highest in the stem and stalk samples (Table IV). In Speight stalk the unsaturated C_{18} fatty acids comprised 56% of the quantitated acids. The high value (5.56%) in Speight flowers as compared to Coker flowers might be attributed to differences in plant maturity.

The plant parts varied slightly in the relative percentage for cholesterol and slightly more in the percentages of campesterol, stigmasterol, and β-sitosterol (Table V). Grunwald (1975) reported increased relative percentages of stigmasterol with increasing leaf maturity. This increase was probably due to an increased concentration of stigmasterol in the stem. As noted in Table V, stigmasterol was the major sterol in all plant parts analyzed.

The data presented here indicate sterols, fatty acids, and hydrocarbons were present in all plant parts although at reduced levels in fibrous tissues. Absence of solanesol in the stem and stalk might indicate that solanesol is localized in the laminal tissue of the leaf. Neophytadiene was found in strip and stem tissue only. If sheet made from close-grown, chopped, whole-plant tobaccos becomes commercially important because of economic considerations, we can expect cigarettes of the future, which contain such sheet material, to have correspondingly lower amounts of the PAH precursors, solanesol, and neophytadiene.

ACKNOWLEDGMENT

The authors thank J. F. Chaplin for supplying the tobacco samples, and Peggy Wilson, Bill Lizzio, and Gwen Noles for their technical assistance.

LITERATURE CITED

Chouroulinkov, I., Lazar, P., Izard, C., Libermann, C., Guerin, M., *J. Natl. Cancer Inst.* **46**, 981 (1969).
Darkis, F. R., Baisden, L. A., Gross, P. M., Wolf, F. A., *Ind. Eng. Chem.* **44**, 297 (1952).
DeJong, D. W., Lam, J., Lowe, R., Yoder, E., Tso, T. C., *Beitr. Tabakforsch.* **8**, 93 (1975).
Ellington, J. J., Schlotzhauer, P. F., Schepartz, A. I., *J. Chromatogr. Sci.* **15**, 295 (1977).
Grunwald, C., *Phytochemistry* **14**, 79 (1975).
Halter, H. M., Ito, T. I., *J. Natl. Cancer Inst.* **48**, 1869 (1972).
Hoffmann, D., Wynder, E. L., *J. Natl. Cancer Inst.* **48**, 1855 (1972).
Ishiguro, S., Satô, S., Sugaware, S., Kaburaki, Y., *Agric. Biol. Chem.* **40**, 977 (1976).
Klimisch, H. J., *Z. Lebensm.-Unters. Forsch.* **150**, 93 (1970).
Schlotzhauer, W. S., Chortyk, O. T., Higman, H. C., Schmeltz, I., *Tob. Sci.* **13**, 153 (1969).
Schlotzhauer, W. S., Chortyk, O. T., *Beitr. Tabakforsch.* **8**, 84 (1975).
Schlotzhauer, W. S., Schmeltz, I., *Beitr. Tabakforsch.* **4**, 176 (1968).
Schlotzhauer, W. S., Severson, R. F., Chortyk, O. T., Arrendale, R. F., Higman, H. C., *J. Agric. Food Chem.* **24**, 992 (1976).
Severson, R. F., Ellington, J. J., Schlotzhauer, P. F., Arrendale, R. F., Schepartz, A. I., *J. Chromatogr.* **139**, 269 (1977).
Tso, T. C., "Physiology and Biochemistry of Tobacco Plants", Dowden, Hutchinson, and Ross, Inc., Stroudsburg, Pa., 1972, p 272.
Wynder, E. L., Hoffmann, D., "Tobacco and Tobacco Smoke Studies in Experimental Carcinogenesis", Academic Press, New York, N.Y., 1967, pp 18, 47.
Wynder, E. L., Hoffmann, D., *J. Am. Med. Assoc.* **192**, 88 (1965).
Zilkey, B. F., Proceedings of the 3rd World Conference on Smoking and Health, Wynder, E. L., Hoffmann, D., Gori, G. B., Ed., DHEW Publication No. (NIH) 76-1221, 1975, p 57.

Received for review July 18, 1977. Accepted October 28, 1977.

Gas Chromatographic Analysis of Tebuthiuron and Its Metabolites in Grass, Sugarcane, and Sugarcane By-Products

Andrew Loh,* Sheldon D. West, and Thomas D. Macy

Analytical procedures are described for determining residues of the herbicide tebuthiuron, *N*-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-*N*,*N*'-dimethylurea, and its major metabolites in forage grass, sugarcane, and sugarcane by-products. Extracts of the plant tissue and sugarcane by-products are purified by liquid–liquid partitioning and alumina column chromatography. Measurement is accomplished by gas chromatography using flame photometric detection (GC–FPD) and by gas chromatography–mass spectrometry (GC–MS) using single ion detection. The methods are capable of determining 0.01–0.3 ppm of tebuthiuron and metabolites.

Tebuthiuron (I), *N*-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-*N*,*N*'-dimethylurea, is the active ingredient in the herbicides SPIKE and PERFLAN (Elanco Products Company, Indianapolis, Ind.). Tebuthiuron is a preemergence and postemergence herbicide for total vegetation control on rights-of-way and industrial sites (Walker et al., 1973). It has exhibited potential for rangeland brush control (Bovey et al., 1975) and for broad spectrum weed control in sugarcane (Pafford and Hobbs, 1974).

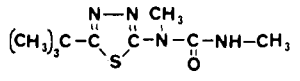
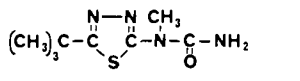
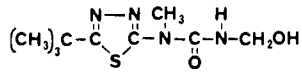
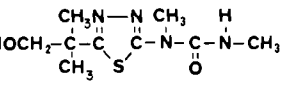
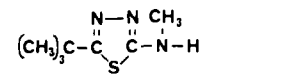
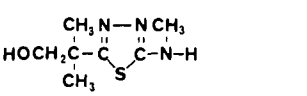
Agricultural Analytical Chemistry, Lilly Research Laboratories, Division of Eli Lilly and Company, Greenfield, Indiana 46140.

Several metabolites of tebuthiuron have been identified in plant tissue (Table I). The major metabolites in grass and sugarcane have been identified as compounds II and III. Compound IV is a major metabolite in grass but is only a minor metabolite in sugarcane (Eaton et al., 1976). The presence of these metabolites requires that analytical procedures for determining tebuthiuron residues in forage and food crops be capable of determining the major metabolites.

Saunders and Vanatta (1974) have reported the derivatization of tebuthiuron with trifluoroacetic anhydride for electron-capture detection by gas chromatography. This derivative exhibits adequate sensitivity for some residue determinations. However, it has not been generally

0021-8561/78/1426-0410$01.00/0

Table I. Compound Designations, Structures, and Chemical Names of Reference Compounds

Compound Designation	Structure	Chemical Name
Tebuthiuron		N-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-N,N'-dimethylurea
I		
II		N-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-N-methylurea
III		N-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-N'-hydroxymethyl-N-methylurea
IV		N-[5-(2-hydroxy-1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-N,N'-dimethylurea
V		5-(1,1-Dimethylethyl)-N-methyl-1,3,4-thiadiazol-2-amine
VI		5-(2-hydroxy-1,1-dimethylethyl)-N-methyl-1,3,4-thiadiazol-2-amine

applicable to grass and sugarcane extracts because of the presence of interfering substances. Tebuthiuron thermally decomposes to compound V in the injection block of a gas chromatograph (Saunders and Vanatta, 1974). This reaction is essentially quantitative at injection block temperatures of 280 °C or greater.

Thus, tebuthiuron can be determined directly by gas chromatography, without prior derivatization, using flame photometric detection of the sulfur moiety of compound V. In addition, two of the metabolites in forage grass and sugarcane (compounds II and III) also thermally degrade to V. The total residue of metabolites II and III in experimental samples may then be determined by separating them from tebuthiuron prior to analysis by gas chromatography. Although metabolite IV thermally degrades to VI, the flame photometric detector response to compound VI is insufficient for a sensitive residue procedure. Consequently, metabolite VI has been analyzed by gas chromatography–mass spectrometry utilizing single ion detection.

In this report analytical procedures are presented for determining residues of tebuthiuron and its major metabolites in forage grass, sugarcane, and processed sugarcane products. Analysis is accomplished by gas chromatography utilizing a flame photometric detector (GC–FPD) or by a mass spectrometer with single ion monitoring of the thermal degradation products of tebuthiuron and its metabolites.

EXPERIMENTAL SECTION

Apparatus, Chemicals, and Reagents. Analytical standard grade tebuthiuron and metabolites were obtained from Lilly Research Laboratories (Greenfield, Ind.). All solvents were reagent grade. The acetonitrile was redistilled in glass prior to use. Anhydrous sodium sulfate was washed with methanol and dried at 50 °C for 16 h. Alumina F-20 (Alcoa) was dried at 110 °C for 16 h, deactivated with 4% water (w/w), and tumbled for 1 h in a closed container.

The gas chromatograph was a Tracor Model 560 equipped with a flame photometric detector and a 394-nm filter to monitor the sulfur emission. The column was a 180 cm × 0.3 cm i.d. borosilicate glass tube packed with 5% Carbowax 20M on 80/100 mesh Chromosorb WHP. The oven temperature was maintained at 215 °C, the detector temperature at 190 °C, and the injection block was operated at 300 °C.

The gas chromatograph–mass spectrometer system was a LKB 9000 (Bromma, Sweden) operated in the single ion mode. The column was a 120 cm × 0.3 cm i.d. borosilicate glass tube packed with 1.5% Carbowax 20M on Chromosorb WHP. The carrier gas was maintained at a flow rate of 40 mL/min. The column temperature was 210 °C and the separator temperature was 250 °C. The accelerating voltage was 3.5 kV, the electron energy was 70 eV, and the trap current was 60 μA.

Glass tubes for column chromatography were obtained from Kimble (a division of Owens-Illinois, Vineland, N.J.) and consisted of a 25 cm × 1.4 cm i.d. tube and a 250-mL reservoir.

Extraction. Sugar, syrup, juice, or molasses (10 g) was weighed into a pint Mason jar. Hydrochloric acid (35 mL, 0.5 N) was added and the sample was shaken for 5 min on a platform shaker to completely dissolve the sample. The sample was transferred to a 125-mL separatory funnel, and the jar was washed with 10 mL of saturated sodium chloride solution, which was then added to the separatory funnel.

Forage grass, bagasse, or sugarcane (10–30 g) was weighed into a quart Mason jar. A mixture of 0.5 N hydrochloric acid–methanol (1:1) was added to make a total of 200 mL of liquid with allowance for the water content of the sample. The fluid level was marked on the jar, the jar was covered with a watch glass, and the sample was boiled for 1 h on a hot plate. It was then allowed to cool and water was added to restore the original volume as marked on the jar. The extract was collected by pouring the supernatant liquid through a funnel containing a plug of glass wool into a graduated cylinder. An aliquot of known volume (25–75 mL) was then transferred to a 125-mL evaporating flask and the methanol was removed by rotary vacuum evaporation in a 50 °C water bath. The remaining aqueous phase was then transferred to a 125-mL separatory funnel. The flask was washed with 10 mL of saturated sodium chloride solution, which was added to the separatory funnel.

Liquid–Liquid Partition. Tebuthiuron and metabolites were extracted from the aqueous phase by shaking with 40 mL of ethyl acetate. The phases were allowed to separate, and the lower aqueous phase was drained into a beaker. An additional 10 mL of ethyl acetate was then shaken with the remaining upper phase to break the emulsion. The lower aqueous phase was again drained into the beaker, and the upper ethyl acetate phase drained through a funnel containing sodium sulfate into a 125-mL evaporating flask. The aqueous phase was returned to the separatory funnel, and the extraction was repeated with a second and a third 40-mL portion of ethyl acetate. The sodium sulfate was washed with 10 mL of ethyl acetate, and the combined extracts were evaporated to dryness with a rotary vacuum evaporator and a 50 °C water bath.

Alumina Column Chromatography. Prior to initial use, each batch of deactivated alumina was standardized to determine the elution pattern of tebuthiuron and its metabolites. The columns were prepared by placing a plug of glass wool in the bottom of the column and adding 30 mL of acetonitrile–isopropanol (99:1), followed by the addition of 13 mL (11.0 g) of standardized, deactivated alumina. The alumina was stirred to remove entrapped air and was allowed to settle. About 1 cm of white masonry sand was added to the top of the alumina to prevent disturbance of the packing during the addition of eluents. The residue was dissolved in 5 mL of acetonitrile–iso-

Table II. Recoveries of Tebuthiuron and Metabolites from Grass, Sugarcane, and Sugarcane By-Products

		1:1 mixture of II plus III								
		Tebuthiuron						IV		
			% recov			% recov			% recov	
Material	No. of fortifications	ppm added	Range	Av	Total ppm added	Range	Av	Added	Range	Av
Grass	22	0.5	69-115	92	0.4	48-120	70	0.5	40-76	60
Sugarcane	9	0.05	48-81	70	0.05	33-81	53			
Bagasse	5	0.075	84-95	91	0.075	56-95	77			
Sugar	5	0.15	50-85	68	0.15	51-67	57			
Juice	5	0.15	47-85	65	0.15	50-74	61			
Syrup	5	0.15	54-91	68	0.15	51-95	71			
Molasses	5	0.15	58-94	77	0.15	54-77	65			

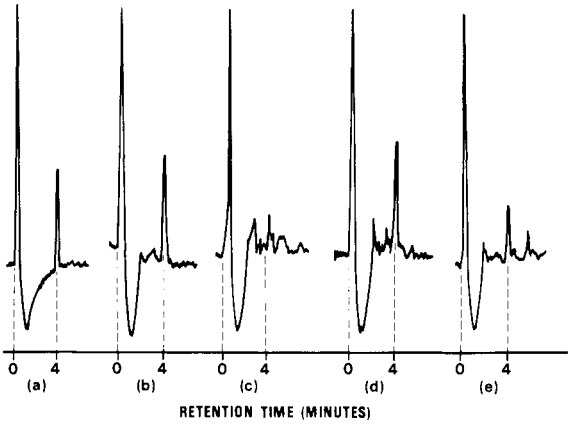


Figure 1. Recovery of tebuthiuron and metablites II and III from grass: (a) tebuthiuron standard, 1.25 ng; (b) metabolite II + metabolite III standards, 0.625 ng of each; (c) control grass; (d) control grass fortified with 0.5 ppm of tebuthiuron, equivalent to 86% recovery; (e) control grass fortified with 0.2 ppm of metablite II and 0.2 ppm of metablite III, equivalent to 62% recovery.

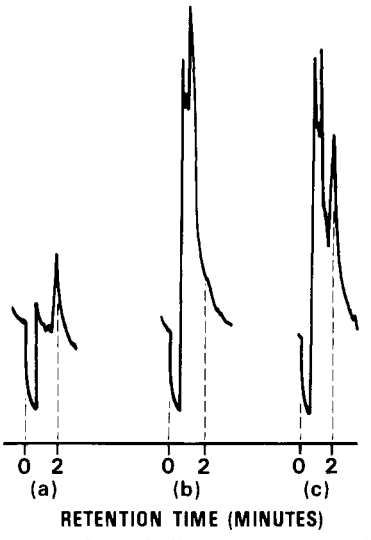


Figure 2. Recovery of metabolite IV from grass: (a) metabolite IV standard, 1.25 ng; (b) control grass; (c) control grass fortified with 1.0 ppm of metabolite IV, equivalent to 54% recovery.

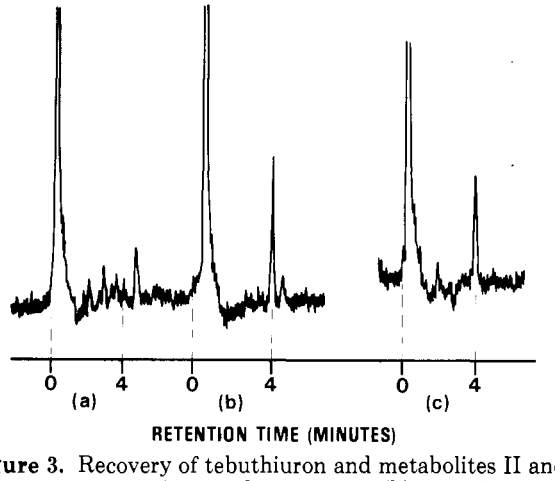


Figure 3. Recovery of tebuthiuron and metabolites II and III from sugarcane: (a) control sugarcane; (b) control sugarcane fortified with 0.05 ppm tebuthiuron, equivalent to 81% recovery; (c) control sugarcane fortified with 0.025 ppm of metabolite II and 0.025 ppm of metabolite III, equivalent to 70% recovery.

propanol (99:1) and added to the top of the column. The evaporating flask was rinsed with two successive 5-mL portions of acetonitrile–isopropanol (99:1) which were added to the top of the column. Each portion was allowed to drain onto the column before the next addition and the eluent was discarded. Tebuthiuron was eluted from the column with an additional 40 mL of acetonitrile–isopropanol (99:1), which was collected in a 125-mL evaporating flask at the rate of 3–5 mL/min. The metabolites were then eluted into a second 125-mL evaporating flask with 60 mL of methanol–water (98:2). The solvents were removed by rotary vacuum evaporation and a 50 °C water bath. The metabolite eluate fraction frequently contained a small amount of water which could not be completely evaporated. Dilution of the water with an additional 20 mL of methanol, followed by rotary vacuum evaporation normally removed the last traces of water. Residues of tebuthiuron and metabolites were dissolved in 1.0–2.0 mL of acetonitrile for measurement by gas chromatography.

Gas Chromatographic Analysis. Tebuthiuron and metabolites II and III were measured by gas chromatography using a flame photometric detector operated in the sulfur mode. The standard response curve was prepared by plotting the detector response (peak height) vs. concentration for a series of standards equivalent to 0.5–5.0 ng of tebuthiuron. The attenuator was set so that a peak height response of 50% of full scale was obtained for 2.5 ng of tebuthiuron.

The amount of tebuthiuron and the total amount of metabolites II and III in various samples were determined from the tebuthiuron response curve since all three compounds thermally degrade to V. Representative chromatograms of tebuthiuron and metabolites in grass extracts are shown in Figure 1.

Gas Chromatography–Mass Spectrometry. Metabolite IV was also eluted from the alumina column in the methanol–water fraction but was analyzed by gas chromatography–mass spectrometry using single ion monitoring. Metabolite IV thermally degraded to compound VI, which displayed an intense fragmentation peak at m/e 156 that was used for quantitative analysis. The standard response curve was prepared by plotting the peak height response obtained by injecting standards ranging from 0.5–5.0 ng of IV. Representative chromatograms of

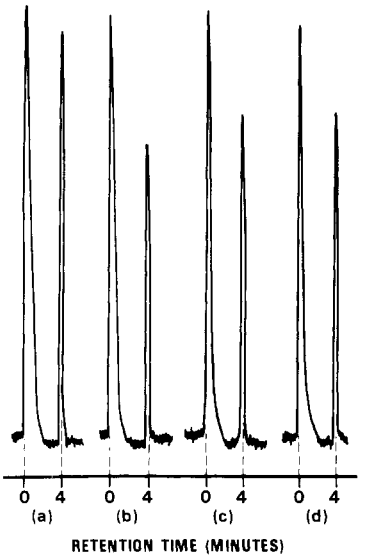


Figure 4. Relative response of the flame photometric detector to metabolites II and III: (a) metabolite II, 2.5 ng; (b) metabolite III, 2.5 ng; (c) metabolite II and III, 1.25 ng of each; (d) tebuthiuron, 2.5 ng.

metabolite IV in grass extracts are shown in Figure 2.

RESULTS AND DISCUSSION

Tebuthiuron and metabolites II and III thermally degrade in the injection block of a gas chromatograph to yield the same product (compound V). Although the alumina column procedure separates tebuthiuron from its metabolites prior to analysis by gas chromatography, it does not separate metabolite II from metabolite III. Consequently, this analytical procedure will not determine the relative amounts of these two compounds, and the residue is determined as the sum of these two metabolites. As shown in Figure 3, the flame photometric detector response is greater for metabolite II (molecular weight, 214) than an equal weight of metabolite III (molecular weight, 244). However, the averaged molecular weight of equal amounts of the two metabolites is very similar to that of tebuthiuron (molecular weight, 288), so that a 1:1 mixture of the two metabolites results in a detector response similar to that of tebuthiuron (see Figure 4). Thus, standard curves of a 1:1 mixture of the two metabolites or a tebuthiuron standard curve can be used for the determination of II and III in the metabolite fraction. If only metabolite II is present in the experimental samples, the calculations will be high by about 20% using the standard curve. Similarly, the calculated results will be low by about 20% if only metabolite III is present in the sample. Metabolites II and III can be separated by thin-layer chromatography (TLC) using Brinkman silica gel G plates and hexane–acetone (60:40) as the eluting solvent (Rainey and Magnussen, 1978). However, the TLC procedure is time consuming and not practical for routine assay of a large number of samples. Studies with [^{14}C]tebuthiuron using this TLC system have shown that both metabolites are present in sugarcane and grass (Eaton et al., 1976), and so the magnitude of the calculation errors should be less than 20% when the metabolites are not separated from one another prior to analysis by GC–FPD. Control samples fortified with tebuthiuron and metabolites were assayed by the same procedure to determine the levels of recovery for the various samples. Some recovery results are summarized in Table II. The method is capable of determining 0.1 ppm of tebuthiuron, 0.1 ppm of metabolites II and III, and 0.3 ppm of metabolite IV in grass. In sugarcane and processed products, 0.01 ppm of tebuthiuron, and metabolites II and III can be detected.

LITERATURE CITED

Bovey, R. W., Meyer, R. E., Baur, J. R., *Abstr. Weed Sci. Soc. Am.*, 54 (1975).

Eaton, B. J., Magnussen, J. D., Rainey, D. P., *Abstr. Weed Sci. Soc. Am.*, 199 (1976).

Pafford, J. L., Hobbs, C. D., *Abstr. Weed Sci. Soc. Am.*, 266 (1974).

Rainey, D. P., Magnussen, J. D., *J. Agric. Food Chem.*, in press (1978).

Saunders, D. G., Vanatta, L. E., *Anal. Chem.* **46**, 1319 (1974).

Walker, J. C., Jones, M. L., Shaw, J. E., *Proc. North Cent. Weed Contr. Conf.* **28**, 39 (1973).

Received for review June 17, 1977. Accepted November 14, 1977.