PAPER CHROMATOGRAPHY AND PAPER ELECTROPHORESIS OF PHENOLS AND GLYCOSIDES

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Although some solvent systems have been reported for the paper chromatography of various phenolic substances[1], the two reported here are valuable alternatives.

PAPER CHROMATOGRAPHY

Solvent systems

Whatman No. 4 paper used; temperature of chromatography room, 20° ± 1°.

(a) Benzene–glacial acetic acid–water, (20:5:just saturated)[2], used for ascending chromatography, gave results within 8 h.

(b) *n*-Butanol–ethanol–borate buffer[3] (9.54 g/l sodium tetraborate), (1:1:1) was used for ascending chromatography, with paper impregnated with the borate buffer which was dried before use. Adequate separation is obtained in 18 h. WACHTMEISTER[4] has used a related solvent system.

2,5-Dihydroxyphenolic substances tend to streak in borate buffers, when used both for paper chromatography and electrophoresis.

Detection reagents

(a) A spray application of the EVANS' quantitative *o*-dihydroxyphenol colour reaction[5] proved useful for detection of catechol substances on paper. Sodium molybdate (10%, w/v)–hydrochloric acid (0.5 *N*), (2:1) mixture was sprayed first; *o*-dihydric phenols gave a weak yellow colour. Sodium nitrite solution (0.5%, w/v) was then sprayed, the spots turning greenish yellow in colour. A final spraying with sodium hydroxide solution (0.5 *N*) resulted in the spots immediately becoming a strong medium-toned pink. The paper should not be made too wet. This test is about as sensitive as the diazotised *p*-nitraniline reaction, but is better for photographic purposes, since background colour is almost absent. 2,5-Dihydroxyphenolic compounds (*e.g.* gentisic or homogentisic acids) give a final medium brown colour, easily distinguished from that of true catechol substances.

(b) Diazotised *p*-nitraniline, prepared in the usual way, followed by sodium carbonate solution[1], was used as a spray for the detection of phenolic substances on chromatograms developed with both solvent systems. Catechol substances gave a typical cherry red to purple colour, with this reagent, except when borate buffers were used in the solvent system or as an electrolyte in paper electrophoresis. In these cases,

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TABLE I

R_F AND COLOUR REACTIONS OF VARIOUS PHENOLS AND PHENOLIC ACIDS

Ascending paper chromatography. (a) Paper: Whatman No. 4. Solvent system: benzene–glacial acetic acid–water (20:5 just saturated). (b) Paper: Whatman No. 4, buffered with borate buffer (9.54 g/l sodium tetraborate). Solvent system: *n*-butanol–ethanol–borate buffer (1:1:1).

Solvent system	*(a)*			*(b)*	
Substance	R_F	*Colour by reagent*		R_F	*Colour by reagent*
		Diazo-p-NA	*Evans'*		*Diazo-p-NA*
Miscellaneous					
Catechol	0.43	Cherry purple	+	0.70	Brown
Homocatechol	0.56	Red-purple	+	0.72	Brown-red
Phloroglucinol	0.00	Yellow-orange	—	0.87	Reddish mustard yellow
Resorcinol	0.12	Yellow-orange	—	0.94	Mustard yellow
Saligenin	0.62	Crimson	—	0.74	Brown-yellow
8-Hydroxyquinoline	0.60	Purple	—	—	—
3,4-Dihydroxy-ω-chloroacetophenone	0.21	Pale yellow	+	0.62	Yellow
DNP-glycine	0.71	—	—	0.70	—
DNP-ethanolamine	0.88	—	—	0.90	—
Monohydroxy acids					
o-Hydroxybenzoic acid	1.00	Bright red	—	0.74	Yellow-red
m-Hydroxybenzoic acid	0.50	Scarlet	—	0.68	Red
p-Hydroxybenzoic acid	0.42	Light crimson	—	0.65	Red
o-Hydroxyphenyl-propionic acid	0.70	Mauve	—	0.79	Red-mauve
m-Hydroxyphenyl-propionic acid	0.55	Pink	—	0.76	Bright crimson
p-Hydroxyphenyl-propionic acid	0.53	Blue-purple	—	0.73	Blue-purple
o-Hydroxy-*cis*-cinnamic acid	—	—	—	0.81	Purple
o-Hydroxy-*trans*-cinnamic acid	0.65	Bluish mauve	—	0.74	Purple
m-Hydroxycinnamic acid	0.53	Crimson	—	0.70	Crimson
p-Hydroxycinnamic acid	0.50	Bright blue	—	0.66	Blue-grey
o-Hydroxyphenyl-glyoxylic acid	0.22	Salmon	—	0.83	Salmon
p-Hydroxyphenyl-pyruvic acid	0.44	Purple	—	0.70	Purple
Dihydroxy acids					
2,3-Dihydroxybenzoic acid	0.43	Cherry purple	+	0.49	Pale brown
2,4-Dihydroxybenzoic acid	0.30	Brown mustard	—	0.53	Reddish brown
2,5-Dihydroxybenzoic acid	0.20	Yellow	—	0.55–0.85	Pale reddish brown
2,6-Dihydroxybenzoic acid	0.16	Yellow	—	0.78	Brown-grey
3,4-Dihydroxybenzoic acid	0.08	Cherry red	+	0.30	Brown
3,5-Dihydroxybenzoic acid	0.02	Bright yellow	—	0.54	Yellow
2,3-Dihydroxyphenyl-acetic acid	0.15	Cherry purple	+		
2,5-Dihydroxyphenyl-acetic acid	0.03	Brown	—	0.65	White
3,4-Dihydroxyphenyl-acetic acid	0.05	Cherry red	+	0.39	Light brown
2,3-Dihydroxyphenyl-propionic acid	0.28	Cherry purple	+	0.45	Medium orange
2,5-Dihydroxyphenyl-propionic acid	0.09	Brown	—		
3,4-Dihydroxyphenyl-propionic acid	0.13	Cherry red	+	0.42	Quenched red
2,5-Dihydroxyphenyl-cinnamic acid	0.04	Yellow	—	0.4–0.6	Pale brown
3,4-Dihydroxycinnamic acid	0.08	Brownish purple	+	0.40	Grey-brown
Monohydroxydicarboxylic acids					
3-Hydroxyphthalic acid*	—	—	—	—	—
4-Hydroxyphthalic acid	0.02	Bright crimson	—	0.41	Red
Dihydroxydicarboxylic acids					
3,4-Dihydroxyphthalic acid	0.02	Red	+	0.20	Quenched orange
4,5-Dihydroxyphthalic acid	0.01	Pink	+	—	—

* 3-Hydroxyphthalic acid does not appear to give a diazo-*p*-NA colour.

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TABLE I (*continued*)

Substance	Solvent system (a) R_F	Colour by reagent: Diazo-p-NA	Colour by reagent: Evans'	Solvent system (b) R_F	Colour by reagent: Diazo-p-NA
Lactones					
2,5-Dihydroxyphenyl-acetic acid lactone	0.03	Light brown	—	Streak	Brown
2,5-Dihydroxyphenyl-pyruvic acid lactone	0.20	Yellow-orange	—	0.79	Mustard yellow
Aldehydes					
2,5-Dihydroxybenzaldehyde	0.63	Pale yellow	—	0.93	White
3,4-Dihydroxybenzaldehyde	0.13	Pale yellow	+	0.62	Yellow

quenched colours (yellowish brown instead of dark cherry red to purple colours) were observed. The borate complexing effect, noted by WACHTMEISTER[4], is presumably responsible for the quenching of the diazo colour of catechol substances. These reactions are useful confirmatory tests for catechol substances. Table I gives R_F values and colours obtained using a variety of phenolic substances.

Effect of substituents in the aromatic nucleus on R_F value

Table I shows the effect on R_F of the position of the hydroxyl group in monohydroxy-aromatic acids (*ortho* $\gg$ *meta* $>$ *para*). The introduction of a second hydroxyl group leads to a reduction of R_F values. Increased separation of the hydroxyl groups reduces the R_F value in the benzene–acetic acid–water, solvent—*e.g.* 2,3- $>$ 2,4- $>$ 2,5- $>$ 2,6-dihydroxybenzoic acids, but increases the R_F values in the *n*-butanol–ethanol–borate buffer solvent. In the former solvent further separation of the substituents in the aromatic nucleus (*e.g.* 3,4- and 3,5-dihydroxybenzoic acids) decreases the R_F even further. This decrease in the R_F values is presumably connected with an increase in solubility of the free acids in water.

The "anomalies" which occur when the *n*-butanol–ethanol–borate buffer system is used are probably due to two factors: firstly, the phenolic acids are partitioned as their sodium salts, and secondly, the complexing effect of the borate ion influences the partition of catechol substances.

PAPER ELECTROPHORESIS

The application of this technique to the separation of phenolic substances using a weak borate buffer (9.54 g/l, sodium tetraborate) also proved to be useful.

Horizontal paper electrophoresis was employed (paper strip: 24″ × 6″) and the time taken was 7 h (10 V/cm; 5–10 mA). The migration values of various phenolic substances are given in Table II. Zero marker: DNP-ethanolamine. The detection reagent used was diazotised *p*-nitraniline.

This buffer can be used for the study of sugars, glycosides and related aglycones. Preliminary results show that this borate buffer is also suitable for the study of plasma proteins (Azocarmine B detection).

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TABLE II

MDG VALUES AND COLOUR REACTIONS OF VARIOUS PHENOLS AND PHENOLIC ACIDS AFTER PAPER ELECTROPHORESIS

Borate buffer: 9.54 g/l sodium tetraborate. Zero marker: DNP-ethanolamine.

$MDG = \frac{\text{Migration of substance}}{\text{Migration of DNP-glycine}}$ Time: 7 h. 10V/cm, 5–7 mA, Whatman No. 4.

Substance	*MDG*	*Colour by diazo-p-NA reagent*
Miscellaneous		
Catechol	1.18	Pale brown
Homocatechol	1.02	Light brown
Phloroglucinol	1.47 (streaky)	Brown
Resorcinol	0.42	Yellow
Saligenin	1.02	Yellow-orange
3,4-Dihydroxy-ω-chloroacetophenone	0.70	Brown
Monohydroxymonocarboxylic acids		
o-Hydroxybenzoic acid	1.28	Yellow
m-Hydroxybenzoic acid	1.28	Crimson
p-Hydroxybenzoic acid	1.39	Red
o-Hydroxyphenyl-propionic acid	1.12	Mauve
m-Hydroxyphenyl-propionic acid	1.05	Mauve
p-Hydroxyphenyl-propionic acid	1.08	Purple
p-Hydroxyphenyl-pyruvic acid	1.13	Purple
o-Hydroxy-*cis*-cinnamic acid	1.08	Red-purple
o-Hydroxy-*trans*-cinnamic acid	1.20	Purple
m-Hydroxycinnamic acid	1.15	Crimson
p-Hydroxycinnamic acid	1.27	Blue
o-Hydroxyphenyl-glyoxylic acid	1.35	Orange
Dihydroxymonocarboxylic acids		
2,3-Dihydroxybenzoic acid	1.46	Brown
2,4-Dihydroxybenzoic acid	1.60	Dull red
2,5-Dihydroxybenzoic acid	1.20	Grey-brown
2,6-Dihydroxybenzoic acid	1.37	Mustard yellow
3,4-Dihydroxybenzoic acid	1.70	Pale steel grey
3,5-Dihydroxybenzoic acid	1.34	Brownish orange
2,3-Dihydroxyphenyl-acetic acid	1.90	Pale orange
2,5-Dihydroxyphenyl-acetic acid	1.1–1.55 (streak)	Pale brown
3,4-Dihydroxyphenyl-acetic acid	1.70	Quenched purple
2,3-Dihydroxyphenyl-propionic acid	1.60	Pale orange
2,5-Dihydroxyphenyl-propionic acid	1.25–1.80 (streak)	Pale brown
3,4-Dihydroxyphenyl-propionic acid	1.55	Pale orange
2,5-Dihydroxycinnamic acid	1.3–1.9 (streak)	Pale brown
3,4-Dihydroxycinnamic acid	1.49	Brownish yellow
2,5-Dihydroxyphenyl-pyruvic acid	0.88	Yellow
Lactones		
2,5-Dihydroxyphenyl-acetic acid lactone	0.9–1.88 (streak)	Medium brown
Aldehydes		
2,5-Dihydroxybenzaldehyde	0.80	Grey
3,4-Dihydroxybenzaldehyde	1.10	Yellow-brown
Monohydroxydicarboxylic acids		
3-Hydroxyphthalic acid	—	—
4-Hydroxyphthalic acid	1.83	Crimson
Dihydroxydicarboxylic acids		
3,4-Dihydroxyphthalic acid	2.00	Quenched orange
4,5-Dihydroxyphthalic acid	2.05	Quenched orange

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TABLE III

R_F VALUES OF VARIOUS SUGARS AND RELATED COMPOUNDS

Ascending paper chromatography. Paper: Whatman No. 4 buffered with borate buffer. Solvent: *n*-butanol–ethanol–borate buffer (1:1:1). Buffer: sodium tetraborate (9.54 g/l). Detection reagent: alkaline $AgNO_3$.

Substance	R_F	*Substance*	R_F
Arabinose	0.22	Inositol	0.24
Arbutin	0.72	Mannitol	0.30
Ascorbic acid	0.28	Quinic acid	0.42
Cellobiose	0.35	Raffinose	0.25
Erythritol	0.27	Rhamnose	0.50
Fructose	0.23	Salicin	0.77
Fucose	0.40	Shikimic acid	0.27
Galactose	0.27	Sorbitol	0.29
Glucosamine	0.24–0.40	Sorbose	0.25
Glucose	0.28	Sucrose	0.42
Glucuronic acid lactone	0.20	Xylose	0.23

TABLE IV

MDG VALUES OF VARIOUS SUGARS AND RELATED COMPOUNDS

Obtained by paper electrophoresis using a borate buffer (9.5 g/l sodium tetraborate). Detection reagent: alkaline $AgNO_3$. Time: 7 h. 10 V/cm, 5–7 mA. Whatman No. 4 paper.

Substance	*MDG*	*Substance*	*MDG*
Arabinose	1.03	Quinic acid	0.51
Cellobiose	0.27	Raffinose	0.30
Fucose	0.91	Rhamnose	0.53
Galactose	1.00	Sucrose	0.19
Glucose	1.12	Xylose	1.05
Inositol	0.53	Ascorbic acid	1.10

Sugars, glycosides and aglycones

The separation of sugars using the *n*-butanol–ethanol–borate buffer solvent was not as satisfactory as the results obtained for phenolic substances and hence it cannot be used as a general solvent system but it may prove useful in the separation of certain mixtures (see Table III). The same can be said of the paper electrophoretic results for the sugars (Table IV). CONSDEN AND STANIER[6] have used similar electrolytes for sugars.

The application of these paper-chromatographic and paper-electrophoretic methods to some glycosides and related aglycones has been carried out (Table V). Good separation of the glycosides and some of the related aglycones has been achieved.

Fractionation of other types of glycosides has been reported elsewhere[3].

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TABLE V

R_F AND MDG VALUES OF VARIOUS GLYCOSIDES AND AGLYCONES

Solvent: *n*-butanol–ethanol–borate buffer (1:1:1). (a) Sodium tetraborate solution (9.54 g/l). (b) CONSDEN's borate buffer[6]. Paper: Whatman No. 4 impregnated with respective buffer. Detection: U.V.

Solvent	*(a)*	*(b)*	*Electrolyte: (a)*
Substance	R_F	R_F	*MDG*
Glycosides			
Quercetrin**	0.44 (0.82)	0.69 (0.95)	0.98 (0.80)
Phloridzin	0.79	—	0.67
Rutin	0.29	0.29	0.88
Aglycones			
d-Catechin**	0.45 (0.49)	—	0.80 (0.98)
Fisetin	0.40	—	0.08
Formononetin	0.91	—	0.25
Genistein	0.86	—	0.01
Khellin	0.92	—	—0.30*
Morin	0.10	—	0.03
Myricetin	0.15	—	0.03
Quercetin**	0.38 (0.84)	0.25 (—)	0.36 (—0.10)
Robinetin	0.12	—	0.25
Tricin	0.50	—	—0.30*
DNP derivatives			
DNP-ethanolamine	0.90	—	0.00
DNP-glycine	0.70	—	1.00

* Did not move from start.
** Commercial samples contained an additional unknown minor constituent; the value for the unknown is given in brackets.

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SUMMARY

The paper chromatography of phenols (especially phenolic acids), aglycones, glycosides and certain sugars is carried out with two new solvents. The authors have also examined these substances with the aid of paper electrophoresis and a borate buffer. A detection reagent for *o*-diphenols on paper is described.

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