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Note

High-performance liquid chromatographic determination of ethiofencarb and its metabolic products

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Ethiofencarb (I) is a systemic insecticide with a specific action against aphids; it is mainly adsorbed by roots and translocated to the aerial part of the plant[1], where it is quickly converted into its sulphoxide (II) and, more slowly, sulphone (III) derivatives (Fig. 1). The same metabolic pathway is observed in soil, whereas in water it is mainly (90%) transformed into the corresponding phenol (IV) by hydrolysis of the carbamic group[2]. Phenolic derivatives, produced by hydrolysis of the carbamic

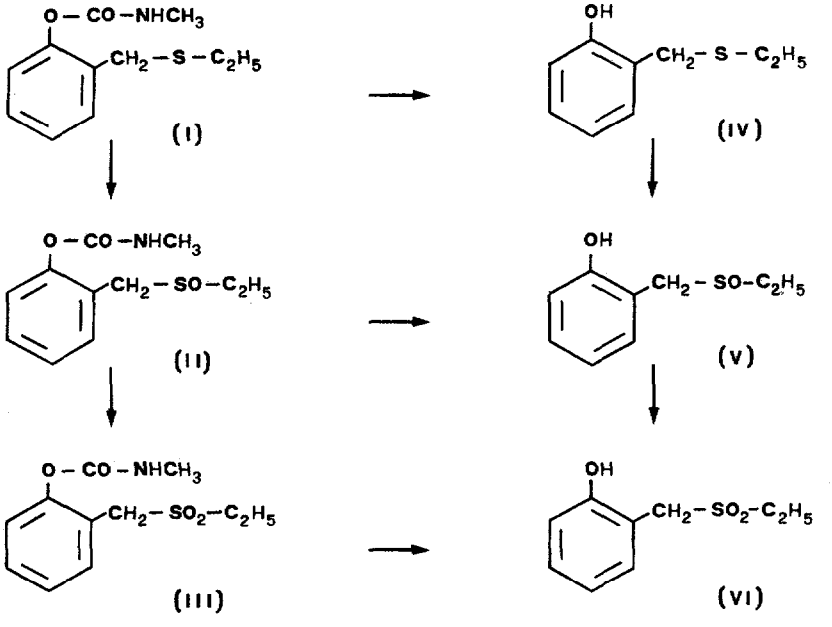

Fig. 1. Metabolic pathway of ethiofencarb in plant, soil, water and animals.

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group, have also been found in animals; the major metabolites are the phenol sulphoxide (V) and phenol sulphone (VI)[3].

Residues of ethiofencarb and its metabolites have been determined by chromatography, by oxidation of the active ingredient and its sulphoxide to the sulphone[4] and by thin-layer chromatography of labelled products[5]. The Drager method was optimized, which allowed the individual determination of ethiofencarb and the sulphoxide and sulphone after separation of the active ingredient from its metabolites with different solvents (light petroleum for ethiofencarb and chloroform for the metabolites). The same oxidative process was used for their determination[6]. Wuest and Meier[7] determined ethiofencarb by capillary chromatography, without any derivatization. A high-performance liquid chromatographic (HPLC) method with electrochemical detection[8] was applied to the determination of the active ingredient after alkaline hydrolysis of its carbamic group to give the phenol derivative (IV).

In this paper the HPLC determination of ethiofencarb and its metabolites (II–VI) without any derivatization or solvent fractioning is described.

EXPERIMENTAL

Apparatus

A Varian (Varian, Palo Alto, CA, U.S.A.) Model 5020 liquid chromatograph equipped with a variable-wavelength UV 100 UV–VIS detector, a Rheodyne injector (50-μl loop) and a Hewlett-Packard 3390A reporting integrator was used.

Chromatography

Hibar RP-18 (Merck, Darmstadt, F.R.G.), Erbasil 10 C_8/H (Carlo Erba, Milan, Italy) and Violet RP-2 (Violet, Rome, Italy) columns (250 × 4.0 mm I.D., 10 μm) were employed. The mobile phase was water–acetonitrile at a flow-rate of 1.0 ml/min. Based on the UV spectra, 190 nm was chosen for the simultaneous determination of all compounds.

Chemicals

Acetonitrile and dichloromethane were HPLC grade solvents (Carlo Erba); water was distilled twice and filtered throughout a Milli-Q apparatus (Millipore, Milan, Italy) before use. Ethiofencarb (2-[(ethylthio)methyl]phenyl methylcarbamate) was an analytical standard purchased from Eherenstorfer (Augsburg, F.R.G.). The 2-[(ethylsulphinyl)methyl]phenyl methylcarbamate (sulphoxide) and the 2-[(ethylsulphonyl)methyl]phenyl methylcarbamate (sulphone) were synthesized by oxidation of the technical active ingredient (extracted by the commercial formulation Croneton 10 Granulare, Bayer AG, containing 10% ethiofencarb) using hydrogen peroxide as oxidizing agent. The sulphoxide was obtained by performing the reaction with stirring at room temperature in methanol for 3 h in the presence of catalytic amounts of ammonium molybdate; the sulphone was obtained in the same way, but with refluxing for 2 h in methanol. Then the reaction mixture was diluted with water and extracted with chloroform. The chloroform layers were dried and evaporated to give the crude sulphone or sulphoxide.

2-[(Ethylthio)methyl]phenol (phenol), 2-[(ethylsulphinyl) methyl]phenol (phenolsulphoxide) and 2-[(ethylsulphonyl)methyl]phenol (phenolsulphone) were ob-

tained by hydrolysis of the corresponding N-methylcarbamate with 3% methanolic potassium hydroxide solution.

All the products (carbamates and phenols) were purified by chromatography on a column of silica gel eluted with suitable benzene–acetone mixtures and their identities were confirmed by IR and ^{1}H NMR spectroscopy.

Extraction procedure

After trituration and homogenization, 25 g of lettuce sample were weighed in a 250-ml screw-capped flask, 50 ml of dichloromethane (or methanol for the phenol IV) were added and the mixture was agitated in a flask-shaker (Stuart Scientific) for 30 min. The organic layer was separated and dehydrated with sodium sulphate; 2 ml were then dried in a thermo-ventilated stove at 30°C, taken up in 1 ml of eluent and injected for HPLC analysis.

RESULTS AND DISCUSSION

A good separation of ethiofencarb (I) and the sulphoxide (II) and sulphone (III) derivatives was achieved using an RP-18 column with water–acetonitrile (50:50, v/v) as eluent (Table I). The separation of the active ingredient and the metabolites was improved by increasing the water content to 60%; only the peaks of the sulphone (III) and the phenol sulphone (VI) derivatives were not enough separated. A further increasing in the water content (to 70%) gave a better separation of metabolites II, III, V and VI, but the retention times of I and IV were too long and their peaks were not sharp enough.

A reduction in retention times was obtained by replacing water with a 10^{-3} *N* sulphuric acid (pH 3.0) in the eluent, but the separation was not improved. The use of methanol instead of acetonitrile in the eluent mixture reversed the elution order of the sulphoxides (II and V) and sulphones (III and VI), but the peaks were less sharp than

TABLE I

RETENTION TIMES OF ETHIOFENCARB (I) AND ITS METABOLITES (II–VI) USING DIFFERENT COLUMNS AND MOBILE PHASE COMPOSITIONS

Column	*Water–acetonitrile composition*	*Retention time (min)*					
		II	*V*	*III*	*VI*	*I*	*IV*
RP-18	50:50	2.45		2.88		6.06	
	60:40	2.65	2.97	3.27	3.40	9.02	10.66
	70:30	3.36	3.76	4.40	4.67	20.02	24.46
C_8/H	60:40	3.32	3.55	4.03	4.23	9.86	11.32
	65:35	3.58	3.88	4.56	4.84	14.09	16.51
	70:30	4.01	4.43	5.39	5.80		
RP-2	60:40	3.52	3.52	3.71	3.92		
	70:30	3.84	4.11	4.48	4.89		
	80:20	4.79	5.55	6.03	6.99		

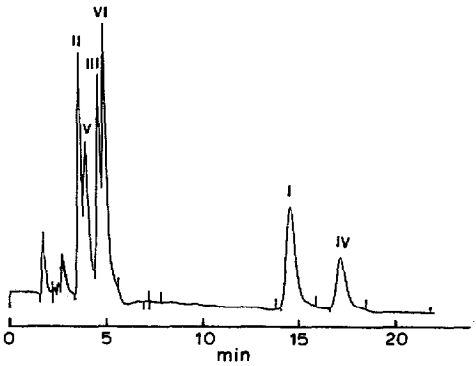

Fig. 2. Chromatography of ethiofencarb (I) and its metabolites: sulphoxide (II), sulphone (III) phenol (IV), phenol sulphoxide (V) and phenol sulphone (VI), on an C_8/H column. Mobile phase, water–acetonitrile (65:35, v/v); flow-rate, 1.0 ml/min; detection, UV (190 nm).

with acetonitrile. This reversal of the peak elution order between sulphones and sulphoxides could be valuable in confirmatory assays.

On a C_8/H column using the same chromatographic conditions the retention times increased, improving the separation, particularly of the sulphone (III) and phenol sulphone (VI). The best conditions for satisfactory separation and good peak sharpness were achieved using water–acetonitrile (65:35, v/v) as eluent (Fig. 2). An increase of only 5% in the water content is sufficient to produce poorer peak sharpness, to increase the retention time differences and to produce worse separations.

The retention times also increased when an RP-2 column was used under the same chromatographic conditions, but a worse separation of metabolites II, III, V and VI was achieved. In order to obtain a better separation it was necessary to increase the percentage of water in the eluent, but this produced a poorer peak sharpness.

Calibration graphs for each compound were constructed by plotting concentrations *vs.* peak areas; good linearities were achieved in the range 0–2.5 ppm, with correlation coefficients between 0.9952 and 0.9977.

Under the optimum conditions the detection limit was 0.02 ppm for all the

TABLE II

RECOVERIES OF ETHIOFENCARB (I) AND ITS METABOLITES (II–VI) BY DICHLOROMETHANE EXTRACTION FROM LETTUCE

Results are means of four experiments.

Compound	*Fortification level (ppm)*	*Recovery ± R.S.D. (%)*
Ethiofencarb (I)	1.50	90.3 ± 5.7
Sulphoxide (II)	1.50	96.4 ± 5.1
Sulphone (III)	1.50	103.1 ± 4.8
Phenol (IV)	1.50	34.9 ± 2.1
Phenol sulphoxide (V)	1.50	93.1 ± 6.7
Phenol sulphone (VI)	1.50	102.4 ± 4.2

compounds. This technique was used to determine ethiofencarb and its metabolites in lettuce. In the extraction procedure, with several organic solvents (benzene, cyclohexane, chloroform and light petroleum) part of the ethiofencarb co-distilled with the solvent during evaporation, making its quantitative determination impossible. Dichloromethane was found to be the only solvent tested that allowed the quantitative extraction of all compounds, except the phenol IV, without any problem of co-distillation and interferences. A good recovery of the phenol IV (>87%) was carried out with methanol as the extraction solvent, using the same procedure as with dichloromethane.

Considering the FAO/WHO specifies the sum of the active ingredient and metabolites II and III as the maximum residue limit allowed in food, the described procedure allows the determination of residues and the study of the metabolites.

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