feces of rats preconditioned on hexazinone and given a single dose of [^{14}C]hexazinone (Rhodes, 1980b).



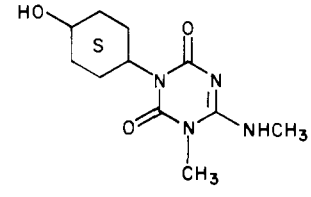
hexazinone



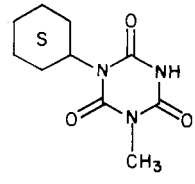
metabolite A



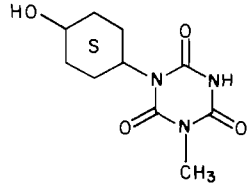
metabolite B



metabolite C



metabolite D



metabolite E

These analytical procedures are based on selective nitrogen-sensitive gas chromatographic measurement after

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reaction of all residues with trifluoroacetic anhydride. This derivatization is necessary to convert metabolites A, B, C, and E to more volatile compounds. Due to major differences in polarity, it was necessary to develop two separate isolation schemes. One was for the determination of the highly polar metabolite C and the other for the less polar parent compound and metabolites A, B, D, and E.

EXPERIMENTAL SECTION

Apparatus and Reagents. The Perkin-Elmer Model 3920-B gas chromatograph (Perkin-Elmer, Norwalk, CT) equipped with a nitrogen–phosphorus detector was used. The chromatographic column was 15% OV-17 on 100/120 Chromosorb W HP (Supelco, Inc., Bellefonte, PA): 2-ft glass; 0.25 in. o.d.; $^1/_{16}$ in i.d. An alternate column packing material was 3% SP-2250 DA on 100/120 Supelcoport (Supelco, Inc.). For the SP-2250 DA packing, acid-treated glass wool was used in the column ends.

Homogenization and extraction were conducted using a blender–centrifuge bottle and adapter base (Pease and Holt, 1971). Conventional bottles and centrifuge tubes may be used but are somewhat more time consuming. Centrifugation was carried out with an International Size 1, Type SB centrifuge capable of holding standard 250-mL centrifuge bottles.

All reference standards of hexazinone and metabolites A–E were obtained from the Biochemicals Department, Agrichemicals Marketing Division, E. I. du Pont de Nemours & Co., Inc., Wilmington, DE. The solvents were distilled in glass purchased from Burdick and Jackson Laboratories, Inc., Muskegon, MI. The trifluoroacetic anhydride was purchased from Pierce Chemical Co., Rockford, IL. The Bio-Beads S-X2 was purchased from Bio-Rad Laboratories, Richmond, CA. The Gas-Chrom S was purchased from Applied Science, State College, PA. The alumina (A-540) was purchased from Fisher Scientific Co., Fair Lawn, NJ.

The 2.5% water-deactivated Florisil was prepared by heating pesticide-grade Florisil (Supelco, Inc.) overnight at 140 °C and allowing it to cool in a tightly stoppered bottle. Two and one-half milliliters of water was added to 97.5 g of dried Florisil, and this was mixed thoroughly. The Florisil was allowed to equilibrate in a sealed bottle for 24 h prior to use.

Isolation. Part I. Moderately Polar Residues (Hexazinone and Metabolites A, B, D, and E). Twenty-five grams of a representative sample was weighed into the blender-centrifuge bottle. Fifteen grams of sodium sulfate and 120 mL of chloroform were added. (Chloroform should be used only in a well-ventilated hood. Skin contact should be avoided. The use of neoprene gloves is suggested.) The bottle was covered, placed in the adapter base, and blended at high speed for ~5 min. The sample was centrifuged at 1500 rpm for 10–15 min and the solvent carefully filtered through cotton into a 1000-mL round-bottomed flask. For liquid samples, the lower chloroform layer may be withdrawn from the blender bottle by using a 200-mL syringe. For samples which tend to become finely ground and pass through the cotton, i.e., grass or alfalfa, it may be necessary to filter the extract under vacuum through Whatman filter paper in a Buchner funnel. The chloroform extraction was repeated 2 more times, each time using additional 100-mL portions of solvent. Fifty milliliters of distilled water and several boiling chips were added to the combined extracts, and the organic solvent was evaporated by using a vacuum rotary evaporator at 60 °C.

For analysis of soils, 25 g of a representative sample was weighed into a 250-mL centrifuge bottle, and 75 mL of an acetone–water solution (80:20 v/v) was added. The bottle was capped and shaken manually for 2 min. The sample was centrifuged at 2000 rpm for 10–15 min, and the solvent was carefully decanted through cotton into a 500-mL round-bottomed flask. The extraction was repeated 2 more times, each time using 75 mL of the acetone–water solution. The extracts were combined and the acetone was evaporated in a vacuum rotary evaporator at 60 °C.

For all samples, the remaining water (40 mL) was transferred from the round-bottomed flask to a 250-mL separatory funnel by using several small volumes of water as rinse. This was then diluted with water to ~60 mL. Fifty milliliters of *n*-hexane was added to the separatory funnel which was shaken gently for ~1 min, and the phases were allowed to separate. Centrifugation may be necessary to obtain a clean separation of phases. The hexane layer was discarded, and the hexane wash was repeated 2 more times using 50-mL portions of solvent, with a more vigorous shaking. The hexane was discarded after each wash.

Seventy-five milliliters of chloroform was added to the aqueous phase in the separatory funnel and shaken for 2 min. The phases were allowed to separate (centrifuge if necessary), and the chloroform was filtered through a 1-in. bed of anhydrous sodium sulfate into a 500-mL round-bottomed flask. The chloroform extraction was repeated 2 more times, using additional 75-mL portions of solvent. (When analyzing for the minor metabolites D and E, we extracted the aqueous phase at this step twice with 75-mL portions of ethyl acetate which were combined with the chloroform extracts by filtering them through the sodium sulfate. However, since these metabolites were minor they were analyzed only intermittently. Omission of the ethyl acetate extraction greatly reduced the overall background interference.) [See Florisil Chromatography: Prederivatization and Gel Permeation Chromatography: Prederivatization.]

The combined solvents were concentrated to dryness in a vacuum rotary evaporator at 60 °C. The residue was immediately dissolved in 50 mL of acetonitrile and quantitatively transferred to a 250-mL separatory funnel by using several small volumes of acetonitrile as wash. Total volume was ~60 mL. Fifty milliliters of *n*-hexane was added to the separatory funnel and shaken for ~1 min, and the phases were allowed to separate. The hexane layer was discarded. The hexane wash was repeated 1 more time using an additional 50-mL portion of solvent. The hexane was again discarded after the wash.

The acetonitrile was quantitatively transferred to a 250-mL round-bottomed flask and concentrated to ~5 mL in a vacuum rotary evaporator at 60 °C. The concentrated solution was quantitatively transferred to a 15-mL graduated centrifuge tube by using several small portions of chloroform as rinse. The sample was evaporated in a water bath (60 °C), under a gentle stream of nitrogen, to dryness. The residue was redissolved in 1 mL of chloroform and 1 mL of trifluoroacetic anhydride was added. The tube was capped and the solution swirled about the tube in order to contact the upper walls. The solution was placed in the water bath (60 °C) for 30 min. At the end of the reaction period, the sample was evaporated to dryness under a gentle nitrogen stream. The residue was dissolved in 1 mL of ethyl acetate and mixed thoroughly. With the absence of any trace quantities of water, the derivatized sample was stable for at least 8 h (for additional cleanup, see Florisil Chromatography: Postderivatization).

Alternate Cleanup. With most of the substrates analyzed, adequate sample cleanup was obtained by contin-

uing the solvent–solvent partitioning procedures outlined under Isolation. However, with some samples (alfalfa; grass; palm oil), additional cleanup was necessary. This was obtained by using one of three chromatographic procedures.

The first two techniques were based on the use of Florisil columns, either before or after derivatization. The third employed a gel permeation column prior to derivatization. Of the three procedures, the postderivatization Florisil technique was the easiest to apply on a routine basis.

(1) *Florisil Chromatography: Postderivatization.* Glass wool was placed at the bottom of a 150 × 10 mm chromatographic column with a coarse fritted disk. The column was then filled to a depth of ~70 mm with Florisil that had been activated at 150 °C for at least 24 h. Just prior to use, the column was washed with 50-mL of *n*-hexane which was discarded.

The *derivatized sample extract* obtained from the isolation procedure was quantitatively transferred to the column. Approximately 1 mL of ethyl acetate was used to rinse the sample onto the column. After adsorbing onto the Florisil, 100-mL of *n*-hexane was passed through the column and discarded. The parent compound and metabolites then were eluted with 150 mL of acetonitrile–H_2O (99:1 v/v) and collected in a 250-mL round-bottomed flask. (In order to ensure consistency in the water percentage, the acetonitrile was dried with anhydrous sodium sulfate before preparing the 99:1 elution solution.)

The elution solvent was evaporated to dryness in a vacuum rotary evaporator at 60 °C. The residues were dissolved in chloroform and quantitatively transferred to a 10-mL centrifuge tube. This was then concentrated to dryness in a water bath at 60 °C under a gentle stream of nitrogen. The residue was redissolved in 1 mL of chloroform and rederivatized by adding 1 mL of trifluoroacetic anhydride. The tube was capped and the solution swirled about the tube in order to contact the upper walls. The solution was placed in the water bath (60 °C) for 30 min. At the end of the reaction period, the sample was evaporated to dryness under a gentle nitrogen stream. The residue was dissolved in 1 mL of ethyl acetate and mixed thoroughly. In the absence of trace quantities of water, the derivatized sample was stable for at least 8 h. (Rederivatization was necessary because trifluoroacetylated metabolite B tends to break down on the column.)

(2) *Florisil Chromatography: Prederivatization.* Glass wool was placed at the bottom of a 40 cm × 25 mm chromatographic column, and ~1 cm of glass beads was added. The column was filled with toluene and any trapped air was dislodged by gently tapping. Ten grams of 2.5% deactivated Florisil was added and allowed to settle. Approximately 3 g of granular sodium sulfate was added to the top of the column, and the solvent was drained to the top of the bed just prior to adding the sample.

After preparation of the column, the sample extract obtained from the extraction of the residues from the aqueous phase (see Isolation) was concentrated to ~5 mL in a vacuum rotary evaporator at 60 °C (instead of concentrating to dryness, dissolving in acetonitrile, and continuing as outlined under Isolation). This was then transferred quantitatively to a 10-mL centrifuge tube with several chloroform rinses and concentrated in a water bath at 60 °C with a gentle nitrogen stream to 1 mL. The sample was next transferred to the column by using several toluene rinses (2–3 mL each). The extract was then introduced onto the column bed by draining the solvent to the top of the sodium sulfate.

The column was eluted with 160 mL of toluene–acetonitrile (50:50 v/v) at the rate of ~5 mL/min. This fraction, which contains hexazinone, was collected in a 500-mL round-bottomed flask.

After total elution of the toluene–acetonitrile, the metabolite residues (A, B, D, and E) were eluted from the column with 200 mL of acetonitrile–H_2O (99:1 v/v). This was collected in a separate 500-mL round-bottomed flask.

Both elution fractions were evaporated to dryness in a vacuum rotary evaporator at 60 °C. The residues were dissolved and quantitatively transferred to separate 10-mL centrifuge tubes with chloroform rinses. Both fractions were then concentrated to dryness in a water bath at 60 °C under a gentle stream of nitrogen. The hexazinone fraction was dissolved in 1 mL of ethyl acetate and chromatographed directly as described under Gas Chromatographic Analyses. Since hexazinone does not react with trifluoroacetic anhydride, derivatization was omitted. The metabolite fraction was dissolved in 1 mL of chloroform and reacted with trifluoroacetic anhydride, prior to chromatography, as described under Isolation. More variability was noted with this technique using water-deactivated Florisil than with the postderivative procedure where dried Florisil was used. Because of this, it was advantageous to recalibrate the elution volumes with each new batch of 2.5% Florisil.

(3) *Gel Permeation Chromatography: Prederivatization.* Twenty-five grams of dry Bio-Beads S-X2 packing was swollen overnight at room temperature in the eluting solvent, cyclohexane–methylene chloride (85:15 v/v). A standard GPC bed was then prepared in a 40 cm × 25 mm GPC chromatographic column partially filled with the solvent system. After settling for 4 h, the column was washed with 300 mL of the eluting solvent. The solvent was discarded.

The retention volumes for hexazinone and metabolites were determined by "spiking" the column with known amounts of the compounds and calibrating their elution volumes relative to the total volume. These calibrated volumes were routinely checked to make sure no changes occurred.

After the preparation and calibration of the GPC column, the prederivatized sample extract from the chloroform extraction of the residues from the aqueous phase (see Isolation) was concentrated to ~5 mL in a vacuum rotary evaporator at 60 °C (instead of concentrating to dryness, dissolving in acetonitrile, and continuing as outlined in the standard procedure under Isolation). This was then transferred quantitatively to a 10-mL centrifuge tube with several chloroform rinses and further concentrated in a water bath at 60 °C with a gentle nitrogen stream to 0.5 mL. The sample was next diluted to 3 mL with the cyclohexane–methylene chloride solvent system and chromatographed on the GPC column. The predetermined elution volumes of hexazinone and metabolites were collected in a 250-mL round-bottomed flask and evaporated to dryness on a rotary evaporator at 60 °C. The residues were redissolved and quantitatively transferred to a 10-mL centrifuge tube by using chloroform rinses. The sample was again evaporated to dryness in a water bath at 60 °C with nitrogen. One milliliter of chloroform was added, the sample was reacted with trifluoroacetic anhydride, and the derivatization was continued as described under Isolation.

Part II. Polar Residue (Metabolite C). Twenty-five grams of a representative sample was weighed into the blender–centrifuge bottle and 100 mL of methanol was added. The bottle was covered, placed in the adapter base,

and blended at high speed for 10–15 min. The sample was centrifuged at 1500 rpm for 10–15 min, and the solvent carefully filtered through cotton into a 500-mL round-bottomed flask. The extraction was repeated once with an additional 100 mL of methanol. Ten milliliters of distilled water was added to the combined extracts, and the organic solvent was evaporated by using a vacuum rotary evaporator at 65 °C. The final volume, which often depended on the water content of the substrate, should be less than 25 mL.

For the analysis of soils, 25 g of a representative sample was weighed into a 250-mL centrifuge bottle and 75 mL of an acetone–water solution (80:20 v/v) was added. The bottle was capped and shaken manually for 2 min. The sample was centrifuged at 2000 rpm for 10–15 min, and the solvent was carefully decanted through cotton into a 500-mL round-bottomed flask. The extraction was repeated once with an additional 75 mL of solvent. The extracts were combined and the acetone was evaporated in a vacuum rotary evaporator at 60 °C. The final volume should be less than 25 mL.

For all samples, the remaining water was quantitatively transferred from the round-bottomed flask to a 25-mL volumetric flask by using several small volumes of water as rinse. The final volume was adjusted to exactly 25 mL and the sample was transferred to a 250-mL separatory funnel, without further water rinses of the volumetric flask. Thirty milliliters of ethyl acetate was added to the separatory funnel which was shaken gently for ~1 min, and the phases were allowed to separate. The organic layer was discarded, and the ethyl acetate wash was repeated once using an additional 30-mL portion of solvent and with a more vigorous shaking. Finally, 30-mL of chloroform was added and the wash repeated. All organic layers were discarded after each wash.

The aqueous phase was transferred to a 250-mL round-bottomed flask, without using any rinse water. The remaining traces of organic solvents were then evaporated in a vacuum rotary evaporator at 60 °C (~3–5 min). After the solution was cooled to room temperature, 10-mL of the aqueous phase was pipetted into a 500-mL round-bottomed flask. Ten grams of Gas-Chrom S was added to the flask, and the flask was stoppered and then shaken vigorously until lump free.

Fifty milliliters of a chloroform–ethanol eluting solution (96:4 v/v) was added to the flask. The flask was stoppered and shaken for 1–2 min. The solution and Gas-Chrom S were then poured into a 300 × 22 mm i.d. chromatographic column containing 2 g of preconditioned aluminum oxide held in place with a $^1/_2$-in. glass wool plug (the alumina was preconditioned by washing thoroughly with methanol and drying for at least 12 h at 150 °C before use). The addition of 50 mL of elution solution was repeated 3 more times. Each addition was used to further rinse out the remaining Gas-Chrom S from the flask onto the column. The eluate from the column was collected in a 500-mL round-bottomed flask and evaporated to dryness in a vacuum rotary evaporator at 60 °C.

The residue was dissolved in methanol and quantitatively transferred to a 15-mL centrifuge tube with methanol (it was advantageous to use the heat of the rotary evaporator bath to ensure dissolution of the flask). The centrifuge tube was next transferred to a water bath at 60 °C and the methanol evaporated to dryness by using a gentle nitrogen stream. After *total dryness*, 1 mL of chloroform and 1 mL of trifluoroacetic anhydride were added. The tube was capped and the solution swirled about the tube in order to contact the upper walls. The solution was placed in the water bath (60 °C) for 30 min. At the end of the reaction period, the sample was evaporated to dryness under a gentle nitrogen stream. The residue was dissolved in 1 mL of ethyl acetate and mixed thoroughly. With the absence of any trace quantities of water, the derivatized sample was stable for at least 8 h.

Gas Chromatographic Calibration. The gas chromatograph is equilibrated under the following conditions: inlet temperature, 230 °C; detector temperature, 280 °C; column temperature, 295 °C; helium carrier gas flow, 35 cm^3/min; nitrogen detector bead wire setting, 600. After the chromatographic column was conditioned at 295 °C with carrier gas flowing for at least 24 h, the initial temperature is set at 200 °C for the determination of hexazinone and metabolites A, B, D, and E. For the determination of metabolite C, the initial temperature should be set at 135 °C. (Note: During the conditioning period, the column exit should not be connected to the detector in order to prevent detector contamination.)

For calibration of the instrument, separate standard solutions of hexazinone and metabolites A, B, D, and E are prepared in chloroform. Metabolite C should be prepared in methanol. These solutions should contain 1.0, 2.0, 5.0, 10 and 25 μg/mL hexazinone or metabolites A, B, D, or E. One-milliliter aliquots of the standard solutions are then transferred by pipet to 15-mL graduated tubes. These five standard calibration solutions can be prepared either as mixtures of several standards or individually. However, since both metabolites B and E have similar retention times, these two standards should be chromatographed separately. Additionally, since the two trione metabolites D and E are less responsive than the other compounds, 2.5 μg/mL is the lower limit of detectability relative to standard solutions. Metabolite C solutions should contain 2.0, 5.0, 10, and 25 μg/mL. These should be derivatized and chromatographed separately from the other five compounds.

For a typical calibration: evaporate the standard solutions to dryness, redissolve in 1 mL of chloroform, react with trifluoroacetic anhydride, and continue with the derivatization as described under Isolation. Inject aliquots (1–2 μL) of the trifluoroacetylated calibration solutions so that each peak stays on scale. Immediately after injection, program the column temperature at 32 °C/min to a maximum of 295 °C. (For the alternate column, SP-225-DA, the final temperature is 275 °C.) Hold the column at this temperature for ~8 min. The approximate retention times from the initiation of the programming, using either column, are 2.5 min for metabolite D, 3 min for metabolites B and E, 4 min for hexazinone, 4.5 min for metabolite A, and 5.0 min for metabolite C. Typical gas chromatographic scans of standard solutions by using both chromatographic columns are illustrated in Figure 1, 2, and 3. Construct calibration curves for each individual compound, i.e., micrograms injected vs. peak height. Chromatograph at least two standard solutions daily to ensure proper calibration. In our laboratory, it has been advantageous to further condition a new column with several 2–3-μL injections of crop extract prior to use. The column is programmed to final temperature and held there for 8–10 min between injections. This process, which tends to tie-up remaining active sites in the column packing, is especially helpful when chromatographing the minor trione metabolites D and E.

Gas Chromatographic Analyses. For a typical analysis: equilibrate the instrument and chromatograph aliquots of the derivatized residue extracts as described under Gas Chromatographic Calibration (in the absence

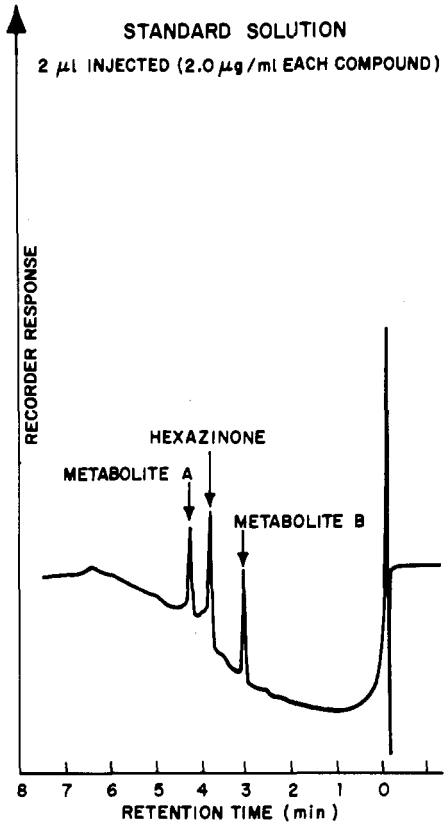

Figure 1. Standard solution of hexazinone and metabolites A and B (15% OV-17 column).

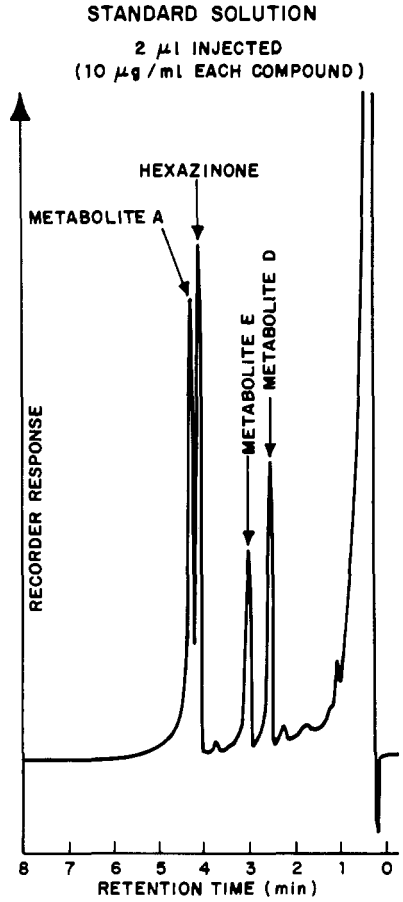

Figure 2. Standard solution of hexazinone and metabolites A, D, and E (3% SP 2250 DA column).

of trace quantities of water, samples are stable after reaction with the derivatization agent for at least 8 h).

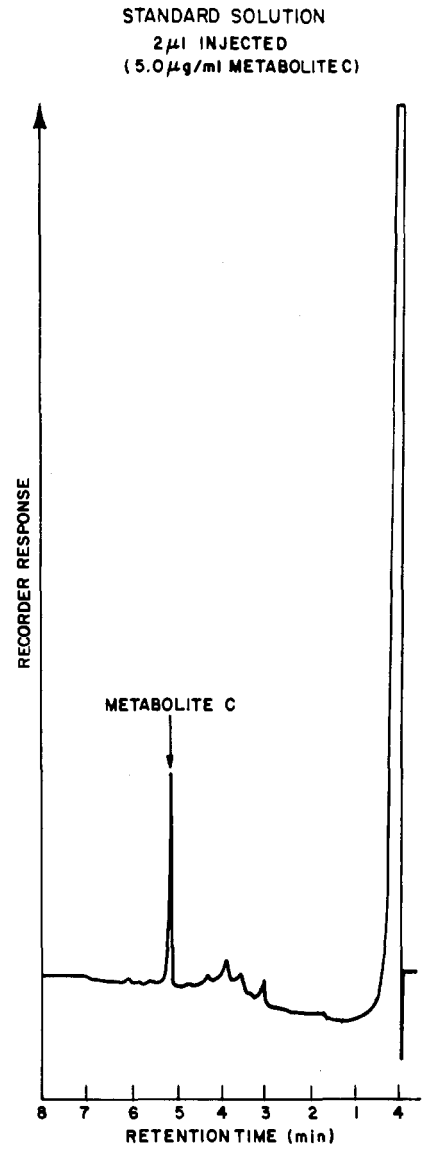

Figure 3. Standard solution of metabolite C (15% OV-17 column).

Measure the peak height of each individual compound and determine the micrograms of each in the aliquot by using the calibration curves previously prepared. Calculate the concentrations of hexazinone and metabolites A, B, D, and E in parts per million by dividing the micrograms found, corrected for the respective average recovery factors, by the sample weight in grams. Calculate the concentration of metabolite C in parts per million by dividing the micrograms found, corrected for the 2.5 aliquot factor and the average recovery factor, by the sample weight in grams.

RESULTS AND DISCUSSION

The gas chromatographic method described for the moderately polar compounds is sensitive to 1.0 μg of hexazinone, metabolite A, amd metabolite B or 0.04 ppm relative to a 25-g sample. It is sensitive to 2.5 μg of metabolites D and E or 0.1 ppm relative to a 25-g sample. The procedure described for the polar metabolite C is sensitive to 5.0 μg or 0.2 ppm relative to a 25-g sample. Recoveries have been demonstrated at various levels on a variety of substrates. The data are summarized in Table I. These recoveries were conducted by adding known amounts of the compounds to an untreated control sample contained in the blender–centrifuge bottle. After evaporation of the solvent, analyses were then initiated by ad-

Table I. Summary of Hexazinone and Metabolites A, B, C, D, and E Recovery Data

crop	recovery levels, ppm	av recovery, %						recovery range, %					
		H[a]	A	B	C	D	E	H	A	B	C	D	E
sugarcane	0.04-1.0	96	91	111	79	96	105	60-130	66-124	90-140	60-104	70-128	70-136
sugarcane—mixed juice	0.04-1.0	90	87	96		100	110	68-124	66-130	60-130		72-140	96-124
molasses	0.20-0.40	93	118	104		105	98	80-110	104-130	100-108		100-110	96-100
pineapple	0.04-1.0	94	88	85	73	91	114	70-130	62-120	74-100	60-86	70-104	86-128
pineapple leaves	0.08-1.0	81	88	80		74	106	72-130	71-130	60-120		66-100	108-108
pineapple bran	0.05-1.0	99	97	79		60	108	78-120	76-118	79-79		60-60	108-108
soil	0.10-20	106	93	104	92			64-124	68-120	60-132	71-96		
alfalfa	0.04-4.0	93	96	82		81	71	82-120	89-125	76-110		48-126	68-100
asparagus	0.04-2.0	104	100	88			87	93-140	76-120	78-120			82-88
blueberries	0.20-1.0	82	75	80		102	68	72-94	65-86	80-80		80-124	68-68
water	0.04-1.0	109	109	113				96-120	96-120	120-130			
muscle	0.04-20	96	99	87		90	99	81-130	75-124	80-116		62-112	91-110
fat	0.20-1.0	81	89	93		82	104	70-88	85-92	85-100		72-92	96-112
liver	0.25-20	95	87	116		100	123	77-110	50-110	101-132		63-130	82-160
blood	0.25-20	108	87	85		104	91	82-120	67-100	60-110		88-120	86-96
urine	0.50-200	84	90	95			100	60-97	51-116	60-124			76-125
feces	0.50-200	92	77	64			102	92-96	50-96	57-71			94-110

[a] H = hexazinone.

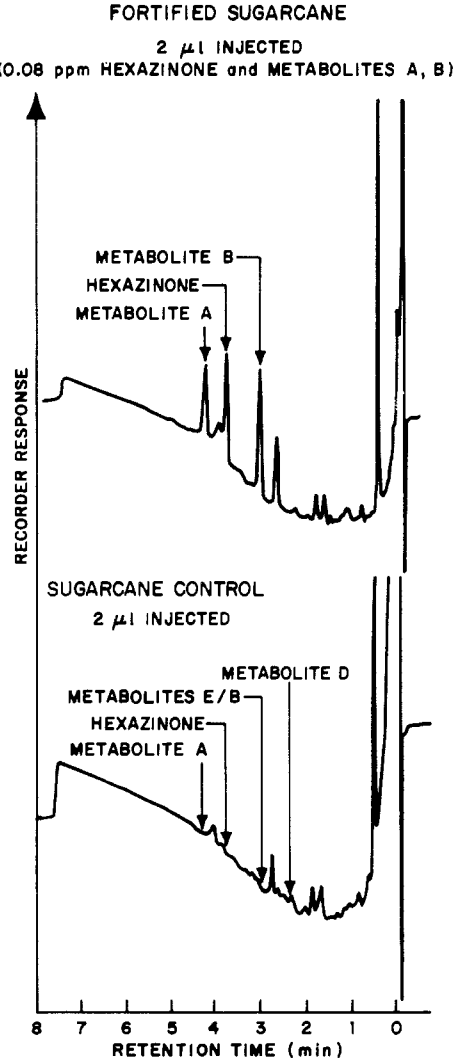

Figure 4. Extract of sugarcane (hexazinone and metabolites A and B; 15% OV-17 column).

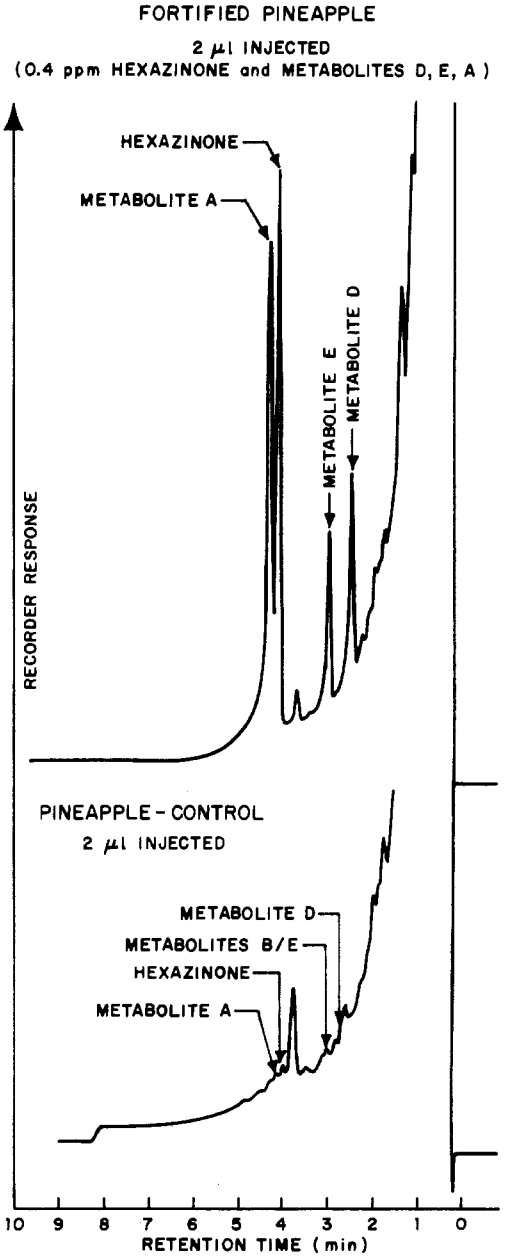

Figure 5. Extract of pineapple (hexazinone and metabolites A, D, and E; 3; SP 2250 DA column).

dition of the first portion of extraction solvent. In Figure 4, typical gas chromatographic scans obtained on extracts of a control (untreated) sample of sugarcane (lower scan) and on a control sample of sugarcane fortified with 0.08 ppm of hexazinone and metabolites A and B are illustrated. Figure 5 shows scans of control pineapple (lower

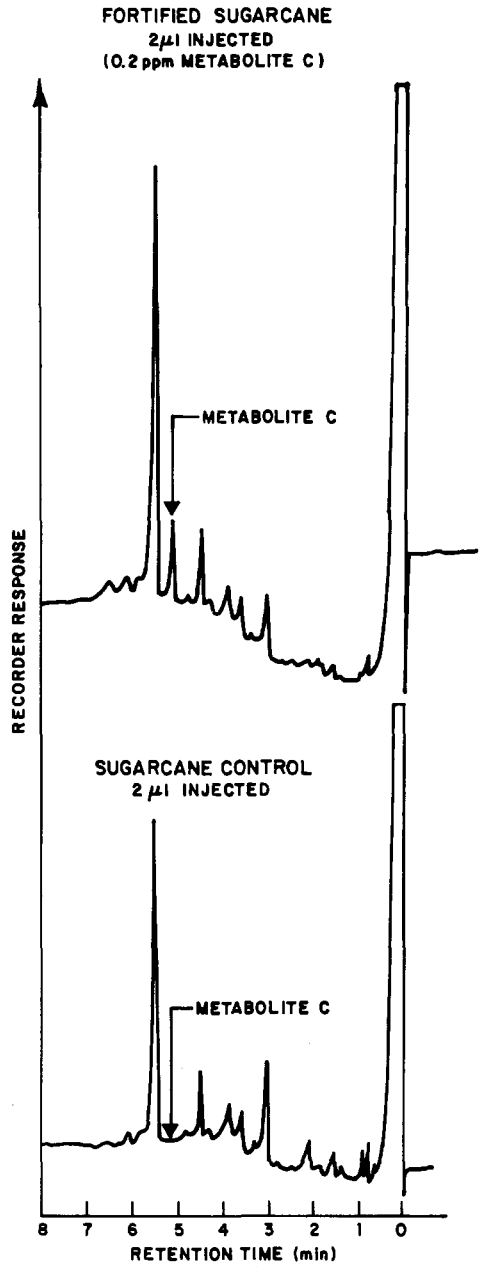

Figure 6. Extract of sugarcane (metabolite C; 15% OV-17 column).

scan) and pineapple fortified with hexazinone and metabolites A, D, and E at the 0.4-ppm level (upper scan). Figure 6 shows scans of control sugarcane (lower scan) and sugarcane fortified with metabolite C at the 0.2-ppm level (upper scan). The sugarcane analyses were conducted using the OV-17 column, while the pineapple samples were analyzed on the alternate SP 2250 packing.

Due to difference in polarity, it was impossible to develop a single isolation procedure for both the highly polar metabolite C and the moderately polar hexazinone and metabolites A, B, D, and E. Therefore, two separate isolation schemes were devised with both using a final derivatization with trifluoroacetic anhydride and with both using nearly identical gas chromatographic detection conditions. Attempts to partially merge the two isolation methods, such as using a single initial methanol extraction to remove all six compounds from a substrate and partitioning out the moderately polar species before continuing with the polar metabolite analysis, were unsuccessful due to background interferences which carried over with the moderately polar compounds. These interferences were not present when the more selective and less polar solvent, chloroform, was used to extract hexazinone and its metabolites A and B.

On the basis of [^{14}C]hexazinone studies [see Craven (1979), Pease and Holt (1971), and Rhodes (1980a,b)], the primary residues of interest are the parent compound and metabolites A and B and, in some substrates, metabolite C. Metabolites D and E are of minor significance and are analyzed only intermittently. Inclusion of these two triones in the analysis scheme requires the addition of ethyl acetate in the aqueous partitioning step of the isolation and thereby results in increased background interference levels. Additionally, these two metabolites are much more difficult to chromatograph, requiring special care to ensure that the gas chromatographic column is totally conditioned. This is necessary due to the tendency of both triones at low levels to adsorb on the packing material.

On the basis of NMR studies, it was confirmed that metabolite B can exist in both the keto and enol forms. However, it was determined that the enol compound predominates in more polar solvents and is the form which is derivatized with trifluoroacetic anhydride and chromatographed.

The experimental procedure described for the moderately polar compounds generally resulted in excellent sample cleanup on most substrates, with minimal background interference. However, if additional cleanup is required, it can be obtained by one of three methods, which were described in detail under Alternate Cleanup. Of the three procedures, the Florisil column, after derivatization of the compounds, was found to be the best technique on a routine basis. It was more reproducible than the prederivatization Florisil approach and less time consuming than the gel permeation column.

As mentioned under Isolation, ethyl acetate is never used for the analysis of soils. This solvent is used primarily to enhance the partitioning of metabolites D and E, but since both compounds were not detected in soil degradation studies with ^{14}C-labeled hexazinone (Rhodes, 1980a), it is not necessary to analyze for either compound.

Relative to a 25-g initial sample, the lower limit of detectability for the trione metabolites D and E is 0.1 ppm, while hexazinone and metabolites A and B are detectable at the 0.04-ppm level. This difference can be attributed partially to one less nitrogen per molecule in the triones.

As mentioned under Gas Chromatographic Calibration, metabolites B and E have similar chromatographic retention times. Therefore, it is recommended that standard solutions of both compounds be chromatographed separately. If residues are detected in the retention interval of metabolite B and E, some resolution and possible residue identification can be obtained by reducing the temperature program rate to 16 °C/min and rechromatographing the extract. (This rate is not recommended for routine analysis because it tends to reduce the response factors for the trione metabolites D and E.) It is also helpful in confirming peak retention times to rechromatograph the extract, fortified with the syringe with known amounts of derivatized metabolite B or E.

The trifluoroacetylated derivatives formed through these procedures are highly water sensitive. This is especially true for metabolite B. Extreme care should be taken to ensure that trace amounts of water are not introduced into the derivatization procedure. This requires dry solvents and high quality trifluoroacetic anhydride. If water is present, the metabolite B derivative will revert to the underivatized form and chromatograph as a shoulder to the metabolite A.

When working with the alternate chromatographic column (SP-2250 DA), we have found it is advantageous to use acid-treated glass wool as column plugs. In addition, for both columns, small amounts of glass wool were placed in the glass inlet to retain nonvolatiles which would otherwise contaminate the top of the column. This insert was changed daily.

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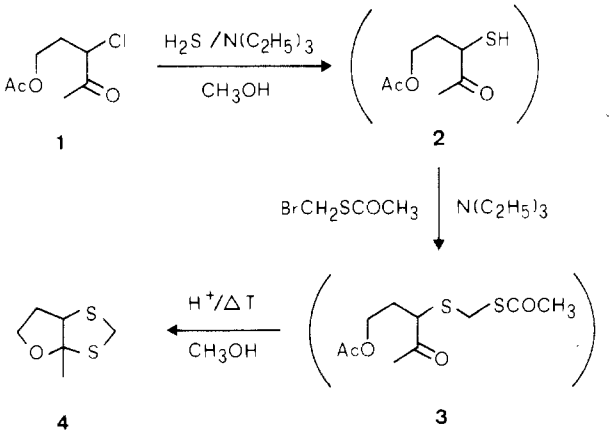
Synthesis of 1-Methylbicyclo[3.3.0]-2,4-dithia-8-oxaoctane, a Degradation Product of Thiamin

Peter Gygax

5-Acetoxy-3-chloro-2-pentanone (1), when treated with hydrogen sulfide/base, gave 5-acetoxy-3-mercapto-2-pentanone (2) which was heated with bromomethyl acetyl sulfide/base to give 5-acetoxy-3-acetyl-2-thiapentane-1-thioacetate (3). Heating 3 with methanol in an acidic medium gave the title compound 4 with a 35–40% overall yield.

Although pure thiamin is odorless, commercial samples possess a characteristic odor, probably arising from decomposition products. As thiamin is used in the flavor industry and is widely spread in foodstuffs, a great number of papers dealing with the decomposition of thiamin (Dwivedi and Arnold, 1973) exist.

Seifert et al. (1978) reported the effect of UV irradiation on thiamin. Among the volatiles, they isolated an oil which seems to be responsible for the characteristic odor. The structure elucidation by means of physical methods showed it to be 1-methylbicyclo[3.3.0]-2,4-dithia-8-oxaoctane (4). Synthetic efforts were unsuccessful, probably due to the instability of the intermediate products. As the structure is only based on physical data, we decided to synthesize this compound. The retrosynthetic consideration shows that the readily available 5-acetoxy-3-chloro-2-pentanone (1) possesses the elements for building up the correctly



substituted tetrahydrofuran ring system. 1 was therefore converted into 5-acetoxy-3-mercapto-2-pentanone (2) by reaction with hydrogen sulfide in the presence of an organic base. This substance was not purified due to the well-known instability of α-mercaptoketones (Mueller et al., 1977), and therefore it was directly converted into the mixed acetal 3 by reaction with bromomethyl acetyl sulfide in the presence of triethylamine. Refluxing the crude acetal 3 in methanol with traces of hydrobromic acid resulted in the formation of the "thiamine odor compound" 4 with a 35–40% overall yield.

EXPERIMENTAL SECTION

Infrared spectra of thin liquid films were obtained on a Perkin-Elmer Model 257 infrared spectrometer.

^1H NMR spectra (360 MHz) were determined with a Bruker WH-360 spectrometer by using $CDCl_3$ as solvent and Me_4Si as an internal standard.

^{13}C NMR spectra were recorded under the same conditions by using a Varian XL-100 spectrometer.

MS spectra were performed on a CEC 21-110 double-focusing spectrometer with an indirect inlet system.

Packed-Column GLC. A 0.3 cm i.d. × 3 m long glass column packed with 80–100 mesh Chromosorb G AWDMCS coated with 2% Carbowax 20 M was used at 180 °C. The injector, the thermal conductivity detector, and the outlet were kept at 220 °C.

Capillary GLC. A 0.2 mm i.d. × 45 m long UCON 50 HB 5100 glass column was used at 140 °C. The injector, collector, and the thermal conductivity system were kept at 220 °C.

1-Methylbicyclo[3.3.0]-2,4-dithia-8-oxaoctane (4). A total of 250 mL of methanol and 15.18 g (0.15 mol) of triethylamine were saturated with hydrogen sulfide at 0 °C. 5-Acetoxy-3-chloro-2-pentanone (26.8 g, 0.15 mol) was added dropwise to this solution while bubbling H_2S through the reaction mixture at the same time. The temperature was kept between 0 and 10 °C by means of an ice bath. After the addition which took 30 min, the stream of H_2S was interrupted and the reaction mixture was stirred for another 3 h without cooling. The reaction flask was then carefully evacuated (foaming) on a water aspirator and stirred under vacuum for 2 h.

After being flushed with nitrogen, 25.4 g (0.15 mol) of the freshly prepared bromomethyl acetyl sulfide (Boehme et al., 1959) was added instantaneously to the solution of crude mercaptoketone 2, followed by the dropwise addition

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