Determination of Residues of Mobil MC-A-600 (Benzo[*b*]thien-4-yl Methylcarbamate) and Its Hydrolysis Product (Benzo[*b*]thiophene-4-ol) in Coastal Bermuda Grass and Milk

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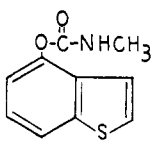
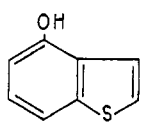
Residues of Mobil MC-A-600 and its hydrolysis product were determined by gas chromatography. The carbamate was unstable under the gas chromatographic conditions used and had to be hydrolyzed to the phenol for analysis. Extracts of milk were analyzed by separation of the carbamate and phenol on alumina, alkaline hydrolysis of the carbamate, and steam distillation of the resulting sulfur-containing phenol, and injection of the phenol into a gas chromatograph equipped to sense sulfur. The carbamate and phenol in the extracts of grass were also separated on alumina, and the effluent concentrates were injected into the gas chromatograph; a short plug containing 85% phosphoric acid, which instantaneously converts the carbamate to the phenol, was included at the head of the chromatographic column. Recoveries of the carbamate (as the phenol) at levels of 0.5 to 5.0 p.p.m. were 92 to 96% from Coastal Bermuda grass and 88 to 92% from milk. Recoveries of the phenol at the same levels were 56 to 64% from grass and 86 to 88% from the milk.

Mobil MC-A-600 (I, hereafter called MC-A-600) has been shown to be effective against a variety of insect pests (Armbrust and Gyrisco, 1966; Brady and LaBrecque, 1966; Brady *et al.*, 1966; Davis *et al.*, 1966; Forsythe, 1966; Pass, 1966; Piquett and Fales, 1966; Wolfenbarger *et al.*, 1966). This insecticide is being considered for use on Coastal Bermuda grass and a sensitive analytical method was required to determine its persistence and that of its phenol hydrolysis product (II) on grass treated with the insecticide. A method of determining residues of the two compounds in the milk of cows consuming the treated grass was also required.



I



II

This paper reported the details of the procedures developed for the required analyses. When carbamate I broke down during gas chromatography, I was separated from II on an alumina column and hydrolyzed to II for analysis. Hydrolysis was accomplished by treating I with alkali or by passing it over a phosphoric acid plug placed at the head of the gas chromatographic column. Sensitivity of the analyses for the compounds was about 0.025 p.p.m. in grass and less than 0.01 p.p.m. in milk.

A gas chromatographic procedure utilizing electron-capture detection has been reported by Gutenmann *et al.* (1964) for analysis of MC-A-600 in milk or urine after hydrolysis, bromination, and acetylation. No attempt was made to separate the phenol from the parent compound.

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EXPERIMENTAL

Apparatus. An F & M Scientific Corp. (Avondale, Pa.) Model 700 gas chromatograph equipped with the Melpar flame photometric detector (394-mμ interference filter) described by Brody and Chaney (1966) was used. The detector responds to the sulfur in the gas chromatographic effluent.

Solvents and Reagents. Reagent grade or C.P. solvents and chemicals were used. Methylene chloride and chloroform were redistilled. Acetone and methanol were used as received. Sodium sulfate was anhydrous. Other chemicals or solutions employed were 10% aqueous sodium chloride, 85% phosphoric acid, and diethylene glycol.

Fisher A-540 adsorption alumina, 80- to 200-mesh, was used as received. It lost 2.48% of its weight after heating at 110° C. overnight.

PROCEDURE

Sample Preparation and Extraction. GRASS. Chop the sample in a Hobart cutter and mix it well. Add 50 grams to a Waring Blendor containing 50 grams of sodium sulfate. [If sample is to be fortified, add appropriate amount of pesticide(s) in 1 ml. of chloroform and allow to stand about 1 minute before adding extraction solvent.] Add 150 ml. of chloroform and blend for 5 minutes. Filter through Whatman No. 1 paper and store the filtrate over sodium sulfate.

MILK. Shake the sample to disperse the cream uniformly, and add 50 grams to a Waring Blendor. [If sample is to be fortified, add the appropriate amount of pesticide(s) in 1 ml. of acetone and blend for 1 minute.] Add 150 ml. of acetone and blend for 3 minutes. Filter through Whatman No. 1 paper on a Büchner funnel, and wash the blender and the filter with an additional 25 ml. of acetone. Extract the filtrate with 100 and then 50 ml. of methylene chloride, and percolate each methylene chloride extract successively through a plug of sodium sulfate (about 4 cm.

in diameter × 5 cm. thick). Evaporate the percolated extract almost to dryness under a Snyder column on a steam bath, and then just to dryness with a jet of dry air at room temperature. Add 60 ml. of chloroform to dissolve the fatty residue.

Separation of MC-A-600 from Its Phenol by Liquid Chromatography. GRASS OR MILK. Prepare columns (2-cm. I.D., Shell type) by adding successively 5 grams of sodium sulfate, 10 grams of alumina, and 10 grams of sodium sulfate. Prewash the column with 50 ml. of chloroform, and discard the eluate. Add to the column 60 ml. of the extract of milk (equivalent to 50 grams) or grass (equivalent to 20 grams). When the extract has percolated into the column, wash the container and the column with a few milliliters of fresh chloroform and then with additional solvent to a total of 100 ml. The chloroform eluate (160 ml. total) contains the MC-A-600. It is processed further as described in the next section.

Next, elute the column with 100 ml. of absolute methanol. This eluate contains the phenol. It is further processed as described under Preparation of Phenolic Fraction for Analysis.

Preparation of MC-A-600 Fraction for Analysis. GRASS. Add 4 drops of diethylene glycol (keeper) to the chloroform eluate, and concentrate under a Snyder column on a steam bath to near dryness. For very low levels of residue, concentrate to 1 ml. (5 μl. is equivalent to 100 mg. of grass) by using a jet of dry air at room temperature. For higher levels, dilute with chloroform as appropriate. The sample is now ready to be injected into the gas chromatograph. The phosphoric acid plug is required for the analysis of this fraction.

MILK. Add 4 drops of diethylene glycol to the chloroform eluate, and concentrate to near dryness under a Snyder column on a steam bath; then evaporate to dryness at room temperature with a jet of dry air. Add 25 ml. of 10% aqueous sodium hydroxide and heat for 30 minutes in a water bath at 40° to 50° C. with frequent stirring. Add 100 ml. of distilled water, 25 ml. of 6*N* hydrochloric acid, and several boiling beads. Distill on a hot plate until 10 to 15 ml. of liquid remains in the flask, collecting the distillate in a 250-ml. flask immersed in an ice bath. Filter the cold distillate through Whatman No. 1 paper into a 250-ml. separatory funnel and extract twice with 50-ml. portions of methylene chloride, percolating each portion successively through a plug of sodium sulfate about 2.5 cm. in diameter by 2 cm. thick. Add 4 drops of diethylene glycol to the percolated extract and concentrate under a Snyder column on a steam bath. Using methylene chloride, transfer the concentrate to a calibrated tube and further concentrate to the desired volume by using a gentle stream of dry air at room temperature. For high sensitivity, concentrate to 0.5 ml. (5 μl. is equivalent to 500 mg. of milk).

Preparation of Phenolic Fraction for Analysis. GRASS OR MILK. Transfer the methanol eluate (100 ml. from the liquid chromatography) to a 500-ml. separatory funnel containing 50 ml. of saturated aqueous sodium chloride, and wash the container with distilled water, adding the washings to the separatory funnel. Extract twice with 100-ml. portions of methylene chloride, and percolate each portion through a plug of sodium sulfate (2.5 cm. in diameter × 3 cm. thick). Add 4 drops of diethylene glycol and evaporate under a Synder column on a steam bath to near dryness. Use methylene chloride to transfer the concentrate to a calibrated tube, and evaporate to the desired volume with a stream of dry air at room temperature. For low residue levels, adjust volume to 1 ml. for grass (5 μl. equivalent to 100 mg.) or to 0.5 ml. for milk (5 μl. equivalent to 500 mg.).

Gas Chromatographic Analysis. Inject 5 μl. per analysis using the following operational parameters:

Column, 75 cm. × 4-mm. I.D. (6-mm. O.D.) glass.

Packing, 10% DC 200 w./w. on 80- to 100-mesh Gas Chrom Q (Applied Science Laboratories, State College, Pa.).

Carrier gas, nitrogen at 160 ml. per minute.

Other gases, oxygen at 40 ml. per minute; hydrogen at 200 ml. per minute.

Column temperature, 140° C.

Other temperatures, injection port 170° C.; detector (external) 160° C.

Precondition the packing overnight at 240° C.

Under these conditions the retention time of MC-A-600 is 7.60 minutes, and that of its phenol is 2.00 minutes.

The MC-A-600 in grass is hydrolyzed to the phenol with a plug of Gas Chrom Q containing phosphoric acid. The plug is not required for the analysis of the phenolic fractions or of the MC-A-600 fraction in milk, which is hydrolyzed to the phenol with alkali. To include the plug, remove about 5 cm. of packing from the front of the column, and in its place insert in sequence some glass wool, about 4 cm. of the acid packing (made by mixing 1.0 gram of Gas Chrom Q with 0.20 ml. of 85% phosphoric acid), and then sufficient glass wool to retain the packing. With the plug in place, allow the system to equilibrate for about 1 hour with several injections of 5 μg. of the phenol followed by injections of 250-ng. portions of the phenol in extract until the response is reproducible and the baseline becomes steady. (Satisfactory operation can be achieved within about an hour.) The system must be continually conditioned to the phenol and extracts to give reproducible results. The phosphoric acid plug lasts 1 day or more, depending on the amount injected. Test the hydrolytic efficiency of the plug by injecting 5 μg. of MC-A-600; if a peak appears at 7.60 minutes, replace the plug.

Prepare a standard curve for the phenol and carbamate (peak height *vs.* concentration) as shown in Figure 1, and determine the concentration of unknowns by reference to the curve.

RESULTS AND DISCUSSION

Samples of Coastal Bermuda grass and milk, untreated and fortified with MC-A-600 and its phenol at several levels, were extracted and analyzed. The results are given in Tables I and II. Recoveries of MC-A-600 (as its phenol) at levels of 0.5 to 5.0 p.p.m. from Coastal Bermuda grass were 92 to 96%, from milk 88 to 92%. The low recoveries of the phenol at the same levels from Coastal Bermuda grass (56 to 64%) were not considered important because the grass is fed to animals, and a rough estimate of the phenol sufficed for our needs. Recoveries of phenol from milk (86 to 88%) were satisfactory.

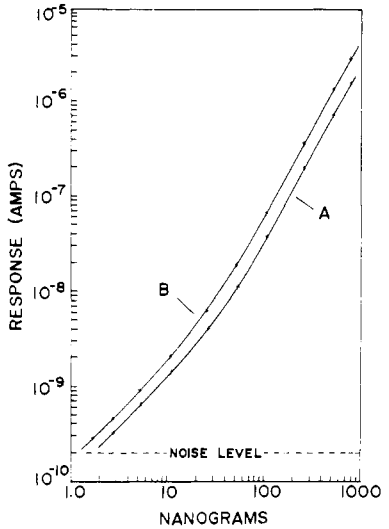

Figure 1. Standard curves of MC-A-600, *A*, and its phenol, *B*, with the flame photometric detector (394-mμ filter)

Typical chromatograms of 25 ng. of MC-A-600 and its phenolic hydrolysis product are shown in Figure 2. The standards are shown in chromatogram *A*. Chromatogram *B* shows the compounds at the 0.5-p.p.m. level in grass. Sensitivity of the MC-A-600 analysis in grass is estimated at 0.03 p.p.m., that of its phenol at 0.02 p.p.m. (twice noise). Chromatogram *C* shows the compounds at the 0.05-p.p.m. level in milk. Sensitivity of the analysis of MC-A-600 or its phenol in milk is estimated at less than 0.01 p.p.m. (4 times noise). In all of the chromatograms, the concentration of MC-A-600, as determined from its response, is about 72% that of an equivalent weight of phenol. This result is in line with the amount of sulfur in the molecules—i.e., the sulfur content of MC-A-600 is about 72% that of its phenolic derivative. As reported by Brody and Chaney (1966) the response of the sulfur flame-photometric detector is not linear with concentration; neither was the response of the compounds linear in a log-log plot over the three decades of concentration shown in Figure 1.

In attempts to chromatograph directly the intact MC-A-600, partial hydrolysis of the carbamate to its phenol always occurred—i.e., the peak of the phenol appeared at 2.00 minutes in addition to the peak of the intact carbamate at 7.6 minutes. Trials with several packings, column lengths, and temperatures failed to eliminate the peak of the phenol; neither could the extent of breakdown be made reproducible. [This instability has been encountered in the gas chromatography of other carbamates—e.g., with Niagara NIA-10242 (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) when using microgram amounts and a flame ionization detector.] Ebing (1966) reported that *N*-monomethylcarbamates were unstable and *N,N*-dimethylcarbamates stable during gas chromatography on two substrates.

The possibility of determining MC-A-600 as its phenolic

Table I. Gas Chromatographic Analysis of MC-A-600 and Its Phenolic Hydrolysis Product in Coastal Bermuda Grass[a]

Trial	Compound	Added		Recovered	
		P.p.m.	μg.[b]	μg.[b]	%
1	MC-A-600	0	0	<1.5[c]	..
	Phenol	0	0	<1.0[c]	..
2	MC-A-600	5.0	250	235	94
	Phenol	0	0	<1.0[c]	..
3	MC-A-600	0	0	<1.5[c]	..
	Phenol	5.0	250	160	64
4	MC-A-600	5.0	250	240	95
	Phenol	0.5	25	14	56
5	MC-A-600	0.5	25	23	92
	Phenol	5.0	250	155	62
6	MC-A-600	1.0	50	46	92
	Phenol	1.0	50	29	58

[a] Mean of duplicate analyses, 50-mg. equivalent of grass injected (5 μl.) per analysis, except as noted.
[b] Per 50 grams of plant material.
[c] 100-mg. equivalents injected (5 μl.) per analysis.

Table II. Gas Chromatographic Analysis of MC-A-600 and Its Hydrolysis Product in Milk[a]

Trial	Compound	Added		Recovered	
		P.p.m.	μg.[b]	μg.[b]	%
1	MC-A-600	0	0	<0.5[c]	..
	Phenol	0	0	<0.5[c]	..
2	MC-A-600	1.0	50	46	92
	Phenol	0	0	<0.5[c]	..
3	MC-A-600	0	0	<0.5[c]	..
	Phenol	1.0	50	43	86
4	MC-A-600	0.5	25	22	88
	Phenol	0.5	25	22	88
5	MC-A-600	2.0	100	90	90
	Phenol	2.0	100	86	86

[a] Mean of duplicate analyses, 125-mg. equivalents of milk injected (5 μl.) per analysis, except as noted.
[b] Per 50 grams of milk.
[c] 500-mg. equivalents of milk injected (5 μl.) per analysis.

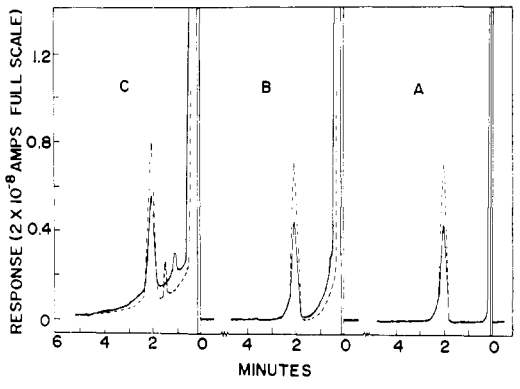

Figure 2. Chromatograms of MC-A-600 (as its phenol) (solid lines) and its phenolic hydrolysis product (broken lines)

A. Standards, 25 ng. each, injected in 5 μl. of chloroform.
B. Extract equivalent to 50 mg. of Coastal Bermuda grass fortified with 25 ng. of MC-A-600 or phenol injected in 5 μl. of solvent
C. Extract equivalent to 500 mg. of milk fortified with 25 ng. of MC-A-600 or phenol injected in 5 μl. of solvent

hydrolysis product was therefore investigated. This approach required the separation of MC-A-600 from its phenol before gas chromatographic analysis and a means of hydrolyzing the carbamate to its phenol. The carbamate was separated from its phenol on a 10-gram alumina column, the carbamate being readily eluted with chloroform and the phenol with methanol. Since additional cleanup of the milk extract was needed to achieve the desired degree of sensitivity (about 0.01 p.p.m.), the carbamate was hydrolyzed with alkali and the resulting phenol steam distilled. The hydrolysis of the carbamate in the grass extract was accomplished most expeditiously by a reaction gas chromatographic procedure (Beroza and Coad, 1966)—i.e., by passage through the phosphoric acid plug. The reaction appears to be practically instantaneous since the liberated sulfur-containing phenol migrates through the column with no appreciable difference in retention time from that of the free phenol similarly injected. This procedure for instant hydrolysis of MC-A-600 may be useful in the analysis of other carbamates that likewise cannot be chromatographed intact.

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