CHROM. 4743

Thin-layer chromatography of some plant phenolics

In the course of studies on the role of phenolics in plant metabolism it became necessary to separate and identify a number of phenolic compounds. Although there have been many reports in the literature on thin-layer chromatography (TLC) used for this purpose[2–5] much of the information available for the compounds of interest has been obtained using paper chromatography[6–8]. In order to obtain the advantages of speed and sensitivity available with TLC we felt it necessary to determine the R_F values of various standards by this technique.

TLC was carried out on 20 × 20 cm MN-polygram CEL 300 cellulose sheets obtained through Brinkmann Instruments, Inc. The solvents used were 2% formic acid (A), 20% potassium chloride (B), isopropyl alcohol–ammonium hydroxide–water, 8:1:1 (C), and 10% acetic acid (D). The solvents were prepared fresh immediately before use. The plates were examined under short-wave UV light after developing and the properties of the compounds were noted. The plates were then sprayed with freshly prepared diazotized *p*-nitroaniline or diazotized sulfanilic acid[1] and the colors which developed were noted within 20 min.

The characteristics of a number of phenolics, coumarins and flavonoids in this system are reported in Table I. Detection of the compounds could be made at the 0.2–1.0 μg range.

TABLE I

CHARACTERISTICS OF SOME PHENOLICS, FLAVONOIDS AND COUMARINS

Compound	R_F				*Detection*		
	A	*B*	*C*	*D*	*UV*	*DSA*	*DNA*
Phenolics							
Acids							
m-Anisic	0.66	0.67	0.49	0.65	F[a]	lt. tan	—
p-Anisic	0.56	0.62	0.49	0.53	Q[b]	tan	tan
Caffeic	0.11	0.16	0.23	0.36	bl. F	tan	grey
Chlorogenic	0.08	0.45	0.49	0.68	bl. F	grn-tan	tan
trans-Cinnamic	0.64	0.57	0.44	0.54	Q	—	—
o-Coumaric	0.34	0.39	0.29	0.51	F	orange	violet
m-Coumaric	0.43	0.26	0.33	0.52	F	orange	violet
p-Coumaric	0.34	0.33	0.28	0.47	Q	red	blue
3,4-Dihydroxyhydrocinnamic	0.18	0.64	0.70	0.76	Q	brown	violet
3,4-Dimethoxybenzoic	0.53	0.32	0.50	0.66	F	tan	violet
Ferulic	0.28	0.21	0.19	0.43	bl. F	purple	blue
Gallic	0.09	0.33	0.31	0.46	F	grn-brwn	tan
Gentisic	0.29	0.37	0.32	0.54	bl. F	yel-brwn	tan
o-Hydroxybenzoic (salicylic)	0.70	0.57	0.44	0.62	bl. F	yellow	red
m-Hydroxybenzoic	0.37	0.63	0.58	0.70	F	yellow	rose
p-Hydroxybenzoic	0.27	0.59	0.52	0.70	Q	yellow	red
3-Hydroxy-4-methoxycinnamic	0.26	0.14	0.21	0.39	F	orange	purple
Isochlorogenic	0.05	0.04	0.10	0.26	F	grn-tan	yel-tan
p-Methoxycinnamic	0.63	0.09	0.14	0.32	Q	—	—
5-Methoxysalicylic	0.07	0.44	0.41	0.60	bl. F	brown	—

TABLE I *(continued)*

Protocatechuic	0.05	0.51	0.44	0.60	bl. F	tan	grey
Pyrocatechuic	0.19	0.48	0.44	0.63	bl. F	tan	violet
α-Resorcylic	0.21	0.48	0.45	0.59	Q	red-orange	yellow
β-Resorcylic	0.30	0.40	0.39	0.54	bl. F	yel-brwn	yellow
γ-Resorcylic	0.71	0.39	0.66	0.81	F	brown	orange-tan
Sinapic	0.18	0.14	0.23	0.38	bl. F	violet	purple
Syringic	0.10	0.46	0.43	0.63	F	red	blue
Vanillic	0.18	0.52	0.44	0.63	F	orange	violet
Aldehydes							
2,4-Dihydroxybenzaldehyde	0.37	0.43	0.54	0.65	F	brown	brown
2,5-Dihydroxybenzaldehyde	0.38	0.52	0.62	0.71	bl. F	grn-brwn	green
3,4-Dimethoxybenzaldehyde	0.94	0.45	0.66	0.78	y. F	—	—
4,6-Dimethoxysalicylaldehyde	0.90	0.08	0.18	0.38	F	yellow	—
m-Hydroxybenzaldehyde	0.79	0.57	0.69	0.78	Q	yellow	—
p-Hydroxybenzaldehyde	0.64	0.56	0.68	0.76	Q	yellow	tan
Protocatechualdehyde	0.36	0.50	0.60	0.68	Q	grn-grey	tan
Salicylaldehyde	0.63	0.52	0.63	0.71	F	orange	—
Syringaldehyde	0.46	0.48	0.62	0.73	Q	pink	bl-grey
Vanillin	0.62	0.53	0.63	0.73	Q	orange	violet
o-Vanillin	0.61	0.49	0.61	0.74	F	yellow	pink
Alcohols and phenols							
Catechol	0.42	0.65	0.69	0.77	Q	brown	brwn-viol
Coniferyl alcohol	0.89	0.38	0.56	0.66	Q	violet	bl-grey
Eugenol	0.98	0.09	0.27	0.32	—	red	blue
Guaiacol	0.95	0.69	0.71	0.79	—	orange	violet
o-Hydroxybenzyl alcohol	0.92	0.71	0.83	0.85	Q	gold	rose
Isoeugenol	0.94	0.06	0.15	0.21	Q	peach	violet
Isovanillyl alcohol	0.83	0.67	0.79	0.83	Q	orange	purple
Phloroglucinol	0.16	0.42	0.53	0.63	—	yel-brwn	brown
Flavonoids							
Apigenin	0.17	0.00	0.00	0.00	Q	gold	yellow
Catechin	0.08	0.20	0.31	0.58	Q	yel-grn	brown
Chrysin	0.64	0.00	0.00	0.04	Q	yellow	orange
Fisetin	0.05	0.00	0.00	0.02	y. F	orange	oran-brwn
Fustin	0.09	0.24	0.33	0.50	F	tan	tan
Hesperetin	0.32	0.02	0.04	0.16	Q	orange	violet
Kaempferol	0.02	0.00	0.00	0.02	or. F	yellow	yellow
Morin	0.02	0.00	0.02	0.13	or. F	orange	yellow
Myricetin	0.00	0.00	0.00	0.00	y. F	grn-brwn	grn-brwn
Naringenin	0.48	0.03	0.04	0.19	—	gold	yel-brwn
Phloretin	0.48	0.00	0.03	0.05	Q	orange	yellow
Quercetin	0.03	0.00	0.00	0.01	gr. F	grey-grn	brown
Rutin	0.04	0.15	0.29	0.49	Q	tan	yel-grn
Coumarins							
Coumarin	0.46	0.43	0.56	0.72	Q	orange	violet
5,7-Dihydroxy-4-methylcoumarin	0.45	0.06	0.10	0.34	F	orange	orange
5,7-Dimethoxycoumarin	0.97	0.06	0.14	0.25	bl. F	yellow	red-brwn
Esculetin	0.16	0.21	0.28	0.46	bl. F	yel-brwn	yellow
Esculin	0.45	0.54	0.61	0.75	F	yellow	—
4-Hydroxycoumarin	0.72	0.41	0.50	0.57	F	yellow	violet
4-Methyldaphnetin	0.21	0.19	0.29	0.47	F	yellow	yellow
4-Methylumbelliferone	0.62	0.21	0.34	0.64	bl. F	tan	tan
Scopoletin	0.47	0.17	0.32	0.49	bl. F	violet	yellow
Umbelliferone	0.60	0.32	0.43	0.61	bl. F	yel-brwn	yel-brwn

[a] F indicates fluorescence.
[b] Q indicates quenching.

The system used proved satisfactory in most respects. Two-dimensional chromatography using the following solvent combinations could be used to separate mixtures of phenolics: A–C, B–C, D–C, and D–B. Solvent C proved to be unsatisfactory for use as the first solvent in a two-dimensional system.

Biological Sciences Research Center,
Shell Development Company, Modesto, Calif. 95352 (U.S.A.)

N. O. JANGAARD*

1 R. M. ACHESON, R. M. PAUL AND R. V. TOMLINSON, *Can. J. Biochem. Physiol.*, 36 (1958) 295.
2 S. ASEN, *J. Chromatog.*, 18 (1965) 602.
3 J. D. CONRADIE AND L. P. NEETHLING, *J. Chromatog.*, 34 (1968) 419.
4 J. W. COPIUS-PEEREBOOM AND H. W. BEEKES, *J. Chromatog.*, 14 (1964) 417.
5 J. W. FRANKENFELD, *J. Chromatog.*, 18 (1965) 179.
6 L. A. GRIFFITHS, *Nature*, 180 (1957) 286.
7 L. A. GRIFFITHS, *Nature*, 182 (1958) 733.
8 J. B. HARBORNE AND J. J. CORNER, *Biochem. J.*, 81 (1961) 242.

Received March 9th, 1970

* Present address: Shell Development Company, Biological Sciences Research Center, P.O. Box 4248, Modesto, Calif. 95352, U.S.A.

CHROM. 4776

A spray reagent for methylated phenolic compounds on thin-layer plates

Phosphomolybdic acid is a nonspecific reagent which gives blue spots with phenolic compounds and their methyl ethers[1] and also with lipids[2] on thin-layer plates. In order to develop a specific spray reagent for some classes of methylated phenols, use has been made of the characterist iccolour reaction of certain phenolic methyl ethers when these are dissolved in conc. HNO_3 (ref. 3). Examination of conc. HNO_3 as a spray reagent with various types of phenolic methyl ethers has shown its suitability as a sensitive spray reagent on thin-layer chromatograms of Silica Gel G (E. Merck). The results are detailed in Table I.

TABLE I

COLOUR DETECTION OF PHENOLIC METHYL ETHERS
The sensitivity in each case was 5 μg.

Sample No.	*Name of the compound*	*Colour produced*	*Time taken for colour development*
1	Phloroglucinol trimethyl ether	Deep blue	Immediately
2	Resorcinol dimethyl ether	Green	Immediately
3	Tetra-O-methyl-ellagic acid	Light blue	Immediately
4	Vanillin	Deep yellow	Immediately
5	Ferulic acid	Deep yellow	2 min
6	Vanillic acid	Yellow	5 min