

## Separation of chlorinated cresols and chlorinated xylenols by thin-layer chromatography

The separation of tar acids by thin-layer chromatography (TLC) has been described recently<sup>1-3</sup>. This paper describes the separation of chlorocresols and chloroxylenols by TLC and its usefulness in following the progress of chlorination of these tar acids. The paper chromatographic separation of these compounds has been reported earlier<sup>4</sup>.

### Materials and methods

The chlorocresols and chloroxylenols used for the separations were obtained from commercial sources<sup>5</sup>.

Using a thin-layer applicator (Desaga, Heidelberg), glass plates (20 × 20 cm) were coated as usual with a well stirred suspension of silica gel G (E. Merck, Darmstadt; 30 g in 60 ml water) to give a layer approximately 270  $\mu$  in thickness. The plates were dried at 105–110° for 30 min and preserved in a desiccator. The compounds were dissolved in acetone and about 10  $\mu$ g was applied with a glass capillary on starting points 2–3 cm from the edge of the plate. The plate was then placed inside the chamber for development. Three solvent systems, A, B and C, were used:

(A) Petroleum ether (80–100°) saturated with formic acid.

(B) Xylene saturated with formamide.

(C) The organic layer from a mixture of benzene–acetic acid–water (2:2:1, v/v).

About 40–60 min were taken for the solvent front to cover a distance of 15 cm. The plates were then sprayed with phosphotungstomolybdic acid (Folin-Denis reagent) and exposed to ammonia, which revealed the compounds as blue spots on a white background.

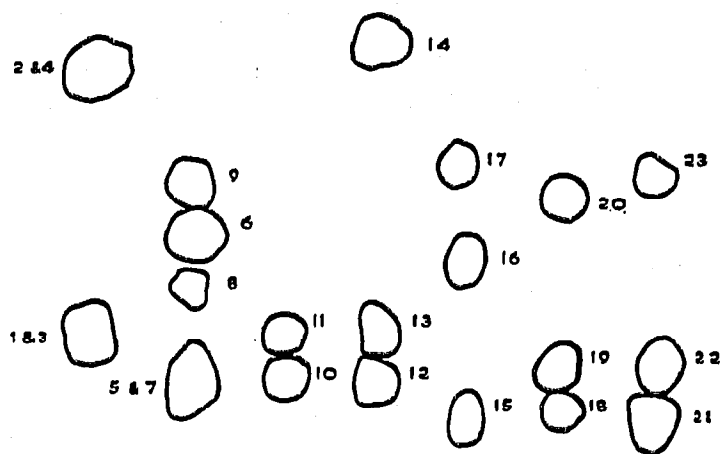
### Results and discussion

Table I lists the  $R_F$  values of the chlorocresols and chloroxylenols in the three solvent systems, and gives the composition of the mixtures spotted. Fig. 1 shows the separation of these mixtures of chlorocresols and chloroxylenols using solvent B, which gave the best separations. Resolution of *p*-chlorocresols from parent cresols and of 6-chloro-2-methylphenol from 4,6-dichloro-2-methylphenol could not be achieved with any of the solvent systems tried.

Generally the  $R_F$  values increased with increasing substitution in the nucleus, an exception being 4,6-dichloro-3-methylphenol in solvent system B. The  $R_F$  values for *o*-substituted compounds were higher than for the corresponding *m*-isomer, probably due to the *ortho* effect<sup>6</sup>. Thus  $R_F \times 100$  values in solvent B were: *m*-cresol = 26, *o*-cresol = 32, 4-chloro-3-methylphenol = 25, 6-chloro-2-methylphenol = 72, 3,4-dimethylphenol = 21, and 6-chloro-3,4-dimethylphenol = 44. The effect was more pronounced in the 2,6-disubstituted products, as seen in the  $R_F \times 100$  values for: 4-chloro-2-methylphenol = 31, 6-chloro-2-methylphenol = 72, 6-chloro-3,4-dimethylphenol = 44, and 2,6-dichloro-3,4-dimethylphenol = 58. In disubstituted products this relative order was followed in all the solvent systems.

The technique was tried in following the progress of chlorination of 2,5-dimethylphenol in carbon tetrachloride with gaseous chlorine at  $20 \pm 2^\circ$ . Samples withdrawn every 30 min were spotted. Fig. 2 reveals that 4-chloro-2,5-dimethylphenol is the

SOLVENT FRONT

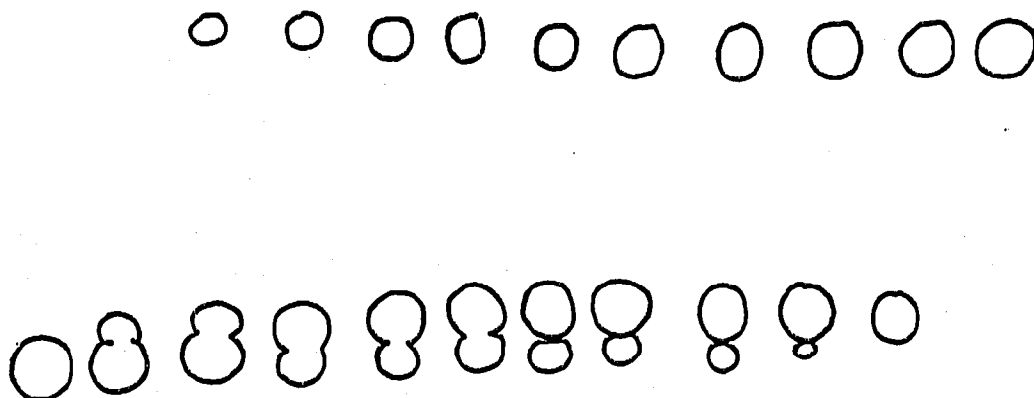


STARTING LINE

a      b      c      d      e      f      g

Fig. 1. Separation of chlorocresol and chloroxylenol mixtures. For the constituents of the mixtures a-g see Table I.

SOLVENT FRONT



STARTING LINE

1      2      3      4      5      6      7      8      9      10      11      12

Fig. 2. Separation of chlorinated 2,5-xyleneol samples withdrawn every 30 min.

TABLE I

 $R_F$  VALUES OF DIFFERENT CHLORINATED CRESOLS AND CHLORINATED XYLENOLSTemperature of chromatography:  $25 \pm 3^\circ$ .

Time of run for all solvent systems: 40-60 min.

Sl. No.	Compounds	$R_F$ values $\times 100$ in various solvent systems			Phenol mixtures	
		A	B	C	Sl. Nos.	Code in Fig. 1
1	2-Methylphenol	11	32	67	1 to 4	a
2	6-Chloro-2-methylphenol	58	72	89		
3	4-Chloro-2-methylphenol	06	31	65		
4	4,6-Dichloro-2-methylphenol	39	74	89		
5	3-Methylphenol	09	26	63	5 to 9	b
6	6-Chloro-3-methylphenol	55	47	89		
7	4-Chloro-3-methylphenol	12	25	63		
8	4,6-Dichloro-3-methylphenol	55	40	83		
9	2,4,6-Trichloro-3-methylphenol	66	56	89	10 and 11	c
10	2,3-Dimethylphenol	12	26	71		
11	4-Chloro-2,3-dimethylphenol	12	31	71	12 to 14	d
12	2,5-Dimethylphenol	10	25	71		
13	4-Chloro-2,5-dimethylphenol	10	31	71		
14	4,6-Dichloro-2,5-dimethylphenol	59	76	89		
15	3,4-Dimethylphenol	09	21	71	15 to 17	e
16	6-Chloro-3,4-dimethylphenol	43	44	88		
17	2,6-Dichloro-3,4-dimethylphenol	49	58	91		
18	3,5-Dimethylphenol	05	22	65	18 to 20	f
19	4-Chloro-3,5-dimethylphenol	22	28	65		
20	2,4-Dichloro-3,5-dimethylphenol	35	53	85		
21	5-Ethyl-3-methylphenol	04	22	66		
22	4-Chloro-5-ethyl-3-methylphenol	04	27	66		
23	2,4-Dichloro-5-ethyl-3-methylphenol	35	56	84		

primary product of reaction. The dichloro (4,6) product appears only after one hour of chlorination. After 5 h, 2,5-dimethylphenol was totally converted to the mono- and dichloro-derivative. Half an hour later the monochloro-2,5-dimethylphenol was completely eliminated and only the dichloro-derivative was present.

### Conclusions

In this study on the separation of chlorocresols and chloroxylenols by thin-layer

chromatography xylene saturated with formamide appeared to be the best solvent system. Resolution of *p*-chlorocresols from the parent cresols and of 6-chloro-2-methylphenol from 4,6-dichloro-2-methylphenol could not be achieved.

The application of TLC technique as an analytical tool in following the extent of chlorination is suggested.

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### **Separation of sterols and corresponding stanols on thin layers of silica impregnated with silver nitrate**

In natural materials, such sterols as cholesterol and  $\beta$ -sitosterol very often occur as inseparable mixtures with the corresponding stanols. IKAN AND KASHMAN<sup>1</sup> have found a mixture of  $\beta$ -sitosterol and  $\beta$ -sitostanol in Israeli peat. Similar observations have been made by McLEAN, RETTIE AND SPRING<sup>2</sup> with Scottish peat and by IVES AND O'NEILL<sup>3</sup> with Canadian peat moss.

In a previous communication<sup>4</sup> we have shown that by bromination of such mixtures, the unchanged stanols were easily separated on thin layers from the brominated sterols. In the present study the method applied by AVIGAN, DE GOODMAN AND STEINBERG<sup>5</sup> and MORRIS<sup>6</sup> for the fractionation of sterols, and by IKAN<sup>7</sup> for the separation of tetracyclic triterpenes on thin layers of silica impregnated with silver nitrate has been extended to include sterol-stanol mixtures. The sterols and the corresponding stanols had been shown to have practically the same  $R_F$  values on thin layers of silica gel G. However, on silica gel G impregnated with silver nitrate, the  $R_F$  values of the sterols were sufficiently different from the stanols. The  $R_F$  values and the colours obtained by spraying with 50 % sulfuric acid are summarized in Table I.

The following mixtures were separated: campesterol-campestanol, cholesterol-cholestanol, cholesterol-desmosterol, allocholesterol-cholestanol, lanosterol-dihydro-lanosterol, agnosterol-dihydroagnosterol,  $\beta$ -sitosterol- $\beta$ -sitostanol, stigmasterol-stigmastanol.

#### *Experimental*

*Preparation of plates.* The suspension for five plates (20 × 20 cm) was prepared by shaking 30 g of silica gel and 60 ml of water for 30 sec and applied uniformly to a thickness of 0.25 mm with a Desaga applicator. After 30 min at room temperature, the plates were heated in an oven at 125-130° for 45 min. After cooling they were

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