CHROMATOGRAPHIC ANALYSIS OF BENZOQUINONES

GÖSTA PETTERSSON

Institute of Biochemistry, University of Lund (Sweden)

(Received March 13th, 1963)

Chromatography is well recognized today as an indispensable technique in the study of all kinds of natural pigments. A large proportion of these pigments, occurring in animals, plants and micro-organisms, consists of quinones. Many systems for separating anthraquinones[1–5] and naphthaquinones[2,6,7] have been described, but except for a number of investigations associated with specific biochemical problems (*e.g.* the ubiquinones[8–10]) little work has been reported on the chromatography of benzoquinones in general[11,12]. In connection with analytical work on mould products, a chromatographic method was required that would give a precise and good separation of mixtures containing isomeric *p*-benzoquinones in different stages of hydroxylation. Thin-layer chromatography was chosen as the principal technique as it was rapid and required a smaller amount of the substance to be analysed, but for special purposes a paper chromatographic method was also required. It often proved advantageous to purify the mould extracts on paper chromatograms, and then, after elution, to identify the different quinones on thin-layer plates.

The compounds investigated could be divided into two groups, which had to be analysed separately:

(1) Benzoquinone derivatives where the substituents consisted of one or more methyl, ethyl or methoxyl groups.

(2) Benzoquinone derivatives where in addition at least one of the substituents was a hydroxyl group.

For quantitative determination of the compounds, the ultraviolet absorption curves of the pure products were determined in anhydrous ether, using 1 cm cells. Maximum absorption is found between 265 and 280 mμ[13], and a wavelength of 270 mμ was chosen to obtain standard curves.

MATERIALS AND METHODS

Samples

The quinones used in the present investigation were synthesized in our laboratory, or obtained through the courtesy of other laboratories. The purity was checked by chromatography. Six of the compounds have not been described previously:

The three isomeric ethyldimethylhydroxybenzoquinones (Nos. 12–14 in Table II) were obtained in good yield from the corresponding hydroquinones[14] by oxidation with air in 0.1 *M* phosphate buffer (pH 8.0). After acidification of the buffer solution with concentrated hydrochloric acid the quinones were extracted with ether and purified by sublimation.

Treatment of the ethyldimethylhydroxybenzoquinones with diazomethane in ether solution and subsequent removal of ether gave a quantitative yield of the corresponding methoxy derivatives (Nos. 9–11 in Table III) in the pure form. Data of these six quinones are given in Table I.

TABLE I

DATA OF SIX SYNTHESIZED BENZOQUINONES

Compound	*m.p.*	*Colour*	
		Crystals	*Alkaline solution*
2-Hydroxy-3,6-dimethyl-5-ethyl-1,4-benzoquinone	106°	yellow	violet
2-Hydroxy-3,5-dimethyl-6-ethyl-1,4-benzoquinone	69°	orange	violet
2-Hydroxy-5,6-dimethyl-3-ethyl-1,4-benzoquinone	79°	yellow	violet
2-Methoxy-3,6-dimethyl-5-ethyl-1,4-benzoquinone	135°	yellow	yellow
2-Methoxy-3,5-dimethyl-6-ethyl-1,4-benzoquinone	82°	yellow	yellow
2-Methoxy-5,6-dimethyl-3-ethyl-1,4-benzoquinone	40°	yellow	yellow

Paper chromatography

The compounds were introduced onto Whatman No. 1 paper by the usual spotting technique, and the papers developed by the descending method for a period of 16–24 h, depending on the solvent system used. During this time the solvent descended to 40 cm from the base line. Greatly increased separation of compounds with relatively low R_F values was obtained by allowing the solvent front to overrun the edge of the paper. In these cases, R_F values were determined by simultaneously running spinulosin (No. 30 in Table III) as a reference substance.

Paper chromatographic purification

The mould extracts were introduced as bands along the base line and the chromatograms developed as above. In the solvent system used, all the compounds of Group I moved with or near the solvent front, and could thereby easily be separated from the members of Group II by elution with ethanol. The quinones of Group II could often be identified directly on the paper chromatogram, but if necessary these were also cut out from the paper, eluted with ethanol and identified by thin-layer chromatography.

Thin-layer chromatography

Smooth glass plates (15 × 15 × 0.3 cm) were covered with a thin, even layer of Silica Gel G (Merck, Darmstadt) by spreading a well-stirred mixture of 30 g Silica Gel G and 60 ml of distilled water with a thin-layer applicator. The plates were activated by heating for 1 h at 105°, and placed in a desiccator over calcium chloride. Five microliter drops containing 1–10 μg of the quinones dissolved in ether or ethanol were applied at a distance of 1.5 cm from the edge of the plate. The origin and a front 10 cm above it were marked off, and ascending chromatograms run. When the solvent front reached the 10 cm mark (20–40 min) the plates were removed and airdried.

Quantitative analysis

After 1 min. exposure to gaseous hydrochloric acid the spots were removed by

scraping the silica gel from the plates and transferred quantitatively to a small column, from which the compounds were eluted, using ether, a total volume of 3 ml being collected. The ultraviolet absorption of each compound at 270 mμ was then determined, and the values obtained interpolated in the standard curves. The results were easily reproducible, giving a recovery of 95–100 % of the compound

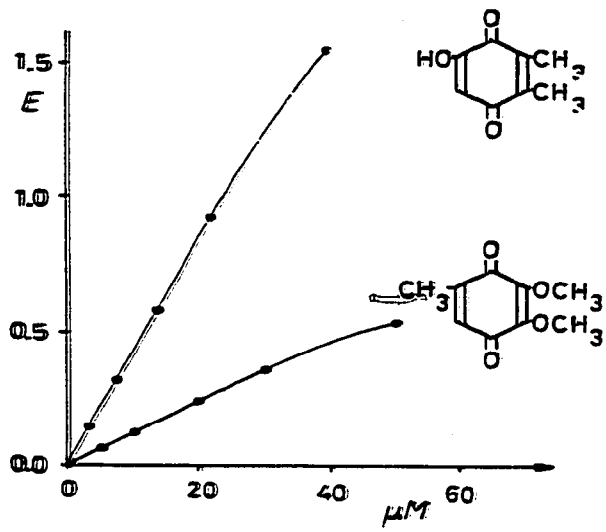

Fig. 1. Extinction at 270 mμ (1 cm cells) as function of concentration for two representative benzoquinones in ethereal solution.

chromatographed. In concentrations lower than 20 μM the quinones obey Lambert-Beer's law, with molecular extinction coefficients ranging from $5 \cdot 10^3$ to $5 \cdot 10^4$ $M^{-1}cm^{-1}$. Fig. 1 shows the standard curves for one representative compound of each group.

Detection

The chromatographic spots are self-indicating. The quinones of Group I all give yellow spots, those of Group II give colours that are characteristic for their structure, passing from orange to purple or blue with increasing number of substituents, and in some cases enable overlapping spots to be identified. The lower limit of detectability is 1 μg for Group I and 0.1 μg Group II. Not more than 20 μg should be applied to the thin-layer plates.

Solvent systems

The following solvent systems proved satisfactory:

A. Chloroform
B. Chloroform–benzene (3:1 by vol.)
C. Chloroform–xylene (3:1 by vol.)
D. Ethanol–concentrated ammonia (5:1 by vol.)
E. Ethanol–*n*-butanol–2 *M* ammonium hydroxide (3:5:3 by vol.)
F. Propanol–*n*-butanol–2*M* ammonium hydroxide (6:1:3 by vol.).

The first three solvents were used for thin-layer analysis of Group I, the others for Group II. Solvent F was also used for paper chromatography.

RESULTS AND DISCUSSION

Tables II and III list the R_F values of Groups I and II respectively of the investigated quinones. These figures are an average of three or more runs, and the reproducibility was established by a statistical analysis of 20 experiments in which the mobility of three compounds of each group was determined in the different solvent systems. On thin-layer plates, using solvents B-F, the standard deviation of the R_F values was in the order of 5 % of the means for all the compounds. With solvent A, and on paper chromatograms (solvent F) the standard deviation was slightly higher (10 %). The major source of error is attributable to temperature fluctuations with their subsequent effect on the mobilities.

The order in which the quinones of Group I appear on the chromatograms is not much affected by the solvent system used. Many systems have been investigated, and the three given above, based on chloroform, showed the best differentiation of the R_F values. Sometimes two-dimensional chromatography is advantageous, and Fig. 2 shows such a chromatogram, where solvent A has been used for the first dimension and B for the second one.

In most of the 90 or so solvents tested the compounds of Group II showed extensive tailing, which could be avoided only by using alkaline systems, the best systems being based on alcohols containing ammonia. The water-free solvent D gives good resolution and generally very distinct, flattened spots. A difference of 0.02 in R_F value is enough to yield two entirely separated spots if not too much substance

TABLE II

100 × R_F VALUES OF 21 DERIVATIVES OF 1,4-BENZOQUINONE BELONGING TO GROUP I

No.	Substituents in position				Solvent system used							Colour
					Thin-layer						Paper	
	2	3	5	6	A*	B*	C*	D	E	F	F	
1	H	H	H	H	66	48	50	73	84	75	85	yellow
2	CH_3	H	H	H	74	53	54	88	91	85	95	yellow
3	CH_3	H	H	CH_3	77	47	52	84	92	81	96	yellow
4	CH_3O	H	H	H	50	54	35	90	92	90	92	yellow
5	CH_3	CH_3O	H	H	54	36	40	87	90	85	93	yellow
6	CH_3	H	CH_3O	H	33	31	26	80	78	80	95	yellow
7	CH_3	H	H	CH_3O	52	22	33	85	87	73	100	yellow
8	CH_3	CH_3	CH_3O	H	43	42	40	76	85	81	100	yellow
9	CH_3	CH_3O	CH_3	H	61	55	67	85	88	85	97	yellow
10	CH_3	CH_3O	H	CH_3	24	21	20	81	78	88	96	yellow
11	CH_3	CH_3	CH_3	CH_3O	63	58	62	90	91	95	100	yellow
12	CH_3	CH_3	C_2H_5	CH_3O	90	66	64	95	96	92	100	yellow
13	CH_3	C_2H_5	CH_3	CH_3O	84	67	62	95	90	91	100	yellow
14	CH_3	C_2H_5	CH_3O	CH_3	87	63	64	92	94	90	100	yellow
15	CH_3O	H	CH_3O	H	15	16	10	83	83	88	92	yellow
16	CH_3O	H	H	CH_3O	17	19	15	75	81	80	95	yellow
17	CH_3	CH_3O	CH_3O	H	39	26	23	78	84	77	100	yellow
18	CH_3	CH_3O	H	CH_3O	36	29	26	83	80	73	98	yellow
19	CH_3	H	CH_3O	CH_3O	46	32	30	79	85	73	95	yellow
20	CH_3	CH_3O	CH_3O	CH_3	43	36	30	82	88	79	100	yellow
21	CH_3	CH_3O	CH_3O	CH_3O	48	39	27	85	89	75	98	yellow

* Solvent systems suitable for separations within the group.

TABLE III

100 × R_F VALUES OF 31 DERIVATIVES OF 1,4-BENZOQUINONE BELONGING TO GROUP II

No.	Substituents in position				Solvent system used							Colour
					Thin-layer						Paper	
	2	3	5	6	A	B	C	D*	E*	F*	F*	
1	OH	H	H	H	6	4	4	74	78	79	38	orange
2	CH_3	OH	H	H	2	1	1	64	70	68	78	orange
3	CH_3	H	OH	H	0	0	0	73	67	63	70	red-orange
4	CH_3	H	H	OH	0	0	0	44	58	52	dec.	orange
5	CH_3	CH_3	OH	H	11	5	3	65	70	67	71	orange
6	CH_3	OH	CH_3	H	11	5	4	76	64	64	79	violet
7	CH_3	OH	H	CH_3	22	8	10	74	62	68	77	red-violet
8	CH_3	CH_3	CH_3	OH	22	9	9	58	63	58	79	red-violet
9	CH_3	CH_3	C_2H_5	OH	29	14	30	66	67	68	77	violet
10	CH_3	C_2H_5	CH_3	OH	23	10	24	65	64	60	77	violet
11	CH_3	C_2H_5	OH	CH_3	17	7	11	72	68	64	80	violet
12	OH	H	OH	H	0	0	0	0	0	0	4	blue-violet
13	OH	H	H	OH	0	0	0	21	25	25	13	orange
14	OH	H	CH_3O	H	5	2	1	50	60	54	56	red-violet
15	OH	H	H	CH_3O	0	0	0	62	62	67	52	orange
16	CH_3	OH	OH	H	0	0	0	42	16	29	32	violet
17	CH_3	OH	H	OH	0	0	0	6	20	10	19	red-violet
18	CH_3	H	OH	OH	1	1	1	63	63	60	65	violet
19	CH_3	OH	CH_3O	H	8	2	5	66	63	67	60	violet
20	CH_3	CH_3O	OH	H	4	2	3	50	38	45	55	orange
21	CH_3	OH	H	CH_3O	0	0	0	64	59	62	54	blue
22	CH_3	CH_3O	H	OH	0	0	0	60	58	58	51	violet
23	CH_3	H	OH	CH_3O	0	0	0	26	38	30	30	violet
24	CH_3	H	CH_3O	OH	1	1	1	49	56	49	45	violet
25	CH_3	CH_3	OH	OH	0	0	0	45	41	41	53	violet
26	CH_3	OH	CH_3	OH	0	0	0	21	15	18	23	red-violet
27	CH_3	OH	OH	CH_3	7	3	4	54	64	63	38	blue
28	CH_3O	CH_3O	OH	H	11	5	6	67	65	55	70	blue-violet
29	CH_3	OH	OH	OH	0	0	0	0	0	0	9	blue
30	CH_3	OH	CH_3O	OH	0	0	0	5	0	0	25	blue-violet
31	CH_3	OH	CH_3O	CH_3O	3	1	1	65	65	58	69	blue-violet

* Solvent systems suitable for separations within the group.

is applied to the plates. There is, however, still some tailing of the quinones with two hydroxyl groups. With solvent E all compounds develop well-defined spots, slightly more diffuse than obtained with D. The resolution is good and the composition of the system can be varied to meet special needs for separation. Increasing the percentage of butanol makes the system more adapted to the chromatography of the less polar quinones, but also increases the time of development. Decreasing the percentage of butanol gives better separation of compounds with low R_F values. Butanol should not be completely omitted, as the spots then tend to tail too much. Variation of the proportion of $2M$ ammonium hydroxide is of no use, as it is chosen to be optimum as regards avoidance of tailing and resolution ability. Solvent F has the same general properties as solvent E.

Paper chromatography was used partly to separate Group I from Group II, partly to identify the members of Group II. For these purposes, solvent F proved superior to other systems, and was exclusively used. It appears from Table III that

paper chromatography gives as good separations within Group II as the thin-layer technique, particularly between the more polar compounds. A better differentiation of the less polar quinones can be effected by increasing the proportion of butanol. The long time of development of the paper chromatograms is, however, a great disadvantage as some decomposition of the less stable quinones (*e.g.* 3-hydroxy-2,5-

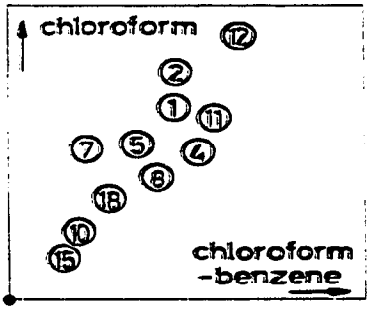

Fig. 2. Two-dimensional chromatography of benzoquinones from Group I. The numbers of the compounds refer to Table II.

toluquinone) occurs in the alkaline medium. Consequently, quantitative analysis should be performed only on thin-layer plates.

No general straight-line relationship between R_F values and number of substituents could be obtained, but in most cases the R_F values are increased by methyl groups, decreased by methoxyl groups, and decreased still more by hydroxyl groups.

ACKNOWLEDGEMENTS

The author is greatly indebted to Prof. J. H. BIRKINSHAW, London, Prof. H. ERDTMAN, Stockholm, Prof. W. FLAIG, Braunschweig and Prof. H. MUSSO, Marburg, for providing samples of quinones.

SUMMARY

A satisfactory method for the separation, identification and determination of *p*-benzoquinone derivatives by adsorption chromatography on plates and partition chromatography on paper, using different solvents as developer, is described, and the dependence of the R_F values on the nature of the solvent system discussed.

REFERENCES

1 J. FRANC, *Chem. Listy*, 49 (1955) 872.
2 J. GASPARIČ, *Mikrochim. Acta*, (1958) 681.
3 J. FRANC, *Collection Czech. Chem. Commun.*, 24 (1959) 250.
4 J. FRANC AND M. WURST, *Collection Czech. Chem. Commun.*, 25 (1960) 657.
5 J. GASPARIČ, *J. Chromatog.*, 4 (1960) 75.
6 L. REIO, *J. Chromatog.*, 1 (1958) 338.
7 M. BARBIER AND E. LEDERER, *J. Chromatog., Chromatog. Data*, 1 (1958) xlii.
8 A. DIPLOCK, J. GREEN, E. EDWIN AND J. BUNYAN, *Biochem. J.*, 76 (1960) 563.
9 H. WAGNER, L. HÖRHAMMER AND B. DENGLER, *J. Chromatog.*, 7 (1962) 211.
10 J. GREEN, S. MARCINKIEWICZ AND D. MCHALE, *J. Chromatog.*, 10 (1963) 158.
11 M. BARBIER, *J. Chromatog.*, 2 (1959) 649.
12 H. SCHILDKNECHT, *Angew. Chem.*, 69 (1957) 62.
13 W. FLAIG, J. C. SALFELD AND E. BAUME, *Ann.*, 618 (1958) 117.
14 J. LJUNGCRANTZ AND K. MOSBACH, *Acta Chem. Scand.*, in the press.