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Note

Separation of pirimicarb and its metabolites by high-performance liquid chromatography

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Pirimicarb, 2-dimethylamino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate (I) (Fig. 1) is a selective aphicide with rapid contact and translaminar actions; it is taken up by roots and translocated in the xylem system[1]. Its highly selective action makes it especially suitable for integrated control programmes. A rapid reduction of pirimicarb occurs in plants after spraying, mainly by volatilization but also by photochemical and metabolic degradation, the major products being compounds II and III (Fig. 1). Some of the residue cannot be extracted from cabbage leaves (14%) or from lettuce (25%) by conventional techniques[2].

Pirimicarb is extensively degraded in soil, the principal route being the hydrolysis of the carbamate moiety either by biological or chemical means. Compounds V, VI and III are the major metabolites (Fig. 1); compound V is the major product (84%) of the photochemical degradation on the soil surface[2].

Even in animals, the principal degradation route of pirimicarb is the hydrolysis of the carbamate moiety: the hydroxypyrimidines V–VII are the major products whereas the carbamate-containing metabolites are absent or in very low amount (8%)[3].

For residue analysis, gas chromatography (GC) has been employed in order to determine pirimicarb[4] and its carbamate-containing metabolites II and III[5]. High-performance liquid chromatography (HPLC) has recently been used with UV–VIS detection[6,7] and with fluorescence detection[8]; with this technique, pirimicarb and its metabolite desmethyl-pirimicarb (III) were determined in various crops.

In this paper an HPLC separation of pirimicarb and its major metabolites is reported.

EXPERIMENTAL

Apparatus

A Varian (Palo Alto, CA, U.S.A.) Model 5020 liquid chromatograph was employed, equipped with a variable-wavelength UV-100 UV–VIS detector and

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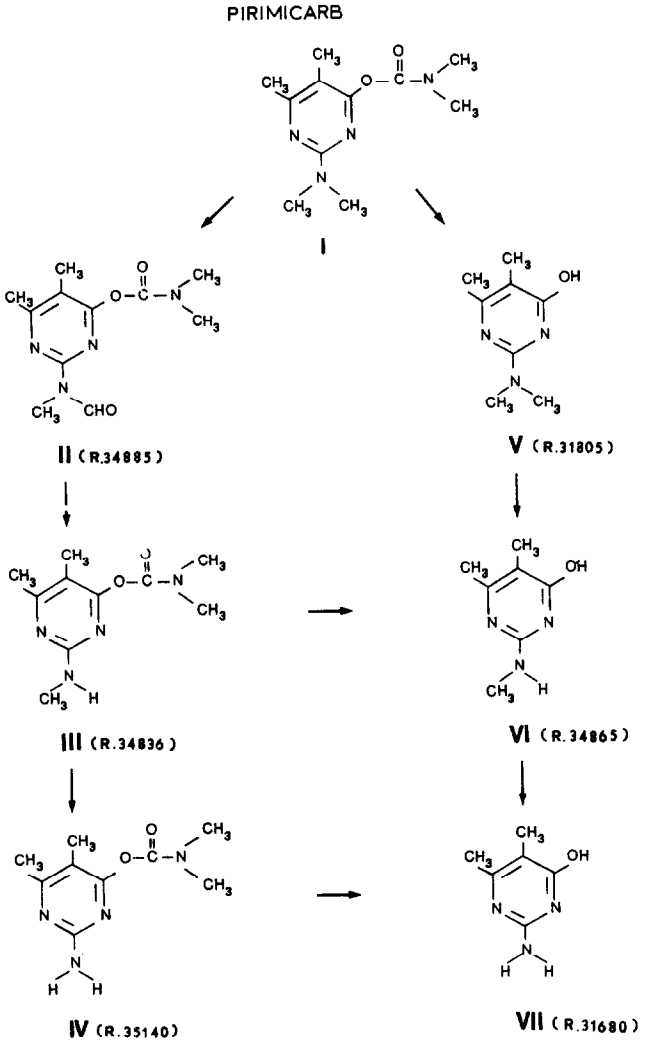

Fig. 1. Metabolic pathway of pirimicarb.

a Rheodyne injector (50-μl loop), connected to an H.P. (Hewlett-Packard, Avondale, PA, U.S.A.) Model 3390 A reporting integrator.

Chromatography

Hibar (Merck, Darmstadt, F.R.G.) RP-8, RP-18 and NH_2 columns (250 mm × 4.0 mm I.D., 10 μm) were used; the mobile phase was water–acetonitrile (NH_2) or phosphate buffer–acetonitrile (RP-8 and RP-18) in various ratios (Table I). The detector wavelength was programmed during the analyses according to the UV spectra of pirimicarb and its metabolites (245 nm for I and II, 236 nm for III, 226 nm for IV, 295 nm for V–VII).

TABLE I

RETENTION TIMES OF PIRIMICARB (I) AND ITS METABOLITES (II–VII) WITH DIFFERENT COLUMNS AND ELUENTS

Column	*Mobile phase*	*Retention time (min)*						
		VII	*VI*	*V*	*IV*	*III*	*II*	*I*
	Buffer–acetonitrile (v/v)							
RP-8	60:40	2.26	2.46	2.75	3.27	4.29	5.80	7.14
	65:35	2.30	2.43	2.81	3.61	4.79	7.26	8.75
	70:30	2.45	2.57	2.92	4.04	5.72	9.91	11.37
RP-18	60:40	2.06	2.25	2.62	3.00	4.23	5.91	8.66
	65:35	2.15	2.30	2.69	3.34	4.89	7.78	11.37
	70:30	2.21	2.48	2.84	3.79	5.98	10.95	15.56
	Water–acetonitrile							
NH_2	10:90	7.89	5.03	3.48				
	8:92	10.59	6.02	3.61				
	5:95	20.07	9.50	4.01				

Chemicals and materials

Acetonitrile was of HPLC grade (Carlo Erba, Milan, Italy); water was doubly distilled and filtered through a Milli Q apparatus (Millipore, Milan, Italy) before use. The buffer solution was made from 10^{-2} *M* KH_2PO_4 with 5 ml/l acetic acid; potassium dihydrogenphosphate and glacial acetic acid were of analytical grade (Carlo Erba).

Pirimicarb (I) and its metabolites II (2-methylformylamino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate, R. 34885), III (2-methylamino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate, R. 34836), IV (2-amino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate, R. 35140), V (2-dimethylamino-5,6-dimethyl-4-hydroxypyrimidine, R. 31805), VI (2-methylamino-5,6-dimethyl-4-hydroxypyrimidine, R. 34865) and VII (2-amino-5,6-dimethyl-4-hydroxypyrimidine, R. 31680) were obtained from ICI Solplant (Milan, Italy).

RESULTS AND DISCUSSION

A water–acetonitrile mixture was initially used as the mobile phase with reversed-phase (RP) columns and a good separation was achieved for the carbamate-containing metabolites I–IV. However, the hydroxypyrimidine (V–VII) peaks were very close but still resolved. After several analyses the columns showed an unaccountable loss of efficiency; compounds V–VII were not resolved and IV and V were overlapped.

Water was then replaced in the eluent mixture by a phosphate buffer solution, so achieving a greater reproducibility of retention times at the same resolution than with water–acetonitrile as the mobile phase.

As shown by the retention times on RP-8 and RP-18 columns (Table I), the carbamate-containing metabolites I–IV were greatly influenced by the percentage of buffer in the mobile phase (in decreasing order from I to IV) whereas the hydroxypyrimidines V–VII appeared less sensitive to such changes. Using mobile phases with a buffer content equal or greater than 65%, a good resolution of compound V from IV was achieved, but peaks V–VII were so close that any loss of efficiency of the column caused them to overlap.

Owing to the incomplete separation of the most polar compounds V–VII on RP columns, a normal phase NH_2 column was used that gave a good separation of V–VII with water–acetonitrile as the mobile phase, whereas I–IV were rapidly eluted and not resolved. The retention times changed considerably by increasing the polarity of the eluent mixture, especially for compounds VI and VII, whereas V was less influenced by changes in the water content of the mobile phase.

Calibration graphs for each compound were constructed by plotting concentrations *vs.* peak areas. An RP-8 column for compounds I–IV and an NH_2 column for V–VII were used, with respectively buffer–acetonitrile (65:35, v/v) and water–acetonitrile (8:92, v/v) as the mobile phase (Fig. 2). Good linearities were achieved in the range 0–1.5 ppm with correlation coefficients between 0.9991 and 0.9997. Under the optimum conditions, the detection limit was 0.005 ppm for each compound.

The method described allows the separation of pirimicarb and its metabolites and may be useful for the determination of these compounds, after appropriate extraction and clean-up in different matrices.

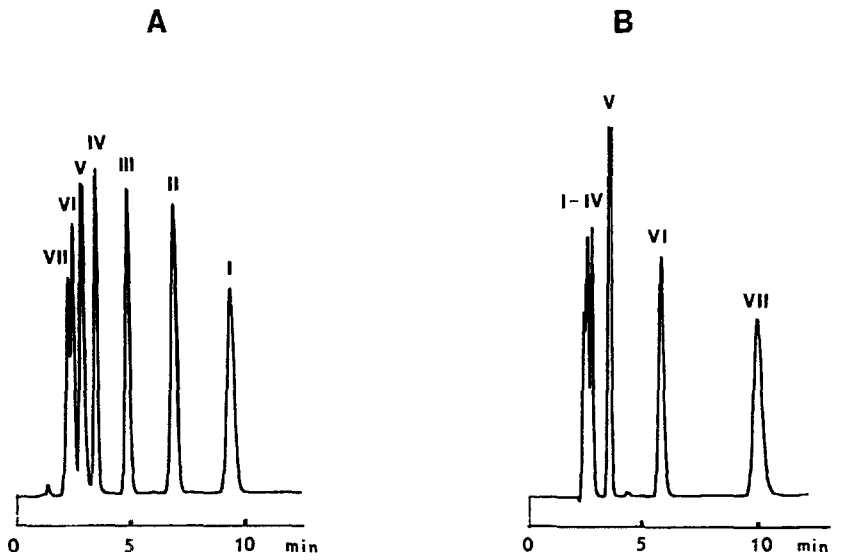

Fig. 2. Chromatography of pirimicarb (I) and its metabolites (II–VII): (A) on an RP-8 column, mobile phase, phosphate buffer–acetonitrile (65:35, v/v), flow-rate 1 ml/min, UV detection at 226, 236 and 245 nm: (B) on an NH_2 column, mobile phase, water–acetonitrile (8:92, v/v), flow-rate 1 ml/min; UV detection at 295 nm.

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