Journal of Chromatography, 391 (1987) 334–337
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 19 300

Note

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

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(Received November 24th, 1986)

Benalaxyl (methyl N-phenylacetyl-N-2,6-xylyl-DL-alaninate) is a systemic fungicide belonging to the acylalanine family. It is particularly effective against fungi of the order Peronosporales[1]. The structure–activity relationships and modes of action of acylalanine have been widely described[2–4].

The degradation of Benalaxyl in soil, both in the laboratory and under natural conditions, produces two main metabolites, I (methyl N-carboxyacetyl-N-2,6-xylyl-DL-alaninate) and II (N-carboxyacetyl-N-2,6-xylyl-DL-alanine) (Fig. 1), depending on the microbiological activity: the parent compound and/or its metabolites are finally transported into plants[5]. A different degradation behaviour was observed when Benalaxyl was sprayed directly on the plants: the main metabolite, III (methyl N-phenylacetyl-N-2-hydroxymethyl-6-methylphenyl-DL-alaninate), is produced by oxidation of a benzene ring methyl group. Another product is formed in very low concentrations by oxidation of the same ring[6].

All these metabolites are partially conjugated to give mono- and diglucosides, and the percentage of the conjugated products is higher than that of the free products. The aglycone can be obtained by chemical or enzymatic (β-glucosidase) hydrolysis.

Benalaxyl has always been determined by gas chromatography in metabolic studies; in this note we describe a reversed-phase high-performance liquid chromatographic (RP-HPLC) procedure for a rapid determination of this pesticide and its metabolites.

EXPERIMENTAL

Chromatography

A Spectra Physics SP 8750 liquid chromatograph equipped with a Model 770 spectrophotometric detector, a Valco AH-20 injector (50-μl loop) and an Hewlett-Packard 3390 A integrator was used. LiChrosorb RP-2 (250 mm × 4.0 mm I.D., 10 μm; Violet Roma, Italy), Partisil 10 C_8 and ODS (250 mm × 4.6 mm I.D., 10 μm; Whatman, Clifton, NJ, U.S.A.) columns were employed. Mixtures of acetonitrile with water, 1% cetrimide aqueous solutions and 10^{-3} *N* sulphuric acid, in different percentages, were used as the mobile phases at a flow-rate of 1 ml/min. The best wavelength for simultaneous determination was found to be 200 nm.

0021-9673/87/$03.50

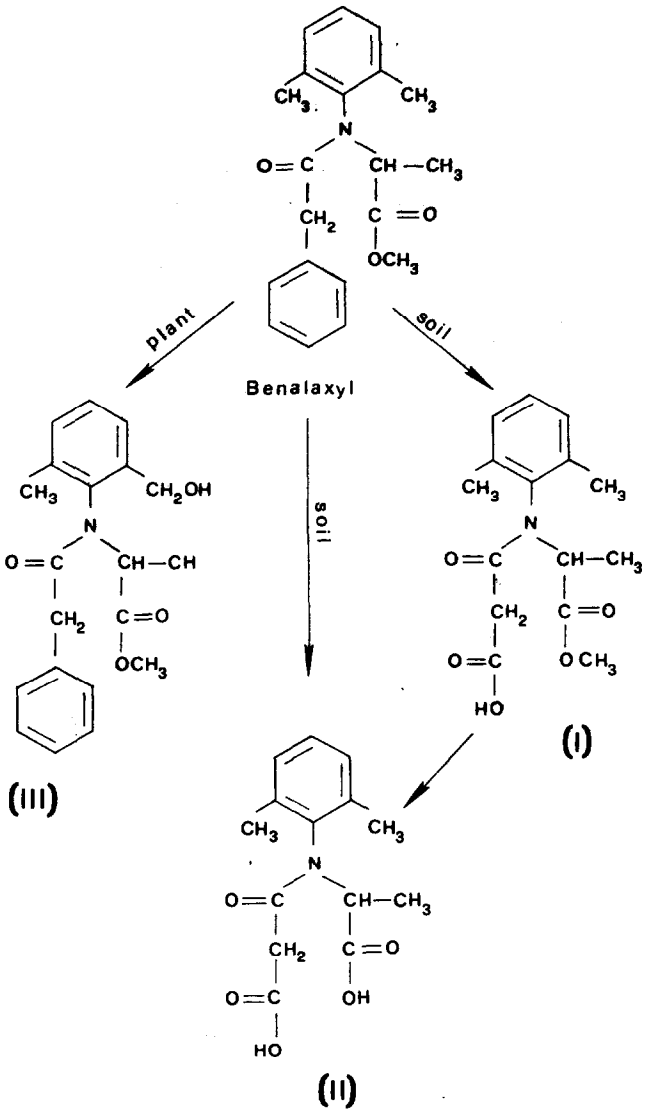

Fig. 1. Metabolic pathways for Benalaxyl in plants and soil.

Chemicals

Benalaxyl and its metabolites I–III were analytical standards, kindly furnished by Farmoplant (Milan, Italy). Acetonitrile was HPLC grade, sulphuric acid and Cetrimide were RPE grade (Carlo Erba, Milan, Italy). Water was distilled twice and filtered through a Millipore apparatus (Milli Q) before use.

RESULTS AND DISCUSSION

Benalaxyl was first determined[7] by HPLC using an RP-8 column eluted with

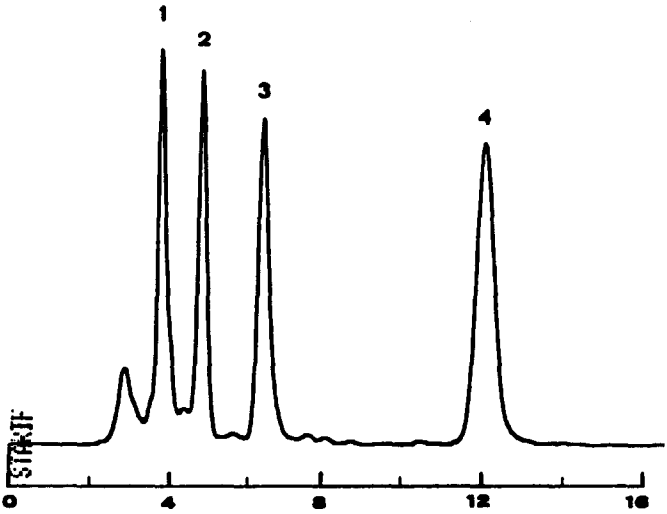

Fig. 2. Chromatogram of pesticides (1 = metabolite II, 2 = metabolite I; 3 = metabolite III; 4 = Benalaxyl) on an RP-2 column. Mobile phase: acetonitrile–10^{-3} *N* sulphuric acid (50:50); flow-rate: 1 ml/min. UV detection: 200 nm.

acetonitrile–water (50:50, v/v). A good separation of Benalaxyl and metabolite III was achieved, but the separation of metabolites I and II was unsatisfactory. The same results were obtained using RP-2 and RP-18 columns, the same eluent mixture and 1% Cetrimide solution instead of water, in different percentages with acetonitrile.

A good separation of all four compounds was achieved using 10^{-3} *N* aqueous sulphuric acid solution in different percentages with acetonitrile, as mobile phase, on RP-2 (Fig. 2) and RP-8 columns. Such a good separation was not achieved on an RP-18 column: in particular, the separation of metabolites II and III and the peak symmetries of metabolites I and II were very poor.

TABLE I

RETENTION TIMES OF PESTICIDES USING DIFFERENT COLUMNS AND MOBILE PHASE COMPOSITIONS

1 = Metabolite II; 2 = metabolite I; 3 = metabolite III; 4 = Benalaxyl.

Column	*Acetonitrile–10^{-3} N sulphuric acid (%)*	t_R			
		1	*2*	*3*	*4*
RP-2	60:40	3.42	4.00	4.75	7.17
RP-8		3.51	4.60	5.60	11.48
RP-2	55:45	3.56	4.37	5.43	9.17
RP-8		3.69	5.08	6.48	15.07
RP-2	50:50	3.79	4.81	6.34	12.07
RP-8		4.15	6.21	8.35	23.01

The best separation of Benalaxyl and its metabolites I–III was obtained with acetonitrile–10^{-3} *N* sulphuric acid mixtures in proportions from 40:60 to 50:50 (v/v), as shown in Table I. Appropriate conditions may be chosen for a particular sample, which may contain interfering materials deriving from starting matrices.

The described procedure will be particularly useful for metabolic and/or degradation studies, since both Benalaxyl and its metabolites can be detected down to concentrations of 0.005 ppm.

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