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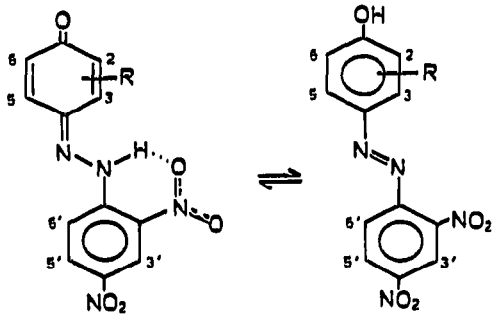
Note

Thin-layer chromatography of mono- and dinitrophenylazoalkylphenols (alkylquinone mono- and dinitrophenylhydrazones)

Thin-layer chromatography (TLC) has been used extensively for separating mixtures of 2,4-dinitrophenylhydrazones (DNPHs)[1]. Surprisingly, this technique does not seem to have been applied to the analysis of quinone DNPHs, in spite of the general use of 2,4-dinitrophenylhydrazine as a reagent for carbonyl compounds. Most 1,4-quinone mono-DNPHs (I) exist as tautomeric mixtures with dinitrophenylazophenols (II)[2]. They move as single spots on chromatograms, owing to the rapid interconversion between the forms[3–4]. The compounds can be prepared by reaction of quinones with 2,4-dinitrophenylhydrazine[5–8] or, more generally, by coupling phenols containing an unsubstituted *para*-position with diazotised 2,4-dinitroaniline[7–10].



(I) (II)

They are well adapted to the TLC analysis of both quinones and phenols, as they give vivid blue colours with bases[7,11] and the necessary standards are easily prepared.

Some free alkylphenols[12,13] and free quinones[14–16] have been separated by TLC, and phenols also by gas–liquid chromatography[17–21], whereas quinones have been subjected only sporadically to GLC separation[22]. The DNPHs of some alkylquinones have been separated on paper[7,23], and of mononitrophenylazophenols on paper[17,24] and on thin-layers[24–28].

In this paper, the TLC of some alkylquinone DNPHs is described. Some insight into the "mechanism" of the chromatography of these compounds was gained by comparison with the corresponding mononitro derivatives and similar compounds.

Experimental

The TLC plates (0.25 mm layer) were prepared from Merck Kieselgel G with water (for the neutral layer) or 0.5 *N* sodium hydroxide[26] (for the basic layer), or from Merck Aluminiumoxide G and water, and dried at 110°. The preparation of the

TABLE I

R_{Bu} · 100 VALUES OF 4-PHENYLAZOALKYLPHENOLS

No.	R in I or II[a]	Nitro position	Colour[b] Dry spot[c]	With Et_2NH[d]		R_{Bu} · 100 values[c] S_1	S_2	S_3	S_4
			2',4'-	2'-	4'- 2',4'-	2',4'-	2',4'-	2',4'-	2',4'-
1	H		r–vi	l–or	p bl	04	00	12	18
2	2-Me		r–vi	r–or	bl–vi l–bl	17	03	33	41
3	2-Et		r–vi	r–or	bl–vi l–bl	24	04	52	43
4	2-Pr		r–vi	r–or	bl–vi l–bl	28	07	59	43
5	2-Bu		b–vi	b–or	bl l–bl	44	25	79	49
6	2-Ph			r–or	bl–vi l–bl	17	01		50
7	3-Me		r–vi	r–or	p l–bl	08	00	15	28
8	3-Et		r–vi	r–or	p l–bl	10	00	22	28
9	3-Pr		r–vi	r–or	p l–bl	10	01		34
10	3-Bu		r–vi	r–or	p l–bl	14	01	40	34
11	3-Ph			r–or	p l–bl	08	01		31
12	$3,5\text{-Me}_2$		r–vi	r–or	p bl	10	00	20	30
13	3-Me-5-Et		r–vi	r–or	p bl	11	01	22	34
14	3-Me-5-Pr		r–vi	r–or	p bl	13	03	25	35
15	3-Me-5-Bu		r–vi	r–or	p bl	10	04		31
16	$3,5\text{-Pr}_2$		r–vi	r–or	p bl	14	07		34
17	$2,3\text{-Me}_2$		b	r–b	bl–vi bl	27	07	52	61
18	$2,5\text{-Me}_2$		b	r–b	bl–vi bl	30	12	71	57
19	2-Me-5-Pr		b	r–b	bl–vi bl	37	22	84	64
20	2-Pr-5-Me		b	r–b	bl–vi bl	44	32	93	65
21	2-Bu-5-Me		ol	r–b	bl bl	73	68	102	87
22	$2,5\text{-Pr}_2$			r–b	bl bl	58	47		56
23	$2,5\text{-Bu}_2$		ol	r–b	bl bl	87	81	104	91
24	$2,3,5\text{-Me}_3$		b	b	bl–vi bl	30	13		55
25	$2,6\text{-Me}_2$		ol	r–b	bl bl	44	37	67	65
26	2-Me-6-Bu		ol	r–vi	bl bl	78	96	99	85
27	$2,6\text{-Pr}_2$		ol	r–vi	bl bl	83	90	101	91
28	3-Cl-2,6-Pr_2		gr	r–vi	bl bl	92	93		93
29	$2,6\text{-Bu}_2$		y	r–vi	l–bl bl	100	100	100	100
30	$2,3,6\text{-Me}_3$		ol	r–vi	bl–vi bl	59	68	98	70
31	$2,3,5,6\text{-Me}_4$		ol	r–b	bl–vi bl	56	68	98	66

[a] Pr = isopropyl; Bu = *tert.*-butyl.
[b] b = brown; bl = blue; gr = green; l = light; ol = olive; or = orange; p = purple; r = red; vi = violet; y = yellow.
[c] On basic silica gel only.
[d] On all layers.

compounds is described elsewhere[2]. About 0.1–1 μg of each substance was chromatographed, using horizontal development[30] in Pyrex glass basins (Sovirel, 310 mm O.D.). The basins were cut to 13–15 mm internal height at the centre so as to reduce the volume. The solvent movement was 180 mm. The colour of the spots was enhanced (*cf.* Table I) by exposing the finished chromatogram to diethylamine vapour[30,31]. The chromatography was performed under ambient conditions, and the results should be considered as a guide only[32]. The mobilities given in Tables I–III are relative to 2,6-di-*tert.*-butylquinone DNPH (No. 29), the R_F values of which in the various systems are given in Table IV.

S_5			S_6			S_7[f]		
2'-	4'-	2',4'-	2'-	4'-	2',4'-	2'-	4'-	2',4'-
12	15	12	36	48	30	24	29	15
21	21	22	43	57	38	29	35	20
24	26	26	44	57	43	30	36	23
28	31	34	48	59	46	33	39	26
43	43	47	52	66	52	38	46	29
42	45	50	34	46	33	24	29	17
15	19	12	36	48	30	26	32	18
19	20	18	38	52	36	29	35	21
22	24	19	44	56	39	32	38	24
23	24	20	46	57	44	36	41	26
16	18	15	34	43	30	21	26	15
16	24	12	38	51	34	29	36	20
20	28	18	43	54	38	33	41	23
24	32	20	44	56	39	36	44	27
24	30	23	46	56	43	38	46	29
32	45	37	51	62	48	46	56	36
24	26	28	43	52	43	35	41	27
28	31	31	38	54	33	32	39	23
41	45	45	44	57	43	38	53	32
46	49	55	43	56	38	39	46	29
66	66	66	48	62	46	39	46	36
62	64	65	48	62	48	76	68	77
81	84	83	41	72	57	74	61	74
26	43	30	38	51	34	99	64	91
57	57	60	43	56	46	49	56	38
80	72	80	71	71	80	65	74	58
83	78	85	71	74	82	55	64	59
97	74	93	90	71	89	56	48	27
102	78	100	115	74	100	114	58	100
64	45	69	53	56	61	58	50	53
62	70	69	56	62	62	59	58	53

[e] S_1 = basic silica gel with $CHCl_3$; S_2 = basic silica gel with CH_2Cl_2; S_3 = alumina with $CHCl_3$; S_4 = neutral silica gel with $CHCl_3$; S_5 = neutral silica gel with CH_2Cl_2; S_6 = neutral silica gel with light petroleum (60–80°)–acetone (4:1); S_7 = neutral silica gel with light petroleum (80–110°)–dioxan (4:1) and developed twice.

[f] Not true R_{Hu} values, since developed twice.

Discussion

The chromatographic behaviour of tautomeric compounds obviously depends on the average structure[3]. In the azophenol tautomer (II), the hydroxyl group constitutes the main centre of activity towards the stationary phase (*cf.*, No. 45 *vs.* No. 1; No. 1 *vs.* No. 40; etc.), and the nitrogen bridge is relatively inert (No. 45 moves rapidly).

In aliphatic 4-nitrophenylhydrazones, the NH-hydrogen is the main centre of activity, whereas in the 2-nitro- and 2,4-dinitro analogues its importance is greatly reduced as a result of the chelation N–H···NO_2 (*cf.*, No. 34, 2-nitro- *vs.* 4-nitro-*vs.*

TABLE II

R_{Bu}·100 VALUES OF 4'-ARYLHYDRAZONES AND RELATED COMPOUNDS[a]

No.	Compounds	Colour[b]			
		Dry spot[c]	With Et_2NH[d]		
	Nitro positions	2',4'-	2'-	4'-	2',4'-
	Nitrophenylhydrazones of				
32	1,4-Naphthoquinone	l-gr	r-vi	bl	l-bl
33	2-Me-1,4-naphthoquinone	y	vi	bl	l-bl
34	Acetone	y	y	y	b
	Nitrophenyl-N-methylhydrazones of				
35	1,4-Benzoquinone				
36	2-Pr-5-Me-benzoquinone				
37	2,6-Bu_2-benzoquinone				
38	Acetone				
	Nitrophenylazoanisoles				
39	2-Pr-5-Me				
40	H				
41	3-Me				
42	2,5-Pr_2				
43	3,5-Me_2				

[a–f] See footnotes to Table I.

dinitro-). Also, in this case the bridge nitrogen atoms are probably relatively unimportant, as shown by No. 34 *vs.* No. 38 and No. 29 *vs.* No. 37; N-methylation increases the activity of one or both nitrogen atoms, probably by the inductive effect, which more than compensates for the loss of NH activity.

The introduction of a nitro group generally should result in a lower mobility, owing to direct interaction with the stationary phase, or to polarisation of other groups (No. 49 *vs.* No. 1; No. 51 *vs.* No. 20; etc.). However, 2-nitroazobenzene moves more slowly than the 4-nitro- compound (Nos. 45–47), perhaps owing to the non-coplanarity of the nitro group in the former, which increases the activity of this group; in hydrazones, the relation is the opposite (2-nitro- > 4-nitro-), as a result of

TABLE III

R_{Bu}·100 VALUES OF AROMATIC COMPOUNDS[a]

No.	Compound	S_4	S_5	S_6	S_7[b]
44	Azobenzene, *cis*	78	52	79	91
45	Azobenzene, *trans*	102	106	107	116
46	Azobenzene, 2-nitro-	90	101	72	75
47	Azobenzene, 4-nitro-	96	103	89	95
48	Azobenzene, 2,4-dinitro-	93	98	66	67
49	4-Phenylazophenol	21	21	55	51
50	4-Phenylazoanisole	93	98	92	101
51	4-Phenylazothymol	57	70	69	75

[a] Solvent systems S_4–S_7; see footnotes to Table I. Detection: UV, 254 nm.
[b] S_7 developed once only.

R_{Bu}·100 values[a]											
S_4			S_5			S_6			S_7[f]		
2'-	4'-	2',4'-	2'-	4'-	2',4'-	2'-	4'-	2',4'-	2'-	4'-	2',4'-
61	07	49	38	04	46	62	29	51	66	16	49
77	16	75	68	12	71	78	39	62	80	21	62
88	37	81	84	49	79	92	51	69	100	33	72
18	12	10	18	02	04	34	11	07	34	07	03
27	33	47	07	15	35	41	46	50	40	32	34
92	92	90	89	88	89	81	88	72	91	88	72
37	32	38	24	19	30	63	44	37	75	45	35
101	100	100	107	103	103	80	100	77	90	103	81
93	94	93	92	92	92	66	81	59	74	84	69
96	93		93	94		71	86		75	90	
		96			103			82			85
		92			94			64			60

the chelation mentioned. Among the dinitrophenylazophenols, Nos. 1–4 and Nos. 6–16 behave as azo compounds (2'-nitro- < 4'-nitro-) in all of the solvents tested; No. 29 behaves as a hydrazone (2'-nitro- > 4'-nitro-) in all of the solvents; and the others behave as azo compounds or as hydrazones, depending on the solvent used. This behaviour largely parallels the ability of the compounds to exist in the hydrazone form in tetrachloroethylene solution[2], and it could be suggested that the hypothetical mobility of the hydrazone tautomer is greater than that of the azo form. The mobility of the actual spot, however, is intermediate between the hypothetical mobilities of the individual forms[3]. It follows that, other things being equal, the keto group of the hydrazone tautomer is probably less active in chromatography than the hydroxyl group of the azo form.

TABLE IV

R_F·100 VALUES OF THE REFERENCE COMPOUND 2,6-DI-*tert.*-BUTYLQUINONE, DNPH (No. 29, R_{Bu} = 100)

Solvent system[a]	R_F·100 *value*
S_1	71
S_2	68
S_3	92
S_4	74
S_5	74
S_6	61
S_7	66

[a] See footnotes to Table I.

Some of the DNPHs, notably Nos. 27 and 28, move on neutral silica gel in solvent S_0 as greenish or blue-green spots; on drying the chromatogram, the colours revert to yellow. Also, the spots of the dinitro compounds change to the anionic colour with lower concentrations of base, or with weaker bases (ammonia, etc.), than spots of the DNPHs of ordinary aldehydes and ketones. This indicates that the quinone DNPHs are more acidic than ordinary DNPHs. Spectrophotometric pK_a values of the dinitro compounds are reported in another paper[2].

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