

chrysanthemum, water spinach, and watercress) and mustard cabbage are of particular interest as they also contain useful levels of  $\beta$ -carotene, iron, potassium, calcium, and dietary fiber.

#### ACKNOWLEDGMENT

We thank J. S. K. Lim and Y. El-Ghetany for assistance with the analyses.

**Registry No.** D-Glucose, 50-99-7; D-fructose, 57-48-7; sucrose, 57-50-1; malic acid, 6915-15-7; citric acid, 77-92-9; oxalic acid, 144-62-7; vitamin C, 50-81-7; thiamin, 59-43-8; riboflavin, 83-88-5; niacin, 59-67-6; sodium, 7440-23-5; potassium, 7440-09-7; calcium, 7440-70-2; iron, 7439-89-6; magnesium, 7439-95-4; zinc, 7440-66-6; starch, 9005-25-8.

#### LITERATURE CITED

- AOAC "Official Methods of Analysis", 13th ed.; Association of Official Analytical Chemists: Washington, DC, 1980.
- AVRDC "Annual Report for 1975"; The Asian Vegetable Research and Development Center: Taiwan, 1976.
- AVRDC "Annual Report for 1978"; The Asian Vegetable Research and Development Center: Taiwan, 1979.
- AVRDC "Annual Report for 1979"; The Asian Vegetable Research and Development Center: Taiwan, 1980.
- Bruemmer, J. H.; Roe, B. *Proc. Fla. State Hort. Soc.* 1979 **1980**, 92, 140.
- Chen, M. L.; Chang, S. C. Guoo, J. Y. *Nutr. Rep. Int.* 1982, 26, 1053.
- Creswell, D. C. *Poult. Sci.* 1981, 60, 1905.
- Dahlen, M.; Phillipps, K. "A Guide to Chinese Market Vegetables"; South China Morning Post: Hong Kong, 1980.
- Del Rosario, R. R.; Lozano, Y.; Noel, M. G. *Philipp. Agric.* 1980, 63, 267.

- FAO "Food Composition Table for Use in East Asia"; Food and Agriculture Organisation and U.S. Department of Health, Education and Welfare: Rome, 1972.
- Gambhir, P. N.; Narain, A.; Tiwari, P. N. *Exp. Agric.* 1979, 15, 411.
- Gebhardt, S. E.; Elkins, E. R.; Humphrey, J. J. *J. Agric. Food Chem.* 1977, 25, 629.
- Grubben, G. J. H. *Meded. Landbouwhoges. Wageningen* 1975, 75, 1.
- Kayukova, V. A.; Rozov, N. F. *Isv. Timiryazevsk. Shk. Akad.* 1977, 1, 217; *Biol. Abstr.* 1977, 64, 022878.
- NH&MRC. "Dietary Allowances for Use in Australia"; National Health and Medical Research Council: Canberra, 1970.
- Nicholas, E. H. "Check List of Hong Kong Plants"; Hong Kong Herbarium: Hong Kong, 1974.
- NRC. "Recommended Dietary Allowances", 9th ed.; National Research Council, National Academy of Sciences: Washington, DC, 1980.
- Rodriguez, D. B.; Lee, T. H.; Chichester, C. O. *Plant Physiol.* 1975, 56, 626.
- Singh, S. B.; Chabra, R.; Abrol, I. P. *Agron. J.* 1979, 71, 767.
- Sreeramula, N. J. *Plant Foods* 1982, 4, 139.
- Wills, R. B. H.; Balmer, N.; Greenfield, H. *Food Technol. Aust.* 1980, 32, 198.
- Wills, R. B. H.; Scriven, F. M.; Greenfield, H. *J. Sci. Food Agric.* 1984, in press.
- Wuensch, A. *Landwirtsch. Forsch.* 1975, 28, 345.

Received for review May 23, 1983. Revised manuscript received October 5, 1983. Accepted November 7, 1983. This work received financial support from the National Health and Medical Research Council Working Party on Food Composition Data.

## COMMUNICATIONS

### High-Pressure Liquid Chromatographic Analysis of Tebuthiuron in Soil

A procedure is described for the extraction of tebuthiuron [*N*-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-*N,N'*-dimethylurea] from soil and its analysis by high-pressure liquid chromatography (HPLC) while using monuron [3-(*p*-chlorophenyl)-1,1-dimethylurea] as an internal standard for the extraction. Tebuthiuron was extracted from soil samples by shaking and sonicating the soil in water-methanol (45:55). The filtrate was recovered by suction filtration and tebuthiuron was partitioned into diethyl ether. Following evaporation of the ether, the tebuthiuron was solubilized into aliquots of water-methanol (45:55) and quantified by HPLC. The extraction efficiency was  $86 \pm 3\%$  and the detector responded linearly to tebuthiuron concentration with high precision ( $r^2 = 0.98$ ). The efficiency of tebuthiuron extraction was not influenced by the cation-exchange capacity (CEC), organic matter content, or clay content of the treated soil.

Tebuthiuron, *N*-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-*N,N'*-dimethylurea, is the active ingredient in the formulated herbicides Spike and Graslan (Elanco Products Co., Indianapolis, IN). Tebuthiuron is applied preemergence and postemergence for vegetation control on rights of way and industrial sites, for rangeland brush control, and for broad spectrum weed control in sugarcane (Pafford and Hobbs, 1974; Scifres et al., 1981; Walker et al., 1973). Tebuthiuron is presently being considered for use in management systems for converting woodland to grazing

land and improved forests in the southeastern United States.

Analytical methods for substituted urea herbicides, such as tebuthiuron, generally include derivatization procedures to achieve thermal stability for gas chromatographic (GC) analysis. Saunders and Vanatta (1974) reported the derivatization of tebuthiuron with trifluoroacetic anhydride for electron capture detection. More recently, Loh et al. (1978) utilized flame photometric detection to quantitatively analyze tebuthiuron by measuring the product(s)

from thermal decomposition in the GC injection block. Procedures for sample derivatization are often time consuming and unreliable. Also, the sample size, used in GC methods, restricts its usefulness for determining low concentrations of tebuthiuron in soil extracts.

The need for rapid and selective methods to monitor the manufacturing processes of tebuthiuron prompted the development of an HPLC-analysis procedure for this compound (Kennedy, 1977). More recently, Lilly Research Laboratories (Greenfield, IN) have developed a procedure for quantifying tebuthiuron in soil samples (Decker, 1983). Generally, these procedures included time-consuming extraction procedures and expensive solvents for the mobile phase and did not include the use of an internal standard. These factors become important when analyzing several thousand soil samples. In this report we present a rapid and accurate analytical procedure for determining tebuthiuron residues in soil samples.

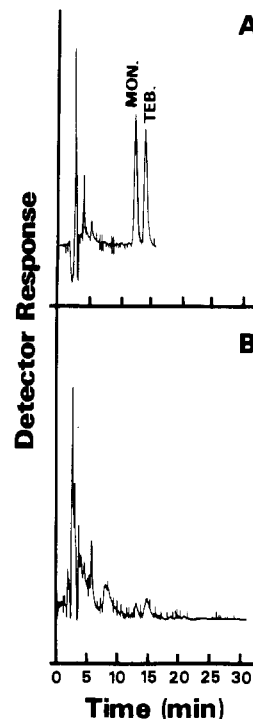
#### EXPERIMENTAL SECTION

**Apparatus, Chemicals, and Reagents.** Analytical standard grade tebuthiuron and monuron [3-(*p*-chlorophenyl)-1,1-dimethylurea] were obtained from Lilly Research Laboratories (Greenfield, IN) and E. I. duPont de Nemours (Wilmington, DE), respectively. All solvents were reagent grade and the water and methanol were glass distilled before use. The mobile phase (water-methanol, 45:55) used in this method was suggested by Lilly Research Laboratories and will be referred to as solvent.

The HPLC system consisted of the following components: (1) a Beckman 340 injector equipped with a 50- $\mu$ L loop (Beckman Instrument Co., Fullerton, CA); (2) a Micromeritics 750 solvent delivery system operated at a flow rate of 1.00 mL/min (ca. 19.0  $\pm$  0.5 MPa) (Micromeritics, Norcross, GA); (3) a precolumn (25  $\times$  4.6 mm) packed with CO:Pell ODS-C18 (Whatman, Inc., Clifton, NJ); (4) a column (250  $\times$  4.6 mm) packed with 10  $\mu$ m of R sil C18 (Alltech Associates, Inc., Deerfield, IL); (5) a Micromeritics 786 variable-wavelength detector operated at 254 nm and 0.05 AUFS; (6) a Hewlett-Packard 3390A integrator/recorder (Hewlett Packard Co., Atlanta, GA) operated at a chart speed of 0.2 cm/min, threshold setting of 3, peak area rejection of 100K, signal voltage output of +0.1  $\rightarrow$  0.4, and attenuation of 4. A Sonic Dismembrator Model 300 (Fisher Scientific, Pittsburgh, Pa.) equipped with the intermediate-size probe and operated at 55% maximum power was used for sample sonication. Soil used in development of the method was a thermic, Typic-Hapudult sandy clay (Cecil series) with an organic matter content of 1%. The soil was air-dried and sieved through a 0.25-cm screen.

**Sample Preparation for HPLC.** Monuron was selected as the internal standard (IS) for this procedure. The relative retention time (RRT) and relative concentration ( $\mu$ g/mL) response (RCR) of tebuthiuron compared to those of monuron were determined with standard solvent solutions of these compounds individually and combined. The IS concentration was maintained at 1  $\mu$ g/mL, whereas the tebuthiuron concentration was adjusted across a range from 0.1 to 100  $\mu$ g/mL.

One-milliliter aliquots of aqueous tebuthiuron solutions were added to 25-g soil samples, resulting in soil samples containing 2.5, 5, and 10  $\mu$ g of tebuthiuron. The soil samples were thoroughly mixed and frozen in closed containers for 72 h. Stored soil samples and freshly prepared samples were freeze-dried in preparation for extraction. The IS (10  $\mu$ g) in 1 mL of solvent was added to each soil sample and the soil was thoroughly mixed. The soil samples were extracted twice with 20 mL of solvent, and following



**Figure 1.** Typical HPLC chromatograms of (A) monuron (MON.) and tebuthiuron (TEB.) in glass-distilled solvent and (B) processed extracts from soil not treated with monuron and tebuthiuron.

each extraction, the filtrate was collected by suction filtration and combined. Twenty-milliliter aliquots of the combined filtrates were partitioned twice with 40 mL of diethyl ether. The ether phases from both partitions were combined and the ether was allowed to evaporate under a fume hood (ca. 22  $^{\circ}$ C) in subdued light. Generally, evaporation occurred over a 3-h period. The residue from evaporation was solubilized in 10 mL of solvent while sonicating for 5 min in an ultrasonicator (Heat Systems Ultrasonics, Inc., Plainview, NY) in preparation for analysis by HPLC.

Fifteen soils having different CEC, organic matter content, and clay content were used to test the method for the influence of these soil properties on the efficiency of tebuthiuron extraction. The ranges for CEC, organic matter content, and clay content were 2.8–12.5 cmol ( $p^+$ )  $kg^{-1}$ , 0.8–3.5%, and 1.8–41.9%, respectively.

All soil- and solvent-sample extractions were replicated at least 4 times and the experiments were repeated at least twice. Statistical analyses were conducted on data when considered appropriate.

#### RESULTS AND DISCUSSION

Monuron was selected for use as IS for tebuthiuron analyses because of the similarities of the two compounds in light absorption spectra, retention time on the HPLC column, solubility in the solvent, and partitioning efficiency into diethyl ether. Both compounds have an absorption peak at 254 nm (Lawrence, 1982). The RRT for tebuthiuron is 1.13  $\pm$  0.01 compared to that for monuron and both herbicides elutriate at a retention time where the chromatogram is void of interfering peaks from soil contaminants (Figure 1). The RCR for tebuthiuron compared to that for monuron was 0.85  $\pm$  0.03 and the detector response to tebuthiuron concentration was linear over a concentration range reported (Figure 2) and maintained a high correlation coefficient. The linear relationship was found to extend up to 100  $\mu$ g/mL (data not included).

The solvent efficiently extracted tebuthiuron and monuron, from the treated soils, whether extraction was

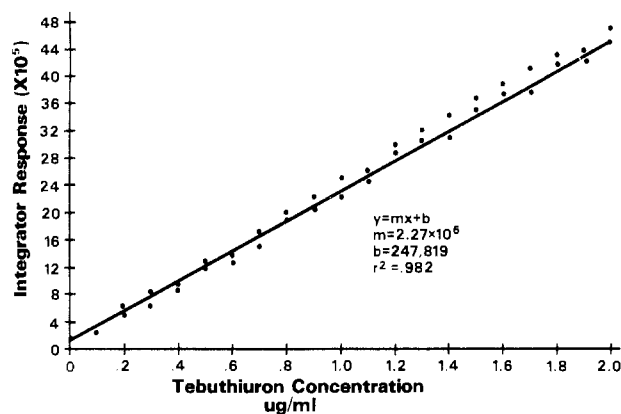


Figure 2. Regression equation and best-fit curve for integrator response to tebuthiuron concentration.

Table I. Recovery of Tebuthiuron and Monuron When 10  $\mu\text{g}$  of Each Herbicide Is Added to Soil Samples and Extracted under Conditions of Shaking, Sonicating, or a Combination of Shaking and Sonicating

herbicide	extraction method	% recovery
tebuthiuron	soil: <sup>a</sup> shake	77 $\pm$ 4
	sonicate	85 $\pm$ 5
	shake and sonicate	86 $\pm$ 5
monuron	ether partition	95 $\pm$ 3
	soil: <sup>a</sup> shake	80 $\pm$ 4
	sonicate	84 $\pm$ 5
	shake and sonicate	86 $\pm$ 5
	ether partition	95 $\pm$ 4

<sup>a</sup> Soil extracts were also partitioned into diethyl ether preceding quantification.

conducted under conditions of shaking, sonicating, or a combination of both (Table I). Generally, the total extraction procedure consistently accounted for more than 86% of both herbicides added to soil samples and 5% of the herbicide loss was accounted for as being lost during partitioning the herbicides into diethyl ether (Table I). The ether-partitioning procedure was considered necessary, as a sample-cleaning step, in preparation for introduction into the HPLC system. Although 86% recovery is low, much of the tebuthiuron loss is adjusted when using the

IS to quantify tebuthiuron in soil samples. Also, use of an IS adjusts for variations in extraction efficiency and instrument sensitivity.

Soil CEC, organic matter content, and clay content did not influence the extractability of tebuthiuron or monuron from soil (data not included). The average tebuthiuron recovery from 15 soils was  $98 \pm 0.05\%$ , when using the IS to adjust for extraction losses, and was not significantly different between soils. Also, the RCR for tebuthiuron compared to that for monuron was  $0.85 \pm 0.03$  and was not significantly different between soils.

This procedure of quantifying tebuthiuron was determined to be simple, inexpensive, accurate, and repetitive. Also, the sensitivity range of this method is adequate for most field studies with tebuthiuron. Assuming an application rate of 2.2 kg/ha tebuthiuron and an even distribution of the applied tebuthiuron within the top 15 cm of soil, the tebuthiuron concentration in the soil would be 1.0  $\mu\text{g/g}$  of soil. Therefore, 25-g soil samples would allow for the detection of a 10-fold decrease in tebuthiuron concentration in soil samples.

Registry No. Tebuthiuron, 34014-18-1; monuron, 150-68-5.

#### LITERATURE CITED

- Decker, O. D., Lilly Research Laboratories, Greenfield, IN, personal communication, 1983.  
 Kennedy, J. H. *J. Chromatogr.* **1977**, *15*, 79.  
 Lawrence, J. F. *Assoc. Off. Anal. Chem.* **1982**, *59*, 1066.  
 Loh, A.; West, S. D.; Macy, J. D. *J. Agric. Food Chem.* **1978**, *26*, 410.  
 Pafford, J. L.; Hobbs, C. D. *Abstr. Weed Sci. Soc. Am.* **1974**, 266.  
 Saunders, D. G.; Vanatta, L. E. *Anal. Chem.* **1974**, *46*, 1319.  
 Scifres, C. J.; Stuth, J. W.; Bovey, R. W. *Weed Sci.* **1981**, *29*, 270.  
 Walker, J. C.; Jones, M. L.; Shaw, L. E. *Proc.—North Cent. Weed Control Conf.* **1973**, *28*, 39.

Albert E. Smith, Jr.\*  
 Larry M. Shuman  
 Nunnally Lokey

University of Georgia  
 College of Agriculture Experiment Stations  
 Experiment, Georgia 30212

Received for review July 21, 1983. Accepted December 6, 1983. This research was supported by State and Hatch funds allocated to the Georgia Agricultural Experiment Stations.

## Identification of Bornyl Acetate in the Leaf Oil from *Parthenium argentatum* A. Gray

Bornyl acetate has been identified by gas chromatography and mass spectroscopy as a component of the leaf oil from *Parthenium argentatum*. Mechanistic schemes for the mass fragmentation of camphor and bornyl acetate are proposed.

Prospects for the foreseeable future (when the fossil fuel supply gets diminished) will require direct utilization of photochemical energy for the production of hydrocarbon fuels. Byproduct processes such as the conversion of phytomass to fuel, as well as to fodder, is presently under vigorous investigation. Calvin (1976) has already suggested that the direct synthesis of hydrocarbon fuel in some plants offers a tremendous potential for the efficient conversion of carbon dioxide to fuel using the free energy obtained from sunlight.

The optimization of latex production by plants has produced in *Hevea brasiliensis* Muell. Arg. an efficient conversion of  $\text{CO}_2$  to rubber by sunlight. Chemical bioinduction of isoprenoids has recently been under investigation by Yokoyama (1977) in *Parthenium argentatum* A. Gray, guayule, a plant under current development as a domestic source of natural rubber. The chemical investigation of dried guayule stem oils dates back to Alexander (1911), who identified  $\alpha$ -pinene, and Haagen-Smit and Siu (1944), who identified  $\alpha$ - and  $\beta$ -pinene, di-