THIN-LAYER CHROMATOGRAPHY OF ISOMERIC HALO AND NITRO DERIVATIVES OF ANILINE AND BENZENE

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Halogenated nitroanilines[1–4] and benzenes[5–8] such as Botran, TCNB and PCNB and isomeric dichlorobenzenes[9] (compounds 28, 55, 56 and 59–61, Tables II and III) have recognized utility as fungicidal agents. A number of dichloroanilines have been reported as growth regulators[10].

Projected photodecomposition and metabolic studies in our laboratory involve these as well as related isomers. The purpose of this preliminary investigation was to assess the overall utility of several pi-electron acceptors and thin-layer chromatography for the identification, differentiation and separation of a variety of isomeric halo- and nitro-anilines and benzenes.

Chloronitrobenzenes have been analyzed by paper[11–14], thin-layer[15] and gas[16–19] chromatography; dinitrobenzenes by paper[20,21], column[22], thin-layer[23] and gas chromatography[24]. Gas chromatographic procedures have been reported for chlorinated anilines[25–27] and chlorinated nitroanilines[28,29]. The analysis of nitroanilines has been accomplished by paper[30,31] and thin-layer[32–35] chromatographic procedures.

EXPERIMENTAL

Thin-layer chromatography

The halo and nitro derivatives of aniline and benzene (from 2–5 μl containing 10–25 μg) were applied in acetone solution to Silica Gel DF-5 chromatoplates (prepared as previously described[36]), 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 developments as well as subsequent color changes noted. The sprayed plates were then exposed briefly to ammonia vapors, with the results described in Table I.

Solvent systems. The developing solvent systems utilized in this work were:

(A) 2.5% ethyl alcohol in benzene[34,35];

(B) benzene–ethyl acetate (5:1)[23];

(C) 2.5% acetone in benzene.

Detecting reagents. The following detecting reagents were used:

(1) DDQ reagent[37], 2% 2,3-dichloro-5,6-dicyano-1,4-benzoquinoneimine in benzene;

(2) TCNE reagent[37] 2% tetracyanoethylene in benzene;

* Short-wave (2537 Å) source. Mineralight Model-UVS-11, obtained from Allied Impex Corporation, New York, N.Y., U.S.A.

TABLE I

SPOT COLORS OF ISOMERIC HALO- AND NITRO-ANILINES ON SILICA GEL DF-5

Detecting reagents: (1) DDQ reagent 2% 2,3-dichloro-5,6-dicyano-1,4-benzoquinoneimine in benzene. (2) TCNE reagent 2% tetracyanoethylene in benzene. (3) Gibbs reagent 2% 2,6-dibromo-N-chloro-*p*-benzoquinoneimine in benzene.
Colors: B = blue, Bg = beige, Bn = brown, Bl = black, C = crimson, G = green, Gr = grey, O = orange, Ol = olive, Mn = maroon, P = purple, T = tan, V = violet, Y = yellow; wk = weak.

Compound	*Before NH_3 exposure*			*After NH_3 exposure*		
	1	*2*	*3*	*1*	*2*	*3*
o-Bromoaniline	B	B (wk)	Bn-V	B	Bg	P
m-Bromoaniline	Bn	Y	V	Bn	Y-G	P-B
p-Bromoaniline	V	Y-Bn	Bn	V-Bn	Bn	P-Bn
o-Chloroaniline	B	B (wk)	C	B	O-C	B
m-Chloroaniline	T	Y	Bn-B	Y-Bn	Y-G	Bn-Bl
p-Chloroaniline	V	T	V	B-Bn	O-C	B-Mn
o-Fluoroaniline	B → Y-Bn	B → Y	V	Bg	Y-G	Bn
m-Fluoroaniline	Y	B → Y	V-Mn	Bg	Y	B-Bn
p-Fluoroaniline	Ol	B → Y	B	Bg	Y	B
2,3-Dichloroaniline	B	V	Bn-V	B	Y-G	B
2,4-Dichloroaniline	G → B → P	Mn	Bn-V	C	Bn	P
2,5-Dichloroaniline	G → B-Bn	Bn-V	Bn-V	C	Y-G	P
3,4-Dichloroaniline	G → B → Ol	Y	Bn-V	Bn-Ol	Ol	Mn
2,4-Dibromoaniline	G → B → P	Mn	Bn-V	C	Bn	P
2,5-Dibromoaniline	G → B	Bn	Bn-V	Bn	Bn	P
2,6-Dibromoaniline	G → B-Bn	V	Bn-V	C	Y-G	P
2,4,5-Trichloroaniline	B	B-G	Bn-V	C	Ol	Bn
2,4,6-Trichloroaniline	B-Gr	B-Gr	Bn-V	C	Ol	B-Gr
o-Nitroaniline	Y-G → Ol-G (wk)	Bn (wk)	Y (wk)	Bg	Y	Bn
m-Nitroaniline	P (wk)	Y (wk)	T (wk)	Bg	Y	Bn
p-Nitroaniline	Gr-Bn (wk)	Ol (wk)	T (wk)	Bg	Y	Bn

(3) Gibbs reagent, 2% 2,6-dibromo-N-chloro-*p*-benzoquinoneimine in benzene.

Materials. 2,3-Dichloro-5,6-dicyano-1,4-benzoquinoneimine, compounds 1–10, 14–28, 31–46, and 56–61 were obtained from J. T. Baker Chemical Co., Phillipsburg, N.J., U.S.A.; compounds 11–13, 29, 30, and 47–55 were obtained from Aldrich Chemical Co., Milwaukee, Wisconsin, U.S.A.; and tetracyanoethylene from Distillation Industries, Rochester, N.Y., U.S.A.

RESULTS AND DISCUSSION

Table I depicts the spot colors utilizing DDQ, TCNE and Gibbs reagents before and after exposure of the chromatoplates to ammonia vapors. The isomeric bromo-, chloro-, fluoro- and nitro-anilines can best be differentiated by the DDQ reagent with the TCNE and Gibbs reagents being of secondary utility. The dichloro- and dibromo-trichloroanilines can be distinguished by the DDQ and TCNE reagents with the former being the reagent of choice. Overall, for the halo- and nitro-aniline derivatives

the utility of the tested pi-electron detectors was in the order: DDQ > TCNE > Gibbs.

Attempts to utilize the above detectors for the differentiation of chloronitro-, dichloronitro- and dinitro-anilines (compounds 23–31, Table II) as well as halo- and nitro-benzene derivatives (compounds 32–61, Table III) were without success. It has been previously shown[37] that electron-withdrawing groups (*meta*-directing) as $-NO_2$ (as in 3,4-methylenedioxynitrobenzene) are generally less reactive toward pi-electron detectors.

Tables II and III list the R_F values of isomeric halo- and nitro-aniline and benzene derivatives, respectively, utilizing three solvent systems. The following comments relate chromatographic behavior to isomeric configuration:

(1) The isomeric bromo-, chloro-, fluoro-, dichloro-, dibromo-, trichloro-, chloronitro-, dichloronitro-, nitro- and dinitro-anilines have been separated by solvents A–C, with solvent C (2.5% acetone in benzene) being the preferred solvent.

TABLE II

$R_F \times 100$ VALUES OF ISOMERIC HALO AND NITRO DERIVATIVES OF ANILINE ON SILICA GEL DF-5

Solvents: (A) 2.5% ethyl alcohol in benzene; (B) benzene–ethyl acetate (5 : 1); (C) 2.5% acetone in benzene.

No.	*Compound*	*m.p.* (°C)	*Solvents*		
			A	*B*	*C*
1	Aniline	184*	39	35	27
2	*o*-Bromoaniline	28–29	60	56	46
3	*m*-Bromoaniline	15–19	54	48	39
4	*p*-Bromoaniline	62–64	46	38	30
5	*o*-Chloroaniline	210.5*	67	59	48
6	*m*-Chloroaniline	227–229*	59	50	40
7	*p*-Chloroaniline	68–70	51	44	31
8	*o*-Fluoroaniline	65–66/13*	47	51	42
9	*m*-Fluoroaniline	74–76/13*	40	43	34
10	*p*-Fluoroaniline	188–190	32	33	25
11	2,4-Dibromoaniline	78–80	41	48	40
12	2,5-Dibromoaniline	53–55	48	57	48
13	2,6-Dibromoaniline	80–82	56	66	57
14	2,3-Dichloroaniline	20–22	61	63	58
15	2,4-Dichloroaniline	61–63	40	48	41
16	2,5-Dichloroaniline	49–50	51	54	49
17	3,4-Dichloroaniline	72–73	29	32	26
18	2,4,5-Trichloroaniline	95–97	55	52	51
19	2,4,6-Trichloroaniline	77–78	64	62	62
20	*o*-Nitroaniline	71–72	35	42	36
21	*m*-Nitroaniline	112–113	23	34	24
22	*p*-Nitroaniline	147.5–148	15	26	16
23	2,4-Dinitroaniline	180–182	21	21	10
24	2,6-Dinitroaniline	139–140	40	42	47
25	3,5-Dinitroaniline	158–160	27	30	17
26	2-Chloro-4-nitroaniline	104–106	30	35	26
27	4-Chloro-2-nitroaniline	115–116	38	41	34
28	4-Chloro-3-nitroaniline	101–102	24	26	20
29	2,6-Dichloro-4-nitroaniline	195–196	41	67	66
30	2,5-Dichloro-4-nitroaniline	148–149	22	48	49
31	4,5-Dichloro-2-nitroaniline	176–178	32	57	58

* Boiling point.

TABLE III

R_F × 100 VALUES OF ISOMERIC HALO AND NITRO DERIVATIVES OF BENZENE ON SILICA GEL DF-5

Solvents: (A) 2.5% ethyl alcohol in benzene; (B) Benzene–ethyl acetate (5 : 1); (C) 2.5% acetone in benzene.

No.	*Compound*	*m.p.* (°C)	*Solvents*		
			A	*B*	*C*
32	Nitrobenzene	210.9*	64	56	67
33	*o*-Dinitrobenzene	116–118	46	46	45
34	*m*-Dinitrobenzene	87–89	52	54	53
35	*p*-Dinitrobenzene	173–175	58	64	59
36	1-Fluoro-2-nitrobenzene	219–220	58	61	60
37	1-Fluoro-3-nitrobenzene	91–93/19*	64	66	66
38	1-Fluoro-4-nitrobenzene	25–27	65	69	69
39	1-Chloro-2-nitrobenzene	32–33	60	60	64
40	1-Chloro-3-nitrobenzene	44–45	66	65	69
41	1-Chloro-4-nitrobenzene	83–85	66	67	70
42	1-Bromo-2-nitrobenzene	40–42	56	55	54
43	1-Bromo-3-nitrobenzene	49–51	60	61	60
44	1,4-Dibromo-2-nitrobenzene	82–84	53	56	62
45	1,2-Dichloro-3-nitrobenzene	59–61	64	60	58
46	1,2-Dichloro-4-nitrobenzene	40–42	69	66	62
47	1,4-Dichloro-2-nitrobenzene	54–55	64	63	61
48	2,3-Dichloro-1-nitrobenzene	61–62	59	61	62
49	2,4-Dichloro-1-nitrobenzene	30–32	62	66	64
50	2,5-Dichloro-1-nitrobenzene	52–54	60	66	66
51	3,4-Dichloro-1-nitrobenzene	40.5–42	60	57	65
52	1,2,4-Trichloro-5-nitrobenzene	56–58	64	65	60
53	2,3,4-Trichloro-1-nitrobenzene	55–56	61	62	52
54	2,4,5-Trichloro-1-nitrobenzene	59–61	59	68	68
55	2,3,4,5-Tetrachloro-1-nitrobenzene	65–67	69	63	74
56	1,2,4,5-Tetrachloro-3-nitrobenzene	98–99	63	56	65
57	Pentachloronitrobenzene	144–145	70	66	71
58	Chlorobenzene	130–132*	45	48	50
59	*o*-Dichlorobenzene	179–180	64	65	63
60	*m*-Dichlorobenzene	172–174	63	60	63
61	*p*-Dichlorobenzene	53–54	63	62	65

* Boiling point.

(2) The order of R_F values for the above isomers was as follows:

bromo-, chloro- and fluoro-anilines: $o > m > p$

dichloroanilines: 2,5 > 2,3 > 2,4 > 3,4

dibromoanilines: 2,6 > 2,5 > 3,4

trichloroanilines: 2,4,6 > 2,4,5

chloronitroanilines: 4-chloro-2-nitro > 2-chloro-4-nitro > 4-chloro-3-nitro

dichloronitroanilines: 2,6-dichloro-4-nitro > 4,5-dichloro-2-nitro > 2,5-dichloro-4-nitro

nitroanilines: $o > m > p$

dinitroanilines: 2,6 > 3,5 > 2,4

(3) The isomeric dinitro-, fluoronitro-, chloronitro-, bromonitro-, dichloronitro-, trichloronitro- and tetrachloronitro-benzenes have been separated on Silica Gel DF-5

plates as shown below. The order of R_F values for the above isomers was as follows:

dinitrobenzenes: $p > m > o$
fluoronitrobenzenes: 1-fluoro-3-nitro; 1-fluoro-4-nitro > 1-fluoro-2-nitro
chloronitrobenzenes: 1-chloro-3-nitro; 1-chloro-4-nitro > 1-chloro-2-nitro
bromonitrobenzenes: 1-bromo-3-nitro > 1-bromo-2-nitro
trichloronitrobenzenes: 2,4,5 > 1,2,4 > 2,3,4 (solvent C only)
tetrachloronitrobenzenes: 2,3,4,5 > 1,2,4,5

It was not possible to effect separation of the isomeric dichloro- and dichloronitro-benzenes with the solvent systems employed.

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SUMMARY

The chromatogenic behavior of a variety of isomeric halo and nitro derivatives of aniline and benzene respectively was elaborated on Silica Gel DF-5 utilizing three solvent systems. The chromogenic behavior of the isomeric bromo-, chloro-, fluoro-, dichloro-, dibromo-, trichloro- and nitro-anilines towards three pi-electron detectors is also described.

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