Detection and thin-layer chromatography of isomeric chlorophenols and their derivatives

I. N-Trichloroacetyl carbamates

Isomeric chlorophenols and their esters have exhibited a broad range of utility as nematicides[1-3], germicides[4], herbicides[5-6], growth regulators[7-8], and insecticides[9].

The analysis of chlorophenols has been accomplished by procedures such as infrared[10-11] and ultraviolet[12] spectrophotometry, non-aqueous titrations[13], potentiometric titrations[14] as well as a number of chromatographic techniques that include ion-exchange[15], gas–liquid[16-19], paper[20-23], pH-paper[24], and silica-gel column chromatography[25].

The purpose of this investigation was to determine the utility of pi-electron acceptors for the detection of isomeric chlorophenols and their derivatives, e.g. N-trichloroacetyl carbamates on thin-layer chromatograms and concomitantly elaborate the effect of structure on their chromatographic behavior in several solvent systems.

Experimental

Thin-layer chromatography. The chromatoplates, silicic acid layers, were prepared according to the method of MORLEY AND CHIBA[26]. Silica-gel DF-5* was applied on 8 × 8 in. plates to a thickness of 280 μ. After air-drying, the plates were activated in an oven for 30 min. Acetone solutions (1–2 μl containing 1–10 $\mu g/\mu l$) of test substance were applied along a line 2.5 cm from the lower end of the plate and developed by the ascending method. After evaporation of the solvent, the spots were located on the plate by U.V. detection, then sprayed with one of the chromogenic reagents and the initial color development as well as subsequent color changes noted.

The sprayed plates were then exposed briefly to ammonia vapors with the results described in Table I.

The developing solvent systems utilized in this work were:

A Chloroform–acetic acid (5:1)
B Benzene–acetic acid (5:1)
C 2.5 % acetone in benzene
D Iso-amyl alcohol–ammonia–water (30:15:5)[21].

Detecting reagents. (1) DDQ reagent[27], 2 % 2,3-dichloro-5,6-dicyano-1,4-benzoquinoneimine in benzene. (2) TCNE reagent[27], 2 % tetracyanoethylene in benzene. (3) Chloranil, 1 % tetrachloro-*p*-benzoquinone in benzene. (4) Gibbs reagent, 2 % 2,6-dibromo-N-chloro-*p*-benzoquinoneimine in benzene.

Materials. The isomeric chlorophenols (compounds 1–13) were obtained from Aldrich Chemical Co., Milwaukee, Wisc., U.S.A. The N-(trichloroacetyl) carbamates (compounds 14–49) were prepared via the reaction of trichloroacetyl isocyanate with the appropriate alcohol or chlorophenol and recrystallized from petroleum ether (30–60°). Trichloroacetyl isocyanate was obtained from Distillation Industries, Rochester, N.Y., U.S.A.

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* Obtained from Camag, Muttenz, Switzerland.

TABLE I

R_F VALUES × 100 AND SPOT COLORS OF ISOMERIC CHLOROPHENOLS ON SILICA-GEL DF-5

(A) After detector application; (B) after detector application and exposure to ammonia vapors.
Designation of colors developed at room temperature: B = blue; Bg = beige; Bn = brown; C = crimson; Cr = cream; G = green; L = lilac;
O = orange; P = purple; Pk = pink; R = rose; T = tan; V = violet; Y = yellow.

No.	Compound	(A) Before NH₃ exposure				(B) After NH₃ exposure				Solvent systems			
		1	2	3	4	1	2	3	4	A	B	C	D
1	o-Chlorophenol	L	O-C	Y-O	O	Pk/B*	R	B-V	O	72	63	58	69
2	m-Chlorophenol	Cr	O	Y	O	B-L/B*	Pk→L	V	Y-O	65	59	53	65
3	p-Chlorophenol	B	O-C →Bn	O	O-C	Y-G/Y-G*	O→YG	B-G	O	62	56	48	63
4	2,3-Dichlorophenol	O	O	Y	Y	Y-G/B*	Y-G	B-V	Y-G →O	74	62	52	66
5	2,4-Dichlorophenol	B	Pk	Y	Pk-O	Y/B-G*	Bg	B	Y-G →O	74	60	50	64
6	2,5-Dichlorophenol	O-C	Y-O	Y-T	Y-O	B/B*	B	B-V	G-B →O	71	56	46	61
7	2,6-Dichlorophenol	T	O	T	Y	B-G/B*	R-O	B	G-B →O	68	51	42	57
8	3,4-Dichlorophenol	B-Bk	O-C	O-C	O-C	T/B*	Y	V	O	62	49	43	54
9	3,5-Dichlorophenol	O-P	O	Y	Y-O	Bg/B*	O	V	O	65	50	44	59
10	2,4,5-Trichlorophenol	B-Gr	O-Bn	T	O	Gr-G	B-G	B	V-G →O	72	57	48	60
11	2,4,6-Trichlorophenol	T-P	G	Y	Cr	B-Gr	Bn-G →Bg	B	B→T	76	60	52	64
12	2,3,4,6-Tetrachlorophenol	T-P	G	Y	Cr	B-Gr	Bn-G →Bg	B	B→Bg	67	53	47	58
13	Pentachlorophenol	O	G	Y	Y	G-T	Bn-G →Bg	Bg	B→Bg	64	50	45	55

* Fluorescence after spraying.

Results and discussion

Table I depicts the spot colors (as well as the R_F values) of the isomeric chlorophenols on silica-gel DF-5 plates obtained with four detecting reagents before and after exposure to ammonia vapors. Tables II and III depict the R_F values of the N-(trichloracetyl) carbamates of isomeric chlorophenols and miscellaneous alkyl and aryl derivatives, respectively.

A number of salient observations can be made in regard to the chromogenic behavior of the isomeric chlorophenols as well as the overall utility of pi-electron detectors employed:

1. The monochlorophenols can be distinguished chromogenically from one another utilizing either DDQ or TCNE reagents with the former being the detector of choice. Differentiation of the above isomers can also be effected utilizing detectors 1, 2 or 3 followed by brief exposure of the plate to ammonia vapors as illustrated in Table I.

2. The isomeric dichlorophenols, *viz.*, 2,3, 2,4, 2,5, 2,6, 3,4 and 3,5 can be distinguished from one another utilizing preferentially the DDQ reagent or secondarily the TCNE reagent followed by subsequent exposure of the sprayed plate to ammonia vapors.

3. The isomeric trichlorophenols, *viz.*, 2,4,5 and 2,4,6 can be differentiated utilizing any of the detecting reagents 1–4 with reagents 1 or 2 preferred.

4. The mono- and dichlorophenols can further be distinguished from the tri-, tetra- and penta-chlorophenols tested utilizing the DDQ reagent followed by exposure of the chromatogram to ammonia vapors. Only the mono- and di-derivatives yield fluorescent spots (see Table I).

5. Overall, in order of decreasing utility for the detection and differentiation of the isomeric chlorophenols (compounds 1–13): DDQ, TCNE > Chloranil > Gibbs.

The utility of the strongest pi-electron acceptors screened in this study, *viz.* DDQ and TCNE (oxidative potential *ca.* 1.0 V) has been previously demonstrated for the detection of 3,4-methylenedioxyphenyl derivatives[27], sulfoxides, sulfones and sulfides[28] and aromatic hydrocarbons[29].

Correlation of R_F values with structure. In addition to the solvents enumerated in Table I (solvent systems A–D), the following solvent systems were screened and rejected because of their less overall utility in effecting separations of at least 0.03 with the majority of the chlorophenols studied:

chloroform–acetic acid–water (4:1:1)
chloroform–acetone–acetic acid (48:2:1)
benzene–acetic acid–water (125:72:3).

1. The isomeric monochlorophenols have been best separated in this study on silica-gel DF-5 plates utilizing solvent C (2.5 % acetone–benzene). The order of R_F values for the monochlorophenols in all the solvent systems tested was $o > m > p$. This same order of separation for the isomeric monochlorophenols has been observed on paper[30] and absorption silica-gel chromatography[31].

2. Separation of isomeric dichlorophenols has best been accomplished utilizing solvent A (chloroform–acetic acid, 5:1). For both solvent systems A and D the following R_F relationship of the above isomers prevails: 2,4 > 2,5 > 3,5 > 3,4. It has not been found possible in this study to effect the separation of 2,3- from 2,4-dichlorophenol with any of the solvent systems employed.

TABLE II

R_F VALUES × 100 OF N-(TRICHLOROACETYL) CARBAMATES OF ISOMERIC CHLOROPHENOLS

No.	R	m.p. (°C)	Solvents			
			A	B	C	D
14	o-Chlorophenyl	94– 95	73	58	54	64
15	m-Chlorophenyl	92– 93	68	52	50	58
16	p-Chlorophenyl	105–106	65	49	46	53
17	2,3-Dichlorophenyl	84– 85	71	54	52	60
18	2,4-Dichlorophenyl	49– 50	69	53	52	58
19	2,5-Dichlorophenyl	95– 97	65	50	49	53
20	2,6-Dichlorophenyl	81– 82	61	46	44	50
21	3,4-Dichlorophenyl	55– 56	54	42	40	46
22	3,5-Dichlorophenyl	123–124	57	44	39	48
23	2,4,5-Trichlorophenyl	55– 56	70	55	56	62
24	2,4,6-Trichlorophenyl	98– 99	75	60	62	66
25	2,3,4,6-Tetrachlorophenyl	58– 59	78	64	67	71
26	Pentachlorophenyl	122–123	74	60	65	68

TABLE III

R_F VALUES × 100 OF N-(TRICHLOROACETYL) CARBAMATES

$$Cl_3—C—C—N—C—O—R$$
$$\qquad \overset{\|}{O} \ \overset{|}{H} \ \overset{\|}{O}$$
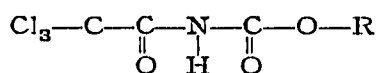
No.	R	m.p. (°C)	Solvents			
			A	B	C	D
27	Methyl	100–101	57	41	37	48
28	Ethyl	57– 58	60	46	42	52
29	n-Propyl	44– 45	64	49	47	55
30	Isopropyl	73– 74	60	45	43	52
31	Allyl	32– 33	68	54	51	60
32	Propynyl	54– 55	67	53	52	62
33	n-Butyl	38– 39	67	53	52	59
34	sec.-Butyl	61– 63	63	50	47	57
35	Isobutyl	80– 81	63	51	46	58
36	tert.-Butyl	99–100	60	47	43	54
37	n-Amyl	34– 35	73	57	56	60
38	Heptyl	28– 29	80	60	59	67
39	2-Chloroethyl	62– 63	67	49	45	55
40	2-Bromoethyl	54– 55	65	46	49	52
41	2,2,2-Trifluoroethyl	95– 97	62	45	41	50
42	2,2,2-Trichloroethyl	101–102	69	48	44	53
43	2,2,2-Tribromoethyl	124–125	66	46	46	51
44	Cyclohexyl	89– 90	64	50	48	58
45	Cyclopentyl	65– 66	61	46	44	54
46	Piperonyl	88– 89	80	64	59	70
47	Phenyl	108–109	68	53	47	59
48	Benzyl	83– 85	73	56	50	63
49	Phenethyl	43– 44	77	60	54	66

3. The isomeric trichlorophenols, *viz.* 2,4,5 and 2,4,6 have been separated utilizing solvents A–D with the order of R_F values being 2,4,6 > 2,4,5. The observed enhancement of the R_F values due to the *ortho* effect shown in this study for mono- and trichlorophenols has been previously reported for halogenated phenols in paper[30], absorption silica-gel[31] and pH chromatography[24].

4. In regard to the N-(trichloroacetyl) carbamates of the isomeric chlorophenols, analogous chromatographic behavior of these derivatives to their parent precursors was observed, *e.g.* the R_F values were as follows: $o > m > p$; 2,4 > 2,5 > 2,6 > 3,5 > 3,4; 2,4,6 > 2,4,5.

5. Utilizing solvent systems A or D, there is essentially a linear relationship when the R_F values are plotted against the number of substituted carbon atoms for the N-trichloroacetyl alkyl carbamates with the R_F values increasing with increasing chain length.

Although the separation of *n-*, *iso-*, and *tert.*-butyl derivatives could be best affected by solvent systems A and C neither system could resolve the respective N-trichloroacetyl *iso-* and *sec.*-butyl carbamates.

Acknowledgements

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Received February 28th, 1966

Concentrating compounds by continuous horizontal thin-layer chromatography

In preparative TLC, the common technique is to place the sample in a small band on the starting line of the plate, and separate the components by single or multiple development, followed by removal of the strip of adsorbent containing one of the compounds, and extraction with a suitable solvent[1-8]. The thus isolated compound can then be identified or determined. Because of band widening during the elution process a relatively large amount of adsorbent must be removed, so that the solution obtained after extraction always contains finely divided adsorbent particles. In quantitative work this is a serious disadvantage, only partly overcome by high-speed centrifuging (15.000 r.p.m.). It is practically impossible to prepare a clear solution which is sufficiently free of background adsorbance for measurement in the U.V. region.

A second disadvantage in quantitative work is the presence of organic compounds in nearly all commercial adsorbents[9]. Dependent upon the elution system, these are more or less eluted, so that on extraction of the separated compounds the solution will be contaminated. It is therefore necessary to pretreat the plates by several elutions with chlorohydrocarbons (*e.g.* trichloroethylene). As the organic compounds are thus concentrated at one end of the plate, their interference in the final solution can largely be obviated in this manner.

The effect of finely divided particles of adsorbent can also be reduced substantially by drastic reduction of the amount of adsorbent required. This, at the same time, would lead to more rapid and more effective extraction.

In the following, a method is described which concentrates separated compounds on a very small surface area by two-dimensional chromatography. Thus only a very small amount of adsorbent need be extracted. Use, in this connexion, is made of the continuous horizontal TLC method according to BRENNER AND NIEDERWIESER[10,11]. Though this method has mainly been applied to the separation of compounds differing very little in R_F value, it appeared to be excellently suited to our purpose: the amount of adsorbent to be extracted could be reduced to 1/50–1/60 that of the amount in normal procedures on 20 × 20 cm plates.

Experimental

As shown schematically in Fig. 1, the set-up for continuous horizontal TLC consists of a chromatoplate covered partly by a second glass plate, kept 2 mm apart