THIN-LAYER CHROMATOGRAPHY OF CHLORINATED GUAIACOLS

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SUMMARY

The thin-layer chromatography of guaiacol and six chlorinated guaiacols has been studied on silica gel with 40 neutral and acidic solvent systems. Standard deviations and relative differences in the R_F values were used for selecting the most suitable solvents for particular separations. For group separation, dichloromethane–benzene–methanol (60:30:10) and acetone were suitable. Light petroleum (b.p. 40–60 °C)–ethyl acetate (70:30) and dichloromethane–chloroform (90:10) separated all components. Some other solvents are recommended for two-dimensional analyses.

INTRODUCTION

Thin-layer chromatography (TLC) is frequently applied for the separation of phenols. Leach and Thakore[1] used preparative TLC on silica gel for the isolation of 4,5,6-trichloroguaiacol and tetrachloroguaiacol from waste liquor from pulp bleaching. The eluents used were light petroleum (b.p. 30–60 °C)–benzene–methanol and benzene–methanol–acetic acid (50:8:4) for group separation and for the separation of the individual components, respectively. Thakore and Oehlschlager[2] separated 3,4,5-trichloroguaiacol, 4,5,6-trichloroguaiacol and tetrachloroguaiacol by TLC with chloroform–light petroleum (9:1). Chloroform[3] and different mixtures of chloroform and ethyl acetate[4] were used in separations of various chlorophenolic compounds. An advanced TLC system for the analysis of 126 different phenols has been presented[5]. In addition, alumina layers have also been applied in the TLC of a large number of *ortho*- and *para*-substituted derivatives of phenol.[6]

Chlorinated guaiacols are formed in pulp bleaching and thus occur as important environmental residues[7]. As they have been found to be extremely toxic to fish[1,8], accumulating[9] and being enriched in natural food chains[10], we have undertaken syntheses of model compounds, structural determinations and the development of analytical methods. Previous work on the TLC of chlorinated cresols[11] and catechols[12] provided a starting point for the present study.

EXPERIMENTAL

Apparatus

Pre-coated TLC plates with a silica gel G60 layer and a concentrating zone

(10 × 20 cm, layer thickness 0.25 mm; Merck, Darmstadt, G.F.R.) were used. Each guaiacol, as a 0.5% (w/v) solution in diethyl ether, was spotted with a 10-μl Hamilton syringe, 2 μl to each spot, on a line 1.5 cm from the bottom of the plate to the concentrating zone with spot intervals of 1.2 cm. Ascending elution in a closed glass chamber (Desaga, Heidelberg, G.F.R.) was applied. Both a Desaga scale plate and a meter scale were used to measure the R_F values of the spots.

Samples

The compounds used (see Fig. 1) were guaiacol (I), 5-chloroguaiacol (II), 4,5-dichloroguaiacol (III), 4,6-dichloroguaiacol (IV), 3,5-dichloroguaiacol (V), 4,5,6-trichloroguaiacol (VI) and tetrachloroguaiacol (VII). Except for guaiacol, which was a commercial sample (Fluka, Buchs, Switzerland), the compounds were synthesized in our laboratory and their structures and purities were checked by infrared, mass, ^{1}H NMR and ^{13}C NMR spectroscopy and by glass capillary gas chromatography.
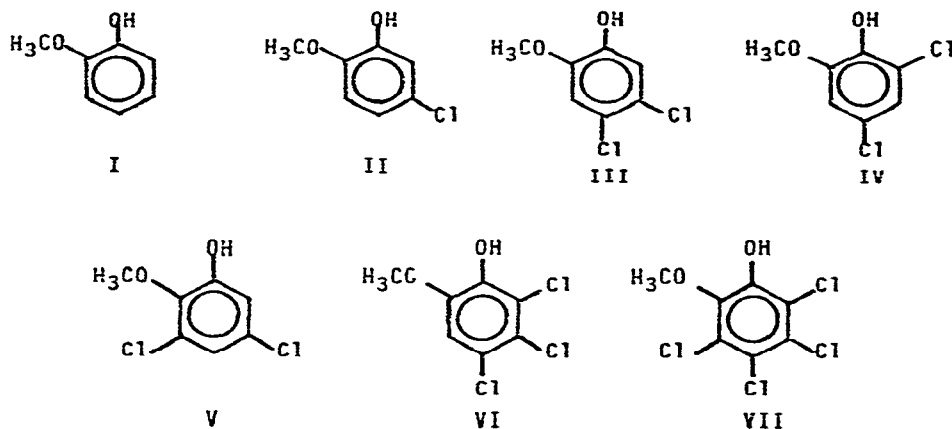

Fig. 1. Structures of guaiacol (I), 5-chloroguaiacol (II), 4,5-dichloroguaiacol (III), 4,6-dichloroguaiacol (IV), 3,5-dichloroguaiacol (V), 4,5,6-trichloroguaiacol (VI) and tetrachloroguaiacol (VII).

Solvent systems

Forty different solvent systems were examined in order to establish which gave the best spots and the most reasonable R_F values with all of the compounds studied. Owing to the use of a concentrating zone the spots were good (narrow) in all instances. The compositions (by volume) of the solvent systems were as follows:

(1) Light petroleum (b.p. 40–60 °C).
(2) Benzene.
(3) Dichloromethane.
(4) Chloroform.
(5) Diethyl ether.
(6) Ethyl acetate.
(7) Acetone
(8) n-Propanol.
(9) Light petroleum (b.p. 40–60 °C)–diethyl ether (70:30)
(10) Light petroleum (b.p. 40–60 °C)–ethyl acetate (70:30).
(11) Light petroleum (b.p. 40–60 °C)–acetone (80:20).
(12) Light petroleum (b.p. 40–60 °C)–n-propanol (90:10).

(13) Dichloromethane–chloroform (90:10).
(14) Dichloromethane–diethyl ether (95:5).
(15) Dichloromethane–ethyl acetate (95:5).
(16) Dichloromethane–acetone (95:5).
(17) Dichloromethane–n-propanol (95:5).
(18) Chloroform–dichloromethane (80:20).
(19) Chloroform–diethyl ether (90:10).
(20) Chloroform–ethyl acetate (95:5).
(21) Chloroform–acetone (95:5).
(22) Chloroform–n-propanol (95:5).
(23) Dichloromethane–chloroform–diethyl ether (85:10:5).
(24) Dichloromethane–benzene–methanol (60:30:10).
(25) Light petroleum (b.p. 40–60 °C)–dichloromethane–ethyl acetate (60:30:10).
(26) Light petroleum (b.p. 40–60 °C)–benzene–n-propanol (40:40:20).
(27) Benzene–acetic acid (85:15).
(28) Benzene–dichloromethane–acetic acid (60:30:10).
(29) Benzene–chloroform–acetic acid (50:40:10).
(30) Benzene–diethyl ether–acetic acid (60:40:10).
(31) Benzene–ethyl acetate–acetic acid (80:15:5).
(32) Benzene–acetone–acetic acid (80:15:5).
(33) Benzene–n-propanol–acetic acid (85:15:5).
(34) Light petroleum (b.p. 40–60 °C)–diethyl ether–acetic acid (80:15:5).
(35) Light petroleum (b.p. 40–40 °C)–ethyl acetate–acetic acid (80:15:5).
(36) Light petroleum (b.p. 40–60 °C)–acetone–acetic acid (80:15:5).
(37) Light petroleum (b.p. 40–60 °C)–n-propanol–acetic acid (80:15:5).
(38) Dichloromethane–ethyl acetate–acetic acid (80:15:5).
(39) Chloroform–ethyl acetate–acetic acid (80:15:5).
(40) Chloroform–acetone–acetic acid (80:15:5).

Chromogenic reagents

A 2% solution of 3,5-dichloro-p-benzoquinonechlorimine in toluene[13] and different concentrations (1–5%) of $FeCl_3 \cdot 6H_2O$ in water were tested for spot detection in order to obtain the most specific colour reaction for each compound studied.

Development of chromatograms

Development was continued until the solvent front had moved 13 cm from the boundary between the concentrating section and the silica gel section of the layer. After development the plates were dried in air at room temperature (24 ± 2 °C) for about 15 min and then sprayed with the chromogenic reagent.

RESULTS AND DISCUSSION

Colour reactions

$FeCl_3$ reagent gave light violet spots for compounds I–VII 1 h after spraying. After 2–3 days the spots changed colour to grey-green or grey-violet.

3,5-Dichloro-p-benzoquinonechlorimine reagent gave more specific colour reactions. The colours of the spots were compared 1 h, 24 h and 10 days after spraying.

The developing solvent influenced the colour reaction only on the basis of its acidity. On the other hand, the influence of time on the colours was substantial. The colour reactions are presented in Table I.

TABLE I

CHARACTERISTIC COLOUR REACTIONS OF GUAIACOL (I) AND CHLORINATED GUAIACOLS (II–VII) IN 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	Neutral developing solvent			Acidic developing solvent		
	1 h	24 h	10 days	1 h	24 h	10 days
I	Violet-brown	Red-brown	Brown	Orange-brown	Brown	Brown
II	Violet-blue	Red-brown	Brown	Red-orange	Brown	Brown
III	Orange-brown	Violet-blue	Red-brown	Orange-brown	Violet-brown	Violet-brown
IV	Grey-green	Red-brown	Brown	Orange-brown	Orange-brown	Orange-brown
V	Violet	Violet	Violet	Light yellow	Light violet	Violet
VI	Grey-green	Red-brown	Red-brown	Orange-brown	Violet-brown	Violet-brown
VII	Light orange	Grey-violet	Light violet	Light yellow	Light brown	Grey-violet

The colour reactions of the 3-chloro-substituted guaiacols were clearly different than those of the others. Firstly, V and VII gave much slower colour reactions and their final colours after 10 days were violet-based, whereas those of the other compounds were brownish.

R_F values

The R_F values of the spots were measured with an accuracy of better than 0.03. To achieve this, most runs had to be carried out three times and average values calculated. The results obtained with neutral (1–26) and acidic (27–40) solvent systems are given in Table II.

The standard deviations of the R_F values (s) of I–VII in each run were calculated for estimation of the separating power of each solvent system (see Table II). Large s values correspond to possible solvents for the analysis of individual components and small s values to solvents suitable for group separation.

Further evaluation of the separation in each experiment was effected by comparing the relative differences (x) of the R_F values as presented by Sattar and Paasivirta[14]:

$$x_{ij} = \frac{R_F(i) - R_F(j)}{R_F(i) + R_F(j)} \cdot 2 \qquad (1)$$

which is the same as the difference between two R_F values divided by their average. From each experiment with seven compounds (each TLC run), 21 different x values were obtained. The results for six solvent systems are presented in Table III.

The averages and sums of the x values ($\bar{x}$ and Σx) for each run were also calculated. These are measures of the relative separating powers of the solvent

TABLE II

R_F VALUES OF GUAIACOL (I) AND CHLORINATED GUAIACOLS (II–VII) ON A SILICA GEL G60 LAYER WITH DIFFERENT SOLVENT SYSTEMS

Solvent system	Compound							Standard deviation of R_F	Development time (min)
	I	II	III	IV	V	VI	VII		
1	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.000	30
2	0.16	0.19	0.19	0.21	0.12	0.20	0.15	0.032	40
3	0.31	0.35	0.37	0.39	0.27	0.39	0.33	0.045	30
4	0.41	0.41	0.38	0.43	0.30	0.40	0.33	0.048	35
5	0.62	0.60	0.56	0.60	0.65	0.53	0.58	0.039	30
6	0.60	0.58	0.57	0.58	0.61	0.54	0.55	0.025	35
7	0.62	0.62	0.62	0.63	0.63	0.61	0.57	0.021	25
8	0.59	0.58	0.59	0.58	0.62	0.59	0.60	0.014	130
9	0.31	0.23	0.18	0.23	0.31	0.14	0.25	0.062	35
10	0.43	0.38	0.34	0.37	0.45	0.29	0.39	0.054	35
11	0.40	0.38	0.37	0.39	0.45	0.35	0.42	0.034	35
12	0.59	0.53	0.50	0.52	0.62	0.47	0.60	0.057	35
13	0.36	0.38	0.41	0.42	0.29	0.44	0.34	0.052	30
14	0.55	0.58	0.58	0.60	0.46	0.62	0.53	0.053	40
15	0.56	0.55	0.56	0.59	0.49	0.58	0.53	0.033	40
16	0.53	0.53	0.52	0.54	0.46	0.52	0.48	0.030	40
17	0.61	0.61	0.60	0.62	0.55	0.62	0.58	0.075	40
18	0.44	0.43	0.42	0.46	0.34	0.44	0.39	0.040	40
19	0.48	0.48	0.45	0.48	0.42	0.46	0.45	0.022	40
20	0.55	0.55	0.51	0.55	0.45	0.51	0.48	0.039	45
21	0.48	0.51	0.48	0.51	0.45	0.48	0.48	0.021	40
22	0.64	0.61	0.57	0.62	0.55	0.58	0.57	0.032	45
23	0.50	0.49	0.48	0.51	0.40	0.52	0.46	0.040	30
24	0.60	0.60	0.57	0.60	0.56	0.58	0.60	0.017	30
25	0.44	0.40	0.36	0.45	0.46	0.34	0.39	0.046	35
26	0.81	0.83	0.85	0.85	0.89	0.83	0.87	0.027	80
27	0.51	0.51	0.49	0.52	0.48	0.51	0.51	0.015	40
28	0.50	0.50	0.49	0.52	0.48	0.50	0.50	0.012	35
29	0.48	0.48	0.48	0.50	0.47	0.49	0.51	0.013	35
30	0.66	0.65	0.65	0.67	0.67	0.65	0.65	0.014	40
31	0.48	0.48	0.45	0.51	0.51	0.45	0.52	0.029	40
32	0.48	0.47	0.45	0.50	0.50	0.45	0.52	0.027	35
33	0.60	0.61	0.58	0.62	0.61	0.59	0.63	0.018	40
34	0.27	0.32	0.31	0.32	0.37	0.28	0.40	0.047	40
35	0.38	0.35	0.33	0.35	0.42	0.33	0.43	0.041	30
36	0.45	0.42	0.42	0.43	0.46	0.42	0.49	0.028	55
37	0.81	0.79	0.80	0.83	0.90	0.79	0.90	0.044	80
38	0.75	0.75	0.73	0.77	0.75	0.75	0.76	0.012	45
39	0.65	0.65	0.63	0.66	0.66	0.64	0.68	0.016	45
40	0.68	0.68	0.68	0.70	0.70	0.68	0.72	0.016	45

systems, whereas the s values give a measure of absolute separation in each experiment. All three values are useful in screening solvents for analysis or group separation purposes. More detailed information for the separation of the components is obtained from the x_{ij} matrixes (examples in Table 3) in which all x values must be other than zero for complete separation to be expected in one-dimensional elution.

The order of the R_F values of different compounds depends on their polarities

TABLE III

RELATIVE DIFFERENCES, x, BETWEEN R_F VALUES OF I–VII ON SILICA GEL G60 WITH SELECTED SOLVENT SYSTEMS

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

Solvent system	x							Average ($\bar{x}$)	Sum (Σx)
	—	II	III	IV	V	VI	VII		
2	I	0.171	0.171	0.270	0.286	0.222	0.065	0.228	4.797
	II		0.000	0.100	0.452	0.051	0.235		
	III			0.100	0.452	0.051	0.235		
	IV				0.545	0.049	0.333		
	V					0.500	0.222		
	VI						0.286		
3	I	0.121	0.176	0.229	0.138	0.229	0.063	0.159	3.337
	II		0.056	0.108	0.258	0.108	0.059		
	III			0.053	0.313	0.053	0.114		
	IV				0.364	0.000	0.167		
	V					0.364	0.200		
	VI						0.167		
8	I	0.017	0.000	0.017	0.050	0.000	0.017	0.026	0.536
	II		0.017	0.000	0.067	0.017	0.034		
	III			0.017	0.050	0.000	0.017		
	IV				0.067	0.017	0.034		
	V					0.050	0.033		
	VI						0.017		
9	I	0.296	0.531	0.296	0.000	0.756	0.214	0.331	6.953
	II		0.244	0.000	0.296	0.486	0.083		
	III			0.244	0.531	0.250	0.326		
	IV				0.296	0.486	0.083		
	V					0.756	0.214		
	VI						0.564		
10	I	0.123	0.234	0.150	0.045	0.389	0.098	0.174	3.659
	II		0.111	0.027	0.169	0.269	0.026		
	III			0.085	0.278	0.159	0.137		
	IV				0.195	0.242	0.053		
	V					0.432	0.143		
	VI						0.294		
13	I	0.054	0.130	0.154	0.215	0.200	0.057	0.171	3.586
	II		0.076	0.100	0.269	0.146	0.111		
	III			0.024	0.343	0.071	0.187		
	IV				0.366	0.047	0.211		
	V					0.411	0.159		
	VI						0.256		

and the polarity of the solvent system. This gives additional structural verification of these guaiacol derivatives. For example, a change from the non-polar chloroform (4) to the polar diethyl ether (5) reverses the order of elution of compounds IV, V and VII with different polarities (see Table II).

CONCLUSIONS

Solvent system 8 (n-propanol) gives almost identical but reasonably large R_F values (0.58–0.62) and the smallest values of s, $\bar{x}$ and Σx. The largest value of x

was only 0.067. Thus n-propanol could be the solvent of choice for the group separation of chlorinated guaiacols. However, the elution time is very long (130 min). Consequently, we recommend the use of dichloromethane–benzene–methanol (60:30:10) (system 24) or acetone (system 7) for the above purpose; the different separation values are almost as low and the elution times are reasoanbly short (see Table II). Small separation values were also obtained for acetic acid-containing solvents, but they cannot be used for analytical clean-up as the acid residues perturb the subsequent derivatization step in the analysis.

The solvent systems light petroleum (b.p. 40–60 °C)–ethyl acetate (70:30) (system 10) and dichloromethane–chloroform (90:10) (system 13) give x_{ij} values different from zero (see Table III) and high s, $\bar{x}$ and Σx values. Hence these solvents are recommended for the separation of the chloroguaiacols by one-dimensional TLC.

The highest overall separation power was observed for light petroleum (b.p. 40-60 °C)–diethyl ether (70:30) (system 9) (see Table III). However, two x values were zero. Hence we conclude that this solvent could be used only as the first stage in a two-dimensional TLC procedure in which the second stage is used to separate the remaining components. Such a second stage could be carried out with benzene (system 2), as from the x_{ij} matrix (Table III) the values corresponding to the zero values with solvent 9 are reasonably large.

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