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Note

Thin-layer chromatography of chlorinated catechols on a silica gel 60 layer

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Thin-layer chromatography (TLC) has been widely applied to phenolic compounds, and the TLC of chlorinated cresols[1], the soil metabolites of MCPA[2], chlorinated guaiacols[3,4] and chlorinated catechols[5] has been carried out previously in our laboratory. In these studies several solvents and solvent mixtures were screened in an attempt to select the most suitable solvent systems for particular separations. Sattar *et al.*[5] studied the TLC of catechol, 3,4-, 3,5- and 3,6-dichlorocatechols, 3,4,5-trichlorocatechol, tetrachlorocatechol and 5-chloro-3-methylcatechol on five different layer materials with 60 different solvent systems. It was found that pure silica gel and the solvent system benzene–ethanol–acetic acid (85:10:5) or benzene–acetone–acetic acid (80:15:5) are suitable for the separation (clean-up) of chlorinated catechols from other compounds. However, for the separation of the individual components an alumina layer has been recommended.

Chlorinated catechols are present in pulp bleachery effluents in the paper industry and thus occur as potent environmental residues[6–10]. As some polychlorinated catechols are known to be toxic to fish[11] and also bioaccumulate in fish[12], the present work was carried out in an attempt to synthesize four new chlorinated catechols, study the characteristic colour reactions of all chlorocatechols and determine their R_F values with several solvent systems. However, the main purpose was to calculate the standard deviations (s)[3,4] and the relative differences[2–4] in the R_F values in order to select the most suitable solvent systems for particular separations of catechol and all possible chlorinated catechols.

EXPERIMENTAL

Samples

The compounds studied were as follows (see Fig. 1): catechol (I), 3-chlorocatechol (II), 4-chlorocatechol (III), 3,4-dichlorocatechol (IV), 3,5-dichlorocatechol (V), 3,6-dichlorocatechol (VI), 4,5-dichlorocatechol (VII), 3,4,5-trichlorocatechol (VIII), 3,4,6-trichlorocatechol (IX) and tetrachlorocatechol (X). II–VI, VIII and IX were synthesized from the corresponding chlorinated salicylaldehydes by the method described by Dakin[13]. VII and X were prepared by direct chlorination of catechol (I) (commercial product, Fluka, Buchs, Switzerland) with SO_2Cl_2 in diethyl ether[14] and with chlorine in glacial acetic acid[15], respectively. The structures of II–X were confirm-

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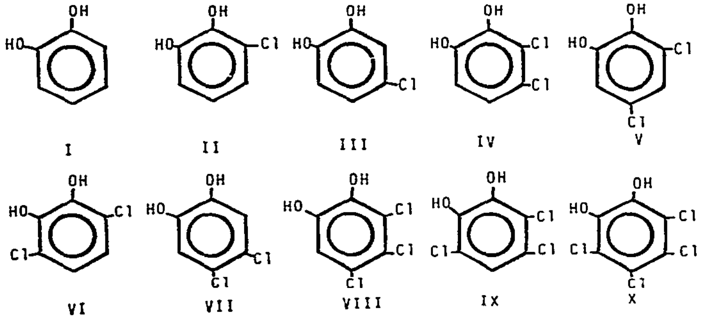

Fig. 1. Structures of catechol (I), 3-chlorocatechol (II), 4-chlorocatechol (III), 3,4-dichlorocatechol (IV), 3,5-dichlorocatechol (V), 3,6-dichlorocatechol (VI), 4,5-dichlorocatechol (VII), 3,4,5-trichlorocatechol (VIII), 3,4,6-trichlorocatechol (IX) and tetrachlorocatechol (X).

ed by ^{1}H nuclear magnetic resonance (NMR) and ^{13}C NMR spectroscopy[16]. The purities of the samples were checked by gas chromatography on an SE-30 glass capillary column (25 m × 0.3 mm I.D.) and flame-ionization detection after derivatization of the samples with ethereal diazoethane solution[6].

Thin-layer chromatography

Pre-coated plates with a silica gel 60 layer and a concentrating zone (20 × 20 cm, layer thickness 0.25 mm; Merck, Darmstadt, G.F.R.) were used. The samples (10 μg of each catechol) were applied on a line 1.5 cm from the bottom of the concentrating zones the first spot being 2 cm from the side of the plate and the following nine spots at 1.7-cm intervals. The other experimental conditions were as used earlier[3].

Solvent systems

Screening of 20 different solvents or solvent mixtures was carried out in order to find those which give the sharpest spots and the most reasonable R_F values. The compositions (by volume) of the ten most suitable solvent systems were as follows:

(1) Benzene–acetone–acetic acid (80:15:5)
(2) Light petroleum (b.p. 40–60°C)–acetone–acetic acid (70:25:5)
(3) Light petroleum (b.p. 40–60°C)–ethyl acetate–acetic acid (80:15:5)
(4) *n*-Hexane–ethyl acetate–acetic acid (70:25:5)
(5) Benzene–chloroform–acetic acid (60:30:10)
(6) Benzene–ethanol–acetic acid (85:10:5)
(7) Chloroform–ethyl acetate–acetic acid (80:15:5)
(8) Chloroform–diethyl ether–acetic acid (85:10:5)
(9) Benzene–acetic acid (85:15)
(10) Chloroform–acetic acid (80:20)

RESULTS AND DISCUSSION

Colour reactions

The colours of the spots were compared 1 h, 24 h and 10 days after spraying the plates with a chromogenic reagent. The colours of the spots are given in Table I.

When the plates with a concentrating zone and solvent mixtures containing

TABLE I

CHARACTERISTIC COLOUR REACTIONS OF CATECHOL (I) AND CHLORINATED CATECHOLS (II–X) DIFFERENT TIMES AFTER SPRAYING TLC PLATES WITH A 2% SOLUTION OF 3,5-DICHLORO-*p*-BENZOQUINONECHLORIMINE IN TOLUENE

Amount of each compound applied: 10 μg.

Compound	*Acidic developing solvent*		
	1 h	*24 h*	*10 days*
I	Red-violet	Brown	Grey-brown
II	Violet	Brown	Grey-brown
III	Red-violet	Brown	Grey-brown
IV	Grey-violet	Brown	Grey-brown
V	Grey-brown	Violet-brown	Grey-brown
VI	Violet-blue	Violet-black	Grey-brown
VII	Yellow-brown	Red-brown	Grey-brown
VIII	Pale green	Pale grey	Pale grey
IX	Pale green	Pale grey	Pale grey
X	Pale green	Pale grey	Pale grey

acetic acid were used, almost all of the compounds studied formed sharp spots. Only with a few solvent mixtures did the spots of trichlorocatechols (VIII and IX) and tetrachlorocatechol (X) show tailing.

One hour after spraying, the spots of II–VII had different colours but, for example after 10 h, their spots were brownish. On the other hand, the spots of VIII–X were much lighter than those of the other compounds studied and their colours changed from greenish to pale grey.

R_F values

The R_F values of the spots are given in Table II. The average R_F values ($\bar{R}_F$), the standard deviations (s) and the averages of the relative differences ($\bar{x}$), Σx values

TABLE II

R_F VALUES OF CATECHOL (I) AND CHLORINATED CATECHOLS (II–X) ON A SILICA GEL 60 LAYER WITH DIFFERENT SOLVENT SYSTEMS

Solvent system	*Compound*										*Development time (min)*
	I	*II*	*III*	*IV*	*V*	*VI*	*VII*	*VIII*	*IX*	*X*	
1	0.35	0.36	0.35	0.35	0.37	0.42	0.34	0.32	0.36	0.34	40
2	0.40	0.42	0.42	0.41	0.47	0.48	0.44	0.41	0.44	0.39	35
3	0.20	0.22	0.18	0.18	0.23	0.29	0.18	0.18	0.25	0.20	35
4	0.28	0.29	0.27	0.25	0.29	0.35	0.25	0.24	0.29	0.26	40
5	0.14	0.22	0.14	0.19	0.20	0.28	0.13	0.15	0.22	0.20	50
6	0.42	0.43	0.42	0.42	0.42	0.45	0.40	0.38	0.42	0.39	50
7	0.37	0.40	0.36	0.36	0.40	0.44	0.35	0.33	0.37	0.35	60
8	0.37	0.39	0.37	0.37	0.39	0.46	0.35	0.32	0.35	0.34	60
9	0.21	0.32	0.22	0.28	0.28	0.39	0.18	0.23	0.33	0.30	40
10	0.45	0.53	0.43	0.49	0.50	0.61	0.42	0.42	0.50	0.48	60

TABLE III

AVERAGE R_F VALUES ($\bar{R}_F$), STANDARD DEVIATIONS (s) OF THE R_F VALUES, THE SUMS (Σx) AND THE AVERAGES ($\bar{x}$) BETWEEN THE R_F VALUES AND THE NUMBER OF ZERO VALUES IN EACH x_{ij} MATRIX OF I–X ON A SILICA GEL 60 LAYER WITH DIFFERENT SOLVENT SYSTEMS

The value of each element, x, in x_{ij} matrixes is calculated by dividing the difference of two R_F values by their average.

Solvent system	$\bar{R}_F$	s	Σx	$\bar{x}$	*Number of* x_{ij} *(= 0.000)*
6	0.42	0.020	2.447	0.054	10
1	0.36	0.026	3.403	0.076	5
2	0.43	0.029	3.558	0.079	3
7	0.37	0.032	4.292	0.095	4
8	0.37	0.038	4.918	0.109	5
4	0.28	0.032	5.519	0.123	4
10	0.48	0.059	6.053	0.135	2
3	0.21	0.037	8.350	0.186	7
9	0.27	0.064	12.595	0.280	1
5	0.19	0.047	13.016	0.289	3

and the number of zero values in each x_{ij} matrix are presented in Table III.

With solvents 1, 2, 6 and 7 the variations in the R_F values in each run were small (small s, Σx and $\bar{x}$ values) (see Table III). With these solvent systems a relatively high elution power was also observed ($\bar{x}$ = 0.36–0.43) and thus they are suitable for the group separation of I–X. The smallest s (0.020), Σx (2.447) and $\bar{x}$ (0.054) and the highest number of the zero values in the x_{ij} matrixes (see Table III) were observed for benzene–ethanol–acetic acid (85:10:5) (solvent 6). Hence, this solvent mixture is the most suitable for the separation of this group from its mixtures with other compounds[5]. Benzene–acetone–acetic acid (80:15:5) (solvent 1) and light petroleum (b.p. 40–60°C)–acetone–acetic acid (70:25:5) (solvent 2) would also be useful for this purpose, although the number of zero values in the x_{ij} matrixes for these solvents are relatively small.

The highest variations in the R_F values were found for solvents 5 and 9 (see Table III). The Σx and $\bar{x}$ values of these systems are nearly as high but the absolute separation power is clearly different. Hence, benzene–acetic acid (85:15) (solvent 9), which gives the highest s value (0.064), can be recommended for the separation of the individual components.

None of the solvent systems studied is suitable for the separation of all of the individual components (I–X) by one-dimensional TLC.

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