Table 4 R_f Values of Anthocyanidins and Anthocyanins on Cellulose (0.1 mm, Merck)

Pigment[a]	S[b]	Substituent position[c]		Solvent system		
		OH	OCH_3	1	2	3
Aglycones						
Delphinidin	a	3,5,7,3′,4′,5′		—	0.11	0.03
Petunidin	a	3,5,7,4′,5′	3′	—	0.20	0.05
Cyanidin	a	3,5,7,3′,4′		—	0.22	0.06
Malvidin	a	3,5,7,4′	3′,5′	—	0.27	0.07
Peonidin	a	3,5,7,4′	3′	—	0.31	0.08
Pelargonidin	a	3,5,7,4′		—	0.35	0.11
Monoglycosides						
Dp-3-glu	b			0.08	0.38	0.13
Pt-3-glu	c			0.13	0.49	0.23
Cy-3-glu	b			0.17	0.51	0.25
Mv-3-glu	c			0.22	0.64	0.34
Pn-3-glu	c			0.25	0.64	0.38
Pg-3-glu	d			0.32	0.65	0.40
Diglycosides (biosides)						
Dp-3-rut	b			0.24	0.69	0.36
Cy-3-rut	b			0.35	0.69	0.49
Pn-3-rut	e			0.47	0.76	0.63
Cy-3-sam	f			0.47	—	0.64
Cy-3-sop	f			0.62	0.81	0.75
Diglycosides						
Cy-3,5-diglu	g			0.38	0.70	0.52
Pn-3,5-diglu	g			0.49	0.81	0.67
Triglycosides						
Cy-3-glurut	f			0.80	0.86	0.88

[a]DP = delphinidin, Pt = petunidin, Cy = cyanidin, Mv = malvidin, Pn = peonidin, Pg = pelargonidin, glu = glucoside, rut = rutinoside, sam = sambubioside, sop = sophoroside, glurut = (2^G-glucosyl)rutinoside.

[b]Pigment source: a, hydrolysis product; b, *Ribes nigrum* berry; c, *Vitis vinifera* fruit; d, *Fragaria* spp. berry; e, *Prunus* spp. fruit; f, *Rubus idaeus* berry; g, *Fuchsia* spp. flowers.

[c]Substituent position refers to the numbering in Fig. 5.

[d]A mixture of conc. hydrochloric acid, formic acid, and water is used as follows: System 1 (19:19:62), system 2 (7:51:42), and system 3 (25:24:51).

jected to cellulose TLC and paper chromatography is comparable, and R_f data for a large number of anthocyanins have been provided by paper chromatography (10).

Anthocyanins are visible at the concentration levels encountered on chromatograms. Examination under UV light is worthwhile, however, because the 3,5-diglycosides of pelargonidin, peonidin, and malvidin are distinguished by their fluorescence from the corresponding 3-glycosides. After the dried chromatograms have been sprayed with aluminum chloride (3% in methanol), anthocyanins with their free adjacent hydroxyls on the B-ring of the aglycone (delphinidin, cyanidin, and petunidin derivatives) turn blue.

C. Practical Experiments

1. Extraction

Anthocyanin extraction should be performed using methanol or ethanol mixed with a weak acid such as acetic acid (5%) or trifluoroacetic acid (3%). The low pH of the extract facilitates the