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THIN-LAYER CHROMATOGRAPHY OF 2-METHYL-4-CHLOROPHENOXYACETIC ACID AND ITS SOIL METABOLITES

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SUMMARY

The thin-layer chromatography of 2-methyl-4-chlorophenoxyacetic acid, 4-chloro-*o*-cresol and 3-methyl-5-chlorocatechol and their pentafluorobenzyl derivatives has been studied on silica gel as adsorbent with 19 solvent systems. The best separation of the individual components occurred with toluene–benzene–acetic acid (2:2:1). Chloroform–diethyl ether–toluene (1:1:1) was suitable for the group separation of the pentafluorobenzyl derivatives.

INTRODUCTION

Thin-layer chromatography (TLC) is one of the most widely used techniques for the separation and analysis of pesticides and their residues[1–8]. Walker and Beroza[9] examined 62 pesticides by TLC with 19 solvent systems on silica gel. TLC has also been recommended for the detection of organochlorine pesticides[10,11]. Numerous reports describe the application of TLC to separation and clean-up processes in the analysis of chlorinated hydrocarbons[12–15] and organophosphorus compounds[16–19]. Organochloride pesticides have been separated on silica gel[20,21] and on silica gel–alumina (7:3) layers[22]. Abbot *et al.*[23] separated herbicide residues by TLC.

In our laboratory, the TLC of chlorinated cresols[24] and catechols[25] was examined on five different layer materials with different solvent systems in order to develop separation and clean-up methods analyses of these potentially important residues from environmental samples. 2-Methyl-4-chlorophenoxyacetic acid (MCPA) (I) is the most commonly used pesticide in Finland[26] and its most important potential residues are its soil metabolites 4-chloro-*o*-cresol (II) and 3-methyl-5-chlorocatechol (III)[27]. We developed a method for simultaneous determination of I, II and III from soil samples[28] in which the crude extract was treated with pentafluorobenzyl bromide in acetone in the presence of potassium carbonate (according to Chau and Terry[29]) to form derivatives IV, V and VI (see Fig. 1). Then the crude derivative mixture was purified by different methods, including TLC, for final determination by gas–liquid chromatography. In this investigation, the TLC of compounds I–VI on silica gel with different solvent systems has been extended to the separation, identification and determination of these important residues and their derivatives.

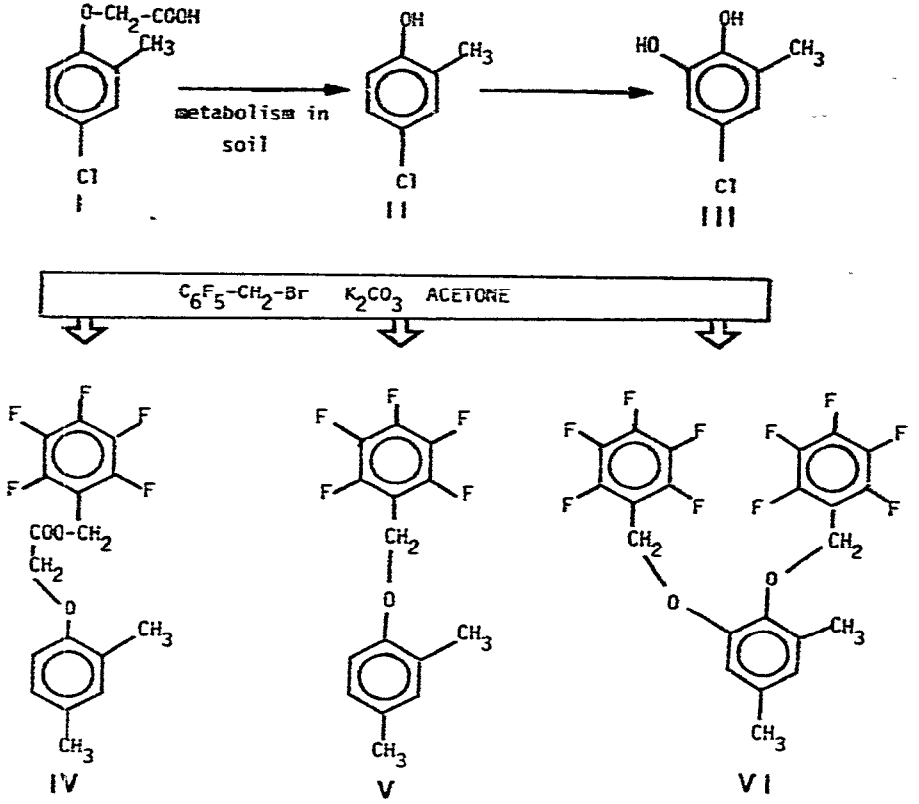

Fig. 1. Structures of MCPA (I), its metabolites in soil, 4-chloro-*o*-cresol (II) and 3-methyl-5-chlorocatechol (III), and their pentafluorobenzyl derivatives (IV–VI).

EXPERIMENTAL

Apparatus and methods

Standard TLC plates (20 × 20 cm; Merck, Darmstadt, G.F.R.) were used. The plates were pre-coated with silica gel G-60 containing a fluorescent indicator, with a layer thickness of 0.25 mm. The samples were spotted with 10–50-μl pipettes (H. E. Pedersen, Denmark) on a line 1 cm from the bottom of the layer, the first spot being 1 cm from the side of the plate and the following four spots at 4-cm intervals. Ascending elution in a closed glass chamber (Desaga, Heidelberg, G.F.R.) was applied. Both a Desaga scale plate and a metre scale were used to measure the R_F values of the spots.

Samples

MCPA was tried as two different samples: a pure acidic sample was MCPA from Pestanol (99%). MCPA as the sodium salt was a commercial formulation obtained from Kemira (Helsinki, Finland) which contained 4% of 4-chloro-*o*-cresol as the main impurity[30]. A commercial sample of 4-chloro-*o*-cresol was obtained from Fluka (Buchs, Switzerland) and purified by vacuum distillation. 5-Chloro-3-methylcatechol

was synthesized in our laboratory by J. Knuutinen. The structures of compounds I–III were confirmed with ultraviolet, infrared, nuclear magnetic resonance and mass spectrometry. The purity of the samples was checked by gas chromatography.

Preparation of derivatives IV–VI was performed following the procedure of Chau and Terry[29]. A 50-mg amount of compound I, II or III was shaken vigorously for 5 min in a glass-stoppered flask with 1 ml of a 1% solution of pentafluorobenzyl bromide in acetone, 500 μl of a 30% solution of potassium carbonate in water and 4 ml of acetone, and allowed to stand for 3 h at room temperature. The sample was mixed with 2 ml of *n*-hexane and evaporated to dryness with a stream of nitrogen and the residue transferred with diethyl ether into a volumetric flask to make 50 ml of a crude derivative stock solution which represents 1 mg/ml of I, II or III as its derivative IV, V or VI, respectively. Derivatives from MCPA and its sodium salt were identical (IV). The solutions (1 mg/ml) of the original substrates I–III to be applied in the TLC experiments were made up in diethyl ether. The sodium salt of MCPA was insoluble in diethyl ether and therefore was not subjected to TLC.

Chromogenic reagent[31]

A 2% solution of 3,5-dichloro-*p*-bezoquinonechlorimine (Merck) in benzene was used for spot detection.

Solvent systems

A preliminary examination of 40 solvents or solvent mixtures was carried out in order to select those giving good spots and reasonable R_F values. All solvent mixtures produced sharp spots. The compositions of the 19 most suitable solvents, all of which except one are mixtures, are listed below (volume ratios):

(1) dichloromethane–acetone–acetic acid (8:1:1);
(2) acetone–toluene–acetic acid (2:2:1);
(3) benzene–ethanol–acetic acid (6:3:1);
(4) acetone–light petroleum (b.p. 40–60°)–acetic acid (2:2:1);
(5) acetone–methanol–acetic acid (6:3:1);
(6) acetone–diethyl ether–acetic acid (2:2:1);
(7) dichloromethane–toluene–acetic acid (2:2:1);
(8) acetone–chloroform–acetic acid (2:2:1);
(9) toluene–benzene–acetic acid (2:2:1);
(10) dichloromethane–*n*-heptane–acetic acid (2:2:1);
(11) dichloromethane;
(12) dichloromethane–acetone–benzene (2:1:1);
(13) acetone–benzene–acetic acid (5:4:1);
(14) dichloromethane–*n*-heptane–ethanol (2:2:1);
(15) chloroform–*n*-hexane–dichloromethane (1:1:1);
(16) chloroform–diethyl ether–toluene (1:1:1);
(17) *n*-hexane–*n*-heptane–ethanol (2:2:1);
(18) dichloromethane–light petroleum (b.p. 40–60°) (2:3);
(19) toluene–*n*-heptane–diethyl ether (1:1:1).

All solvents for free substrates I–III needed acetic acid to give good results. The derivatives, however, could be tested with other, mainly neutral, solvents (11–19) also.

Development of chromatograms

The development with each solvent system was continued until the solvent front had ascended 17 cm. The plates were then dried in air and sprayed with chromogenic reagent.

RESULTS AND DISCUSSION

All compounds (I–VI) formed sharp spots with all solvent systems used. The spots were yellow with MCPA (I), 4-chloro-*o*-cresol (II) and their pentafluorobenzyl derivatives (IV) and V). The spots from 3-methyl-5-chlorocatechol (III) and its pentafluorobenzyl derivative (VI) were ash grey. The development time, which varied at room temperature from 30 to 90 min, had no marked effect to the spots.

The R_F values obtained with different solvent systems are collected in Table I.

TABLE I

R_F VALUES OF MCPA (I), TWO OF ITS SOIL METABOLITES (II AND III) AND THEIR PENTAFLUOROBENZYL DERIVATIVES (IV–VI) ON A SILICA GEL G-60 LAYER WITH SOLVENT SYSTEMS 1–19

Solvent	*Compound*						*Elution time (min)*
	I	*II*	*III*	*IV*	*V*	*VI*	
1	0.54	0.82	0.76	0.94	0.83	0.76	60
2	0.56	0.52	0.59	0.71	0.59	0.59	48
3	0.74	0.85	0.79	0.94	0.82	0.82	90
4	0.85	0.88	0.88	0.94	0.91	0.91	90
5	0.79	0.88	0.88	0.91	0.88	0.88	64
6	0.94	0.97	0.90	0.97	0.97	0.97	85
7	0.56	0.59	0.50	0.59	0.59	0.50	45
8	0.88	0.94	0.94	0.94	0.94	0.94	30
9	0.50	0.41	0.56	0.76	0.56	0.44	70
10	0.56	0.59	0.50	0.65	0.62	0.50	55
11				0.62	0.57	0.54	35
12				0.57	0.55	0.57	35
13				0.94	0.88	0.82	50
14				0.62	0.52	0.47	60
15				0.50	0.57	0.52	45
16				0.71	0.73	0.71	50
17				0.41	0.20	0.16	40
18				0.35	0.55	0.41	50
19				0.65	0.41	0.24	47
Colour	Yellow	Yellow	Ash grey	Yellow	Yellow	Ash grey	

The separation in each experiment can be evaluated from these results by comparing the relative differences, x:

$$x = \frac{R_F(\mathrm{I}) - R_F(\mathrm{II})}{R_F(\mathrm{I}) + R_F(\mathrm{II})} \cdot 2 \qquad (1)$$

where x is the difference of two R_F values divided by their average. From each three-component TLC experiment, three values of x and their average ($\bar{x}$) are

obtained and the results are given in Table II. It can be concluded that the best separation of the original substrates I–III is achieved with dichloromethane–acetone–acetic (8:1:1) (solvent 1) and toluene–benzene–acetic acid (2:2:1) (solvent 9). The latter is to be preferred because the separation of all three components is greater than $x = 0.1$. Solvent 9 gives a good separation of derivatives IV–VI. However, the acid-free solvents 17, 18 and 19 also give good separations of derivatives IV–VI.

TABLE II

RELATIVE DIFFERENCES (x) BETWEEN R_F VALUES OF I, II AND III OR IV, V AND VI ON SILICA GEL G-60 WITH SOLVENT SYSTEMS 1–19

The value of x is calculated by dividing the difference of two R_F values with their average. Averages of x ($\bar{x}$) for each run are also given.

Solvent	*I/II* (x)	*I/III* (x)	*II/III* (x)	*I–III* ($\bar{x}$)	*IV/V* (x)	*IV/VI* (x)	*V/VI* (x)	*IV–VI* ($\bar{x}$)
1	0.412	0.338	0.076	0.275	0.124	0.212	0.088	0.141
2	0.074	0.052	0.126	0.084	0.185	0.185	0.000	0.123
3	0.138	0.065	0.073	0.092	0.136	0.136	0.000	0.091
4	0.035	0.035	0.000	0.023	0.032	0.032	0.000	0.021
5	0.108	0.108	0.000	0.072	0.034	0.034	0.000	0.023
6	0.031	0.043	0.075	0.050	0.000	0.000	0.000	0.000
7	0.052	0.113	0.165	0.110	0.000	0.165	0.165	0.110
8	0.066	0.066	0.000	0.044	0.000	0.000	0.000	0.000
9	0.198	0.113	0.309	0.207	0.303	0.533	0.240	0.359
10	0.052	0.113	0.165	0.110	0.047	0.261	0.214	0.174
11					0.084	0.138	0.054	0.092
12					0.036	0.000	0.036	0.024
13					0.066	0.136	0.071	0.091
14					0.175	0.275	0.101	0.184
15					0.131	0.039	0.092	0.087
16					0.028	0.000	0.028	0.019
17					0.689	0.877	0.222	0.596
18					0.444	0.158	0.292	0.298
19					0.453	0.921	0.523	0.632

For the use of TLC as a clean-up stage prior to the gas chromatographic determination of MCPA and its metabolites[28,32,33], small values of x and $\bar{x}$ are required, which allows to take a narrow zone of the layer for the purified group extract. On the other hand, acetic acid is an unsuitable component of the solvent as it contaminates the sample and thus affects electron-capture detection. As the gas chromatography of compounds I–III is difficult and the sensitivity of the electron-capture detector towards them is low, only the TLC of derivatives IV–VI with acid-free solvents (11, 13–19) can be considered in the clean-up. Tables I and II indicate that chloroform–diethyl ether–toluene (1:1:1) (solvent 16) is the best, and consequently it is recommended for the group separation (clean-up) of the pentafluorobenzyl derivatives IV–VI of MCPA and its metabolites. In our previous analyses, dichloromethane (solvent 11) was used[28] succesfully, which is confirmed by the present results through the low x values (Table II).

CONCLUSIONS

The herbicide MCPA can be analysed together with its metabolites 4-chloro-*o*-cresol and 3-methyl-5-chlorocatechol by TLC on silica gel with developing solvents containing acetic acid, or as their pentafluorobenzyl derivatives with neutral organic solvent mixtures. The best overall separation of all components is achieved with toluene–benzene–acetic acid (2:2:1). For group separation of the pentafluorobenzyl derivatives, chloroform–diethyl ether–toluene (1:1:1) is recommended.

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