m/e 162 for both compound B and reference umbelliferone.

RESULTS AND DISCUSSION

Umbelliferone (7-hydroxycoumarin) was found to be present in the seeds of *D. odorata*. The rather low yield of 6.3 mg (0.01%) may well explain why umbelliferone had not been previously isolated from the tonka bean.

A total of 725 mg of coumarin (2*H*-1-benzopyran-2-one) was also isolated from the tonka bean. This yield (1.15%) was found to be considerably lower than those reported in the literature. Yields of 2.1–3.5% have been reported (Follett-Smith, 1936; Pound, 1938; Coomber et al., 1952). This lower yield might be attributed to the extraction procedure employed in this study or simply because of inferior tonka beans.

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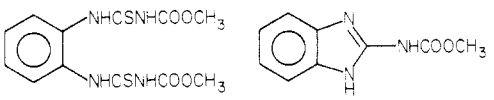
Determination of Thiophanate Methyl as Carbendazin by High-Pressure Liquid Chromatography: Application to Onions and Cabbage

Thiophanate methyl was extracted from onion and cabbage with ethyl acetate and was converted quantitatively to carbendazin (II) in hot 50% aqueous acetic acid. The determination of II was carried out after separating II from interfering plant materials with an LC strong cation exchange column by measuring the absorbance of II at 275 nm. With a 50-g sample spiked at 0.08 ppm, the signal-to-noise ratio was 12 and the recovery 75%. The detection limit was approximately 0.02 ppm.

The title compounds are systemic fungicides and are related in that carbendazin (II) is the principal metabolite



I II

of thiosphanate methyl (I). Carbendazin is also the principal metabolite of benomyl. Analytical schemes for I and II in plant materials and soils have been reviewed recently (Gorbach, 1980). These schemes are principally of two kinds: (1) direct determinations of both I and II simultaneously, for example, by measuring absorbance at two wavelengths (Chiba, 1977) or by separating them chromatographically (Austin et al., 1976), and (2) a direct determination of II (or a derivative of II) accompanied by a separate step in which I is converted quantitatively to II (Shiga et al., 1977). The procedure reported here is of the latter type and includes the extraction of I from onion and cabbage samples, the quantitative conversion of I to II, and the determination of II by high-pressure liquid chromatography (LC). This study was carried out since, in earlier work, low recoveries and interferences were experienced from onions and cabbage. The introduction of an ion-exchange column was necessary for the separation of II from naturally occurring interferences. Our analytical conditions were similar to those of Kirkland et al. (1973).

EXPERIMENTAL SECTION

Materials and Apparatus. Solvents used were Fisher reagent grade redistilled in glass stills. The high-pressure liquid chromatograph utilized was a Micromeritics Model 7000 with a variable-wavelength detector. The column was a Zipax SCX (strong cation exchange), 1 m × 2.2 mm i.d. Instrument conditions were as follows: column temperature, 60 °C; mobile phase water containing 0.025 M tetramethylammonium nitrate and 0.025 M nitric acid; carrier flow rate, 0.5 mL min^{-1}; absorbance detector at 275 nm and 0.02 absorbance unit full scale. The noise level was about 10^{-4} absorbance unit; the sensitivity was 4.8×10^{-3} absorbance unit/μg of II. Signals were evaluated from areas, not peak heights. Samples were injected with a 100-μL syringe. For the most dilute samples, 10 injections were made for a total sample volume of 1000 μL. The elution volume of II was 8.5 mL. Typical chromatograms of these most dilute samples are shown in Figure 1.

Procedure. Samples of onion and cabbage were treated as follows: A 50-g sample was blended twice with 200 mL of ethyl acetate for 2 min. After filtration, the solution was evaporated to 5 mL and mixed with 20 mL of 50% aqueous acetic acid and 100 mg of copper(II) acetate. After the remaining ethyl acetate evaporated, the solution was covered loosely and held just below its boiling point on a hot plate for 0.5 h. The mixture was transfered and diluted with 40 mL of 0.1 M HCl and was washed with petroleum ether until the petroleum ether was clear (about 3 × 50 mL). The aqueous layer was made basic with 25 mL of 6.5 M NaOH and was extracted 4 times with 75-mL portions of ethyl acetate. The combined extracts were dried with Na_2SO_4 and evaporated to 50 mL. This solution was extracted 3 times with 10-mL portions of 0.1 M HCl. The acid extraction was then brought to pH 8–10 with about

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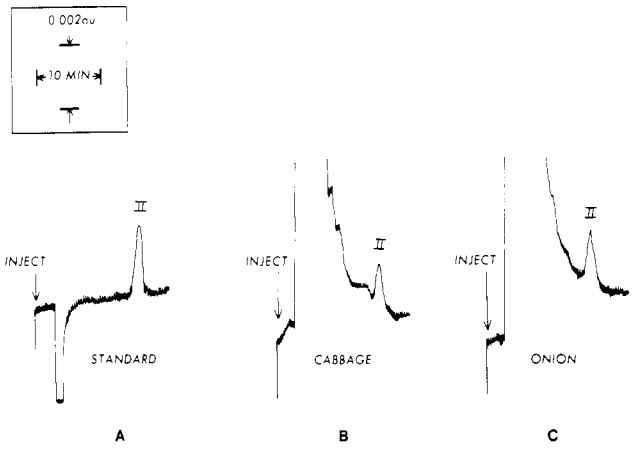

Figure 1. Liquid chromatograms of II. (A) Calibration standard; 0.5 μg in 100-μL injection. (B) 10 × 100 μL injections of sample obtained from 50 g of cabbage spiked with 4 μg of I; extract diluted to 5.0 mL finally. (C) Same as (B) except that sample was onion. See the text for chromatograph conditions. The noise shown in this figure is schematic and has been exaggerated in two ways: the amplitude is too large by about a factor of 2 and the frequency is too low. We did not observe distinct noise spikes as drawn.

Table I. Recovery of Carbendazin (II) from Thiophanate Methyl (I)

sample	mass of I, μg[c]	recovery, %[d]
standard[a]	30 (2)	
	10 (1)	103 ± 4
	4 (2)	
onion[b]	100 (1)	91
	30 (4)	83 ± 5
	10 (3)	72 ± 3
	4 (2)	74 ± 3
cabbage[b]	100 (2)	82 ± 2
	30 (3)	72 ± 4
	10 (4)	70 ± 1
	4 (3)	77 ± 3

[a] The conversion of I to II with no vegetable matter present. [b] Samples were 50 g of vegetable matter. [c] Mass of I added to sample and number of replicates in parentheses. [d] Average ± one standard deviation.

4 mL of 6.5 M NaOH and was extracted with four 50-mL portions of ethyl acetate saturated with water. The ethyl acetate solution was evaporated to 3–5 mL, 1 mL of 0.03 M H_3PO_4 was added, and the remaining ethyl acetate was removed. Finally, the volume was adjusted to 5.0 or 10.0 mL, depending on the mass of I added to the sample, by the addition of 0.03 M H_3PO_4. Samples of this solution were analyzed for II with an LC fitted with a cation-exchange column and an absorbance detector as described above.

RESULTS AND DISCUSSION

Analyses were carried out for standard samples with no plant material present and for check samples in which various known amounts of I were added to 50-g samples of onion or cabbage. The results are listed in Table I. The standard samples gave conversions and recoveries of 103 ± 4%. The recoveries from onion and cabbage are incomplete, 68–91%, but are acceptable. There is a trend toward lower recoveries as the concentration of I in the vegetable matter decreases. At our lowest concentrations, 0.080 ppm, the signal-to-noise ratio was approximately 12 (see Figure 1) and the recovery was near 75%, comparable to recoveries reported by Shiga et al. (1977) for soils and by Ono et al. (1975) for vegetables. The limit of our method corresponds to samples with a concentration of 0.02 ppm for which the results suggest a signal-to-noise ratio of 3 and a recovery of about 65–70%. This detection limit is about the same as the limits reported by Shiga et al. (1977) for both I and II in soil, 0.03 ppm, by Ono et al. (1975) for vegetables, 0.02 ppm for I and 0.01 ppm for II, and by Gorbach (1980) for various plant materials, 0.02–0.06 ppm for II. The selectivity of our method is good, as can be seen in Figure 1.

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Natural Rubber from Sunflower. 2

The rubber content of the air-dried leaves of 31 wild species of sunflower (*Helianthus*) was determined by a gravimetric method. Ten species with a rubber content > 0.93% were further analyzed by ^{13}C NMR spectroscopy. The extracts from the four species with the highest rubber content were >92% pure rubber. The benzene extract from the remaining species was contaminated by high molecular weight straight-chain hydrocarbons. Little or no rubber was found in the benzene extract from stems of these 10 species. Among the four species with the highest rubber content was *H. annuus*, the species from which commercial sunflower hybrid varieties are derived.

The profitable production of natural rubber from cultivated domestic plants is of considerable interest to agriculturalists to ensure an uninterrupted supply of this critical raw material. This is exemplified by the renewed research effort in guayule production. General screening programs have discovered that members, other than guayule, of the Compositae family are capable of producing rubber. A prominent rubber-producing member of this