CHROM. 5834

CELLULOSE THIN-LAYER CHROMATOGRAPHY OF PHENOLIC SUBSTANCES

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(First received May 3rd, 1971; revised manuscript received November 24th, 1971)

SUMMARY

R_F values of most common phenolic compounds were worked out on the commercially available cellulose powders MN-300 and Merck-2330 (Avicel), using a new system of thin-layer chromatography, very useful for serial analysis in chemotaxonomy. Colours of these phenolic compounds as intensified by spraying with different chromogenic sprays were also recorded under daylight and long-wave ultra violet light.

It was observed that R_F values and colours of various phenolic substances varied much depending on the type of cellulose powder, the solvents and chromogenic sprays used and thus are useful in identifying these substances. Importance of identification of the phenolic substances for meaningful interpretation in chemotaxonomy is discussed.

INTRODUCTION

Paper chromatography of phenolic substances has been well worked out in detail after BATE-SMITH initiated work on this aspect[1-6]. Usefulness of phenolic substances in solving taxonomic problems[7-9] led to further search for improved and efficient techniques, like thin-layer chromatography (TLC), for better separation, especially when these substances are present in very minute quantities in plant material[10,11].

Since then, separation of certain phenolics and coumarins was attempted by STAHL AND SCHORN[12], MINAMIKAWA *et al.*[13] and COPENHAVER AND CARVER[14] on silica gel and by CHELACK AND RAYNER[15] on aluminium oxide layers. Further, GRANT AND WURTTER[16] used silica gel for separating secondary phenolic compounds employing a multi solvent system which is cumbersome, and apart from this, a vast array of substances such as phenolics cannot be identified on one-dimensional chromatography. Moreover, silica gel is very rarely used for separating phenolics in biochemical systematics because of unsatisfactory detection due to poor colour reactions obtained with diazotized reagents on these layers, VAN SUMERE *et al.*[17] obtained

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improved separation by steaming the cellulose–silica gel mixed layers. However, because of difficulty in steaming and in handling the soft silica gel layers, cellulose layers alone have been preferred and have been found useful in several types of plant material by many workers, *i.e.* JAWORSKA AND NYBOM[18], OLDÉN AND NYBOM[19], BRUNSBERG[20], ISING AND FRÖST[21], DASS AND NYBOM[22], DASS[23], DEDIO *et al.*[24] and WEIMARCK[25] for chemotaxonomic purposes. This is mainly because cellulose layers are very firm, can withstand rough handling, don't require any activation, and are thus most suitable for large scale screening of a number of species in a genera or a number of genera at a time which is required in this type of work.

However, in most of the above papers, phenolic substances have not been identified. Therefore it was thought to be quite worthwhile to work out R_F values of most common phenolic substances on cellulose powders commercially available, so that meaningful interpretation can be done in chemotaxonomic manuscripts.

EXPERIMENTAL

Material

Most of the phenolic compounds were obtained from Eastman Organic Chemicals, Rochester, N.Y.; Nutritional Biochemical Corporation, Cleveland, Ohio; Sigma Chemical Company, St. Louis, Mo.; Aldrich Chemical Company Inc., Milwaukee, Wisc.; all in U.S.A. Apart from this, the authors are grateful to Dr. A. C. NEISH, At-

TABLE I

DETAILS OF SOLVENT SYSTEMS USED FOR DIFFERENT CELLULOSE POWDERS

Cellulose powders	*Solvent systems*	*Running times (min)*
MN-300	FW = formic acid–water (2:98)	37–40
	ACW = *n*-amyl alcohol–acetic acid–water (10:6:5)	188–190
Merck-2330 (Avicel)	FW = formic acid–water (2:98)	69–71
	BPW = benzene–propionic acid–water (20:45:15)	236–240

TABLE II

R_F VALUES OF PHENOLIC COMPOUNDS ON MN-300 AND MERCK-2330 (AVICEL) CELLULOSE POWDERS

Solvent systems FW, ACW and BPW.

Phenolics	*MN-300 layer*		*Merck-2330 layer*	
	FW	*ACW*	*FW*	*BPW*
Apigenin[a]	0.0	0.89	0.0	0.70
Apigenin[b]	—	—	—	0.78
Arbutin	0.22	0.59	0.25	0.53
Benzoic acid	—	—	0.73	—
Catechin	0.34	0.59	0.40	0.38
Catechol	0.75	0.89	0.75	0.84
Caffeic acid[a]	0.22	0.78	0.21	0.62
Caffeic acid[b]	0.58	—	0.57	0.66

TABLE II (*continued*)

Phenolics	*MN-300 layer*		*Merck-2330 layer*	
	FW	*ACW*	*FW*	*BPW*
Chlorogenic acid[a]	0.51	0.66	0.52	0.53
Chlorogenic acid[b]	0.72	0.76	0.71	0.57
Coumarin	0.69	0.96	0.66	—
Cyanidin chloride	—	—	0.00	—
2,4-Dimethoxycinnamaldehyde	0.63	0.96	0.66	1.00
2,6-Dihydroxybenzoic acid[a]	0.75	0.71	0.72	0.65
2,6-Dihydroxybenzoic acid[b]	—	—	—	0.72
2,5-Dihydroxybenzoic acid	0.54	0.86	0.49	0.67
Ellagic acid	0.00	0.40	0.30	0.11
Esculin hydrate[a]	0.63	0.64	0.49	0.55
Esculin hydrate[b]	—	—	—	0.65
Ferulic acid[a]	0.24	0.89	0.20	0.91
Ferulic acid[b]	—	—	0.53	0.95
Gallic acid	0.33	0.57	0.32	0.37
Hesperidin[a]	0.42	0.74	0.42	0.65
Hesperidin[b]	—	—	—	0.75
Hesperetin	0.10	0.94	0.08	0.91
Hydroquinone	0.76	0.84	0.74	0.72
p-Hydroxycinnamic acid	0.27	0.91	0.27	0.87
o-Hydroxycinnamic acid	0.38	0.95	0.35	0.92
p-Hydroxybenzoic acid	0.50	—	0.64	0.85
m-Hydroxybenzoic acid	0.60	0.95	0.65	0.83
p-Hydroxyphenylacetic acid	0.85	0.91	0.80	0.82
o-Hydroxyphenylacetic acid	0.87	0.94	0.82	0.86
Kaempferol	0.0	0.91	0.0	0.71
Malvin chloride	0.33	0.43	0.25	—
Myricetin	[b]	0.54	[b]	0.22
Mandelic acid	0.89	—	0.90	—
Phloridzin	0.30	0.75	0.29	0.61
Phloretin	0.05	0.94	0.05	0.73
Quercetin[a]	0.0	0.72	0.00	0.44
Quercetin[b]	—	—	—	0.65
Rutin[a]	0.30	0.64	0.31	0.40
Rutin[b]	—	0.70	—	0.55
Syringic acid	0.50	0.88	0.51	0.92
Scopoletin	0.29	0.87	0.25	0.84
Sakuranetin	0.05	0.96	0.073	0.99
Sakuranin[a]	0.29	0.79	0.20	0.72
Sakuranin[b]	0.39	—	0.40	—
Sinapic acid[a]	0.20	0.86	0.18	0.87
Sinapic acid[b]	0.49	—	0.45	—
Taxifolin[a]	0.29	0.75	0.32	0.58
Taxifolin[b]	—	—	—	0.68
Vanillic acid	0.48	0.89	0.50	0.91
Vanillin	0.65	0.93	0.63	0.98
Vitexin[a]	0.05	0.57	0.02	0.14
Vitexin[b]	—	—	—	0.39

[a] Division in a, b under different compounds refers to splitting of a compound into its isomers, and thus R_F values of different isomers are given.
[b] Streaked.

TABLE III

EFFECT OF DIFFERENT CHROMOGENIC SPRAYS ON THE COLOUR OF PHENOLIC SPOTS AS OBSERVED IN DAYLIGHT AND UV LIGHT

Spots are obtained by TLC on cellulose. Further details in text and in Tables I and II.

Phenolics	*No spray*		*Spraying compounds*						
	In daylight	*In UV light*	*Turnbull's blue reagent; in daylight*	*Fast Blue RR + NH_3; in daylight*	*Fuming with NH_3; in UV light*	*Methanolic NaOH; in UV light*	*Methanolic $AlCl_3$; in UV light*	*Methanolic zinc acetate; in UV light*	*Flavone reagent; in UV light*
Apigenin	—	violet to black	—	yellow	black	grey	light yellow	grey	yellow
Arbutin	—	light violet	blue	—	light violet	grey	light violet	—	white grey
Benzoic acid	—	—	whitish	—	—	—	—	—	—
Catechin	blackish tinge	light violet	blue	brown	light violet	grey to blue violet	violet	violet	light violet
Catechol	—	light violet	blue	brownish tinge	light violet	light brown to violet	light to dark violet	violet	light violet
Caffeic acid	—	blue	blue	light brown	blue	bluish	blue	whitish blue	greenish white to bluish
Chlorogenic acid	—	blue	blue	light brown	blue	greenish bluish	blue	whitish blue	greenish white
Coumarin	—	—	—	—	—	—	—	—	—
Cyanidin chloride	—	light violet to grey	—	—	violet	grey to blackish	grey to black	black	blackish
2,4-Dimethoxy-cinnamaldehyde	yellow	greenish yellow	blue	yellow	dark green	dark to yellowish green	pinkish yellow	dark green	dark yellow
2,6-Dihydroxy-benzoic acid	—	green to light violet	blue	light violet	light violet	bluish violet	blue	grey	blue
2,5-Dihydroxy-benzoic acid	—	blue	blue	brownish tinge	blue	grey	blue	blue	intense blue
Ellagic acid	—	grey tinge	light blue	brown	—	brownish yellow	grey	light yellow	grey
Esculin hydrate	—	blue	blue	brown	blue	blue	blue to violet	intense blue	blue
Ferulic acid	—	dark blue	blue	light yellow	blue	blue	blue	blue	bluish tinge
Gallic acid	—	—	blue	light brown	—	muddy yellow	bluish grey	violet	blue violet
Hesperidin	—	—	—	—	—	grey	grey	grey	—
Hesperetin	—	grey to light violet	blue	brown	light violet	light to dark grey	greenish yellow	grey	yello
Hydroquinone	—	grey to light violet	blue	—	light violet	brownish	grey to violet	—	—
p-Hydroxy-cinnamic acid	—	bluish	blue	orange	bluish	intense blue	violet	tinge of blue	—
o-Hydroxy-cinnamic acid	—	whitish grey	blue	whitish	whitish grey	light green	bluish grey	white grey	grey
p-Hydroxy-benzoic acid	—	—	yellow	whitish	—	light violet	light violet	violet tinge	—

Hesperidin	—	grey to light violet	blue	brown	light violet	grey light to dark grey	grey greenish yellow	grey grey	— yellow
[illegible]	—	grey to light violet	blue	—	light violet	brownish	grey to violet	—	—
p-Hydroxy-cinnamic acid	—	bluish	blue	orange	bluish	intense blue	violet	tinge of blue	
o-Hydroxy-cinnamic acid	—	whitish grey	blue	whitish	whitish grey	light green	bluish grey	white grey	grey
p-Hydroxy-benzoic acid	—	—	yellow	whitish	—	light violet	light violet	violet tinge	—
m-Hydroxy benzoic acid	—	—	bluish white	light yellow	—	blue	light violet	—	—
p-Hydroxyphenyl-acetic acid	—	light violet	blue	light yellow	light violet	light violet	bluish violet	violet tinge	—
o-Hydroxyphenyl-acetic acid	—	light violet	blue	light yellow	light violet	grey to violet	violet	violet tinge	light blue
Kaempferol	light yellow	muddy yellow	—	dark brown	yellow	orange yellow	green to yellow	yellow	yellow
Malvin chloride	violet	pinkish brown	—	violet	pink	—	pink	pink	—
Myricetin	yellow	yellow	—	yellow	grey	black to violet	yellow	yellow	orange
Mandelic acid	—	—	whitish blue	—	grey	—	—	—	—
Phloridzin	—	—	blue	brown	—	brownish	grey to bluish	—	grey
Phloretin	—	—	blue	dark brown	light violet	grey	grey to light yellow	light violet	muddy yellow
Quercetin	yellow	muddy yellow	blue	muddy yellow	yellow	orange brown	yellow to green	yellow	orange
Rutin	yellow	black violet	blue	yellow	dark grey	yellow	yellow	muddy yellow	orange
Syringic acid	—	blackish blue	blue	light yellow	grey	light violet	violet	grey	bluish grey
Scopoletin	—	blue	—	brown	blue	light blue	blue	intense blue	dark blue
Sakuranetin	—	—	light blue	—	—	grey	grey to greenish	grey	muddy yellow
Sakuranin	—	grey	light blue	—	grey	orange	grey	grey	grey
Sinapic acid	—	blue	blue	light yellow	blue	light blue to blue	dark blue to blue	blue	blue
Taxifolin	—	violet grey	blue	light brown	violet	brownish	muddy yellow	grey	dark brown to black
Vanillic acid	—	light violet	blue	tinge of yellow	light violet	light violet	light violet	—	—
Vanillin	—	—	blue	—	blue	violet	light blue	light blue	grey
Vitexin	—	light violet	—	—	light violet	light yellow	whitish grey to yellow	light yellow	yellow

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lantic Regional Laboratory, Halifax, Canada, for the gift of cyanidin chloride, malvin chloride, sinapic acid and sakuranetin; to Dr. T. J. MABRY, Cell Research Institute, University of Texas, Austin, U.S.A., for the gift of myricetin, rutin, sakuranin, apigenin, hesperidin; and to Dr. M. K. SEIKEL, Forest Products Laboratory, Madison, Wisc., U.S.A., for the gift of vitexin and taxifolin.

Solutions of different phenolic compounds were made in ethanol.

Thin-layer plates of the size 16 × 12 cm with a 350 μ-thick cellulose layer were prepared by the method of NYBOM[26]. For preparation of plates usually 17 g of cellulose powder (MN-300 or Merck-2330 (Avicel)) along with 120 ml of water were used to make a slurry in a fast electric mixer. The plates were allowed to dry overnight.

Methods

All the chromatographic work was carried out at a temperature of 24–25°. Solvent systems and their running times for the two cellulose powders are given in Table I. In each run plates were equilibrated for 1 h. Chromatographic glass jars were lined with filter paper.

Detection of phenolic substances was done by observing the chromatograms in daylight before and after spraying with Fast Blue RR salt (0.5 % solution in water) and fuming with ammonia, or with Turnbull's blue reagent (equal portions of 0.5 % solutions of ferric chloride and potassium ferricyanide in water are mixed just before spraying) and also by observing in long-wave UV light before and after spraying with methanolic solutions of NaOH, $AlCl_3$, zinc acetate, Flavone reagent (diphenyl boric acid ethanol amine complex) and fuming with ammonia. The solutions of NaOH, $AlCl_3$ and Flavone reagent were prepared as 1 % in methanol. Zinc acetate solution was made by dissolving 2.0 g of zinc acetate in a few milliliters of acetic acid and water and then making up the volume to 100 ml with methanol.

R_F values of different phenolic substances were averaged from four chromatograms.

RESULTS AND DISCUSSION

Table II shows how the solvent systems used have proved good to separate and spread the phenolics over most of the area of the chromatograms. However, the solvent systems FW and BPW used for Merck-2330 powder proved better in spreading the spots over a greater area of the chromatogram, instead of concentrating them to one portion of the chromatogram.

The colour of different phenolic substances as intensified by different chromogenic sprays also showed that these sprays can be quite useful in identifying these substances (Table III). This identification of phenolic substances can be used in the interpretation of evolutionary pathways as studied by biochemical systematics in *Lemnaceae*[27] and *Baptisia*[28].

ACKNOWLEDGEMENT

Senior author (H.C.D.) is grateful to the National Research Council of Canada for the award of post-doctoral fellowship (1967–1968) during the tenure of which this work was done.