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THE FLOWER PIGMENTS OF THE BELGIAN HYBRIDS OF *RHODODENDRON SIMSII* AND OTHER SPECIES AND VARIETIES FROM *RHODODENDRON* SUBSERIES OBTUSUM

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Abstract—The flower pigments of about 100 different varieties of the Belgian hybrids of *Rhododendron simsii* and other representative species and varieties from the *Rhododendron* subseries obtusum were studied by TLC and absorption spectroscopy. The most common anthocyanins are cyanidin 3-monoglucoside and cyanidin 3,5-diglucoside. Normally both pigments co-occur; the concentration of either one may strongly exceed that of the other by 4- to 10-fold. Several varieties also contain peonidin 3,5-diglucoside. The violet-coloured species and varieties contain malvidin 3,5-diglucoside. Azaleatin 3-rhamnosylglucoside is the most common flavonol. The violet flowers also contain myricetin 5-methyl ether. *R. indicum* and some Kaempferi-Malvatica hybrids contain quercetin instead of azaleatin.

INTRODUCTION

FLOWER bud variation in the Belgian hybrids of the evergreen *Rhododendron simsii* Planch. (syn. *Azalea indica* or Chinese Azalea) is a well-known phenomenon. Those sports introduced as clonal strains have played a very important role in the creation of new varieties. In order to elucidate the true nature of the sporting process one should first know the nature of flower pigments. A new flower colour can be the result of new pigment synthesis but it may also be caused by a change in either anthocyanins or flavonols. Therefore, the flower pigments of about 100 different varieties (hybrids and sports) have been studied. *R. simsii* Planch. belongs to the subseries obtusum (Azalea series) and other representatives of the same subseries that have been included in our study are: (*a*) from the Kurume group: *R. obtusum* Planch. var. *kaempferi* Wils., *R. obtusum* f. *japonicum* Wils. (syn. *R. kiusianum*) and *R. obtusum* f. *amoenum* Wils.; (*b*) from the Ryukyu group: *R. mucronatum* G. Don. f. *ripense* Wils. and *R. pulchrum* Sweet. var. *maxwellii*; (*c*) the Indica Azalea: *R. indicum* Sweet. (macrantha) and the clone Kokinshita; and (*d*) the Korean Azalea: *R. yedoense* Rehder (syn. *Azalea yodogawa* Grignan) and its form *R. yedoense* f. *poukhanense* Nakai. Several varieties belonging to the Kurume- and the Kaempferi-Malvatica hybrids were also included.

Robinson and Robinson,[1,2] using distribution tests, recorded the presence of cyanidin 3,5-dimonoside in the bluish-red flowers of the variety Mme. Van der Cruyssen, of a mixture of cyanidin 3-glycoside and cyanidin 3,5-diglycoside in the variety "Hindu Girl" and of cyanidin 3-monoside in a salmon-red variety. Hayashi[3] found quercetin derivatives as co-pigments of the anthocyanins in flowers of *Azalea hinodigeri* and Wada[4] later isolated from

[1] G. M. ROBINSON and R. ROBINSON, *Biochem. J.* **15**, 1692 (1931).
[2] G. M. ROBINSON and R. ROBINSON, *Biochem. J.* **16**, 1650 (1932).
[3] K. J. HAYASHI, *Pharmazie* **5**, 245 (1957).
[4] E. WADA, *J. Am. Chem. Soc.* **78**, 4725 (1956).

white flowers of *R. mucronatum* G. Don. a quercetin 5-methyl ether, azaleatin, as the 3-rhamnoside (azalein), the structure of which was confirmed by Jurd and Horowitz.[5]

In the present work, TLC on cellulose has been used for isolation and purification. Absorption spectral measurements and R_f values were used for pigment identification.

RESULTS AND DISCUSSION

1. *Belgian Hybrids of* Rhododendron simsii

A. Anthocyanins. The flower colour of the Belgian hybrids of *Rhododendron simsii* ranges from white, pink, rosy red, orange, scarlet to magenta and violet. Most red varieties only contain one anthocyanidin, i.e. cyanidin, that occurs either as the 3-glucoside or as the 3,5-diglucoside. Cyanidin 3-glucoside is accompanied by a second pigment provisionally identified as the 3-glucosylglucoside and likewise cyanidin 3,5-diglucoside by the 3-glucosylglucoside-5-glucoside (Table 1). For simplicity in the following account, the terms 3-glucoside and 3,5-diglucoside are used to include the secondary pigments. Normally both 3-mono- and 3,5-diglucosides of cyanidin are present in one variety but the concentration of either one may strongly exceed that of the other (4–10-fold). A few varieties also contain peonidin (as the 3,5-diglucoside) and the few violet ones malvidin (as the 3,5-diglucoside).

TABLE 1. SPECTRAL CHARACTERISTICS AND R_f VALUES OF ANTHOCYANINS ISOLATED FROM BELGIAN HYBRIDS OF *Rhododendron simsii*

	λ_{max} (nm) in MeOH—		R_f Descending chromatography on Whatman No. 1			
Pigment	0·01 % HCl	+ $AlCl_3$	BAW	Bu–HCl	1 % HCl	HAc–HCl
Cyanidin 3-glucosylglucoside-5-glucoside	526	565	0·25	0·06	0·56	0·34
Cyanidin 3,5-diglucoside	524	559	0·31	0·09	0·13	
Cyanidin 3-glucosylglucoside	526	564	0·35	0·23	0·43	0·22
Cyanidin 3-monoglucoside	526	558	0·40	0·24	0·05	
Peonidin 3,5-diglucoside*	522	522	0·26	0·13	0·16	0·42
Malvidin 3,5-diglucoside*	534	534	0·22	0·09	0·15	0·38

* The peonidin and malvidin 3,5-diglucosides when run in BAW on cellulose-coated glass plates also separate in two spots in the same manner as the cyanidin glucosides. The R_fs are between those of cyanidin 3,5-diglucoside and cyanidin 3-glucosylglucoside.

B. Flavonols. Azaleatin occurs in fluctuating amounts in the different varieties; normally the concentration is high in deep-red and blue–red varieties and very low in scarlet, salmon and stone-red varieties. This flavonol usually occurs bound to glucose and rhamnose but a 3-glucoside is also present in white varieties (Table 2). Myricetin 5-methyl ether has been found in the few varieties with violet flowers. Egger[6] previously reported the presence of the latter flavonol in violet flowers of *R. catawbiense* Michx., *R. japonicum* Suring. and *R. obtusum* Planch. var. *amoenum* Rehd.

C. Survey of the flower pigment composition of the different varieties. I. Varieties containing one anthocyanidin (cyanidin). A. The amount of cyanidin 3-monoglucoside strongly exceeds that of the 3,5-diglucoside (4–10-fold).

(*a*) Deep red → blue red (high flavonol content): Olympia, Ambrosius, Ambrosiana, Knut Erwin, Martin Winter, Carmen, Prosper Van den Dael, Leuchtfeuer,

[5] L. JURD and R. M. HOROWITZ, *J. Org. Chem.* **22**, 1618 (1957).
[6] K. EGGER, *Z. Naturforsch.* **17b**, 489 (1962).

TABLE 2. SPECTRAL CHARACTERISTICS AND R_f VALUES OF FLAVONOLS ISOLATED FROM BELGIAN HYBRIDS OF *Rhododendron simsii*

Pigment	λ_{max} (nm) in				R_f in*			
	EtOH	EtOH–$AlCl_3$	EtOH–NaOAc–H_3BO_3	EtOH + 0·002 M NaOEt	BAW	60% HAc	Forestal	H_2O–HCO_2H–HCl (60:30:3)
Azaleatin 3-glucoside†	346, 248	428	368	n.d.‡	0·62	0·64	n.d.	0·38
Azaleatin 3-rhamnoglucoside	338, 294, 247	428	357	n.d.	0·79	0·63	n.d.	0·56 (0·60 on paper)
Azaleatin (hydrolysis product)	366, 252	428	385	326, 440, 276	0·58	0·27	0·45	0·03 (0·12 on paper)
Myricetin-5-methyl ether (hydrolysis product)	372, 252	431	391	n.d.	0·32	0·19	0·29	0·03

* R_fs *determined on purified pigments by descending chromatography on* Whatman No. 1 paper (BAW, 60% HAc and Forestal) or on crude extracts by ascending chromatography on cellulose plates (H_2O–HCO_2H–HCl). Quercetin was run as a reference and it had R_f 0·78 in BAW and 0·36 in Forestal.

† Spectral measurements were made on all flavonols in EtOH–NaOAc; Band II disappeared and an inflection appeared at 260–280 nm.

‡ Not determined.

TABLE 3. FLOWER PIGMENTS OF SOME SPECIES AND VARIETIES FROM THE *Rhododendron* SUBSERIES OBTUSUM

Species or varieties	Relative amount of anthocyanins*	Pigments							
		Cyanidin 3-mono-glucoside†	Cyanidin 3,5-di-glucoside‡	Peonidin 3,5-di-glucoside	Malvidin 3,5-di-glucoside	Azaleatin-3-rhamno-glucoside	Myricetin 5-methyl ether	Quercetin	Kaempferol
SPECIES									
Rhododendron obtusum Planch. f. *amoenum* Wils.									
Red sport	100	(+)	+++	+++	−	+++	−	−	−
Violet	58 (71C)	−	+++	−	++	++	+	−	−
R. obtusum Planch. var. *kaempferi* Wils.	54	(+)	+++	−	−	(+)	−	−	−
R. obtusum Planch. f. *japonicum* Wils.									
Violet	29	−	−	−	++	+++	++	−	+
White sport	0	−	−	−	−	++	−	−	+
R. pulchrum Sweet. var. *maxwellii*	51	(+)	+++	+++	−	+++	−	−	−
R. mucronatum G. Con f. *ripense* Wils.	3	−	−	−	+	+++	++	−	+
R. indicum Sweet.	50 (62A)	+++	+	−	−	−	−	++	−
Kokinshita	76	+++	+	−	−	−	−	(+)	−
R. yedoense Rehder	32	(+)	(+)	−	+++	+++	++	−	+
R. yedoense f. *poukhanense* Nakai	25 (78C)	(+)	(+)	−	+++	+++	++	−	+
Malvatica	26 (72C)	−	−	−	+++	+++	++	−	+
VARIETIES									
Violet colour									
Beethoven (Vuykiana hybrid)	39 (72C)	−	−	−	+++	+++	++	−	+
Hatsugiri (Kurume hybrid									
Violet	54 (72D)	−	++	−	+++	++	+	−	−
Red sport	51 (67C)	(+)	+++	+++	−	+++	−	−	−
Deep Red to Pink colour									
Moederkensdag (Kurume × *indicum*)	95 (53D)	+++	+	+	−	+++	−	−	−
Mr. Wuyts	95	+++	+	++	−	−	−	(+)	−
Toreador	91 (58B)	+++	(+)	−	−	+++	−	−	−
Hinodigeri (Kurume hybrid)	83 (57D)	(+)	+++	++	−	−	−	+	−

Brazier	73	+++	++	(+)	–	–	–	+	–
Alice (Kaempferi × Malvatica)	74 (52B)	+++	++	++	–	–	–	(+)	–
Rosy Red colour									
Betty (Kaempferi × Malvatica)									
Rose	41 (61D)	+++	+	–	–	–	–	(+)	–
Salmon	68 (46D)	+++	++	–	–	–	–	(+)	–
Splendide	63 (47D)	+++	+	+	–	+	–	–	–
Mikado	52 (61D)	+++	++	+	–	–	–	(+)	–
Cangallo (Kurume hybrid)	39 (66B)	–	+++	–	–	–	–	–	–
Hinomayo (Kurume hybrid)									
Red	33 (63C)	(+)	+++	+	–	+++	–	–	+
Salmon	39 (62A)	(+)	+++	+	–	++	–	–	+
Rex	31 (48C)	+++	+	–	–	–	–	–	–
Royal Pink	27 (63C)	+++	+	(+)	–	–	–	(+)	–
Esmeralda (Kurume hybrid)	25 (62A)	–	+	+	–	+++	–	–	–
Blauw's Pink (Kurume hybrid)	23 (52CD)	+	+++	++	–	–	–	–	–
Pink Pearl	15 (55C)	(+)	+++	+++	–	(+)	–	–	–
Kirin (Kurume hybrid)	12 (55BC)	+++	+	+	–	(+)	–	–	–

Key: –, absent; (+), very low relative amount (for anthocyanins: within one and the same variety) (for flavonols: also between varieties and species); + + +, high relative amount.

* The relative anthocyanin amounts have been determined by mixing 3 g of fresh flower petals and anthers in a volume of 250 ml HCl 1 % in water. The extinction was measured on a suitable aliquot at $\lambda_{max} = 512$ nm. The highest value thus obtained was equalled to 100 % (*R. obtusum* form *amoenum* with red flowers). Figure in bracket refers to the R.H.S. Horticultural Colour Chart Rating.

† This refers to the mixture of cyanidin 3-glucosylglucoside and cyanidin 3-glucoside.

‡ This refers to the mixture of cyanidin 3-glucosylglucoside-5-glucoside and cyanidin 3,5-diglucoside.

Perle de Melle, Glas 6, Simplet, Mme. Henny, Gloire de Claude Goyet, Mme. Van Acker, Adventsglöcke, Kees Bier, Mme. Petrick.

(*b*) Scarlet → salmon (low flavonol content): Herman Superba, Excelsior, Glas 9, Ilse Kärger, Glas 10, Willem van Oranje, Prinses Margriet, Apollo, Oranje Boven, Dr. Bergmann Feu, R. Van Oost, Mme. Coty, Benelux, Prinses Beatrix, Mme. G. Toebaert.

(*c*) Rosy red (low flavonol content): Mme. Maenhout, Comtesse de Kerckhove.

(*d*) With white-edged corolla (high flavonol content): De Waele's Favoriet.

B. The amount of cyanidin 3,5-diglucoside strongly exceeds that of the 3-monoglucoside (4–10-fold).

(*a*) Deep red → magenta → blue red (high flavonol content): Etoile de Belgique, K. Glaser, Memoria Sanders, Lentegroet, Rubis de Meirelbeke, Gloire St. Georges, Glas 1, Euratom, G. Nicolai, Mme. Van der Cruysse, John Haerens, Josiane Maeseele, Mme. Buyle, Red Wing.

(*b*) Violet (high flavonol content): Pink Dream.

(*c*) Brick red → salmon (low flavonol content): Prinses Juliana, Mme. Collumbien, Findeisen, Avenir, Haerens Lorraine, Mme. Van Gele, Melanie, saumonea and rubra, Vervaeneana rubra, Mme. Leon Moreux, Haerensiana.

(*d*) Rosy red → pink → light pink (low flavonol content): P. Schäme, Marie Claude Truffaut.

(*e*) With white-edged corolla (or red periphery) (low flavonol content): Dresden, Capt. Gau, Mme. Gau, Mme. Hartlieb, Mme. Mestdagh, Mme. Van Damme, Charles Encke, Mme. August Haerens, Dr. Bergmann Saumonea, Eri, G. J. Bier, Perle de Noisy, Souvenir de Theophile Piens.

II. Varieties containing two anthocyanidins (cyanidin and peonidin). The following varieties contain peonidin 3,5-diglucoside in addition to cyanidin 3-glucoside and 3,5-diglucoside (normally high in flavonol content): Alice Erauw, Eclaireur, Dr. Stahnke, Ernest Eeckhaute, Ernest Thiers, Haerens Lorraine, Hexe, J. Rhoers, Mme. Bier, Mémoire John Haerens, Milda Glaser, Prof. Wolters, Vervaeneana and its sports.

III. Varieties containing malvidin. The following varieties with violet flowers contain malvidin 3,5-diglucoside: Violacea (high flavonol content); Tempérance (high flavonol content); Concinna (+cyanidin 3,5-diglucoside) (high flavonol content); and Adrien Steyaert (+cyanidin 3-glucoside+cyanidin 3,5-diglucoside) (low flavonol content).

2. *Other Species and Varieties from the* Rhododendron *Subseries Obtusum*

Table 3 gives the results of a survey of the flower pigments of these plants. Malvidin 3,5-diglucoside clearly occurs regularly in the violet-flowered species and varieties and is accompanied by myricetin 5-methyl ether. The few known red sports from these violet varieties (*R. obtusum* Planch. f. *amoenum* Wils., red-flowered type and "Hatsugiri" with red flowers) contain peonidin instead of malvidin 3,5-diglucoside and are not able to synthesize the myricetin derivative (azaleatin is the only flavonol present). However, the presence of azaleatin is not necessarily linked to the synthesis of peonidin, for cyanidin types (3- or 3,5-diglucoside) containing no peonidin may also contain azaleatin. In the two representatives from *R. indicum* Sweet. and in some varieties with very low flavonol content, azalatin is replaced by quercetin. Kaempferol only occurs (in very low amounts) in a few violet varieties and species.

EXPERIMENTAL

Flavonoids were separated and identified by standard procedures.[7–10] TLC was carried out on cellulose MN 300. The solvent used for primary separation of anthocyanins was H_2O–HCO_2H–HCl (60 : 30 : 3). Sugars were identified by paper chromatography[11] and TLC.[12]

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[7] J. B. Harborne, *J. Chromatogr.* **1**, 473 (1958).
[8] J. B. Harborne, *Biochem. J.* **70**, 22 (1958).
[9] J. B. Harborne, *J. Chromatogr.* **2**, 581 (1959).
[10] F. J. Francis and J. B. Harborne, *Proc. Am. Soc. Hort. Sci.* **89**, 657 (1967).
[11] H. F. Linskens, *Papierchromatographie in der Botanik*, p. 86, Springer-Verlag, Berlin (1959).
[12] E. Stahl, *Dünnschichtchromatographie*, pp. 770 and 775, Springer-Verlag, Berlin (1967).