Phytochemistry, 1970, Vol. 9, pp. 875 to 879. Pergamon Press. Printed in England.

FLAVONOID GLYCOSIDES IN THE PETALS OF SOME *RHODODENDRON* SPECIES AND HYBRIDS

R. DE LOOSE

I.W.O.N.L. Irradiation Laboratory, Government Ornamental Plant Culture Station, Melle (Ghent), Belgium

(*Received* 4 *September* 1969, *in revised form* 26 *September* 1969)

Abstract—The following anthocyanins were identified in flowers of *Rhododendron simsii* hybrids and other species and varieties from the *Rhododendron* subseries obtusum: cyanidin 3-glucoside, cyanidin 3-galactoside, cyanidin 3-arabinoside, cyanidin 3,5-diglucoside, cyanidin 3-galactoside-5-glucoside, peonidin 3,5-diglucoside (acylated with caffeic acid) and malvidin 3,5-diglucoside (acylated with caffeic acid). The flavonol glycosides identified were kaempferol 5-methyl ether 3-galactoside, quercetin 3-galactoside, quercetin 3-rhamnoside, azaleatin 3-galactoside, azaleatin 3-rhamnoside, myricetin 5-methyl ether 3-rhamnoside, myricetin 5-methyl ether 3-galactoside and gossypetin 3-galactoside. Caffeoylgalactose was also detected in these species.

INTRODUCTION

IN A PREVIOUS paper,[1] two anthocyanin flower pigments, based on cyanidin, were provisionally identified in *Rhododendron* as 3-glucosylglucoside and 3-glucosylglucoside-5-glucoside. Their further characterization forms part of the subject of this paper. The glycosidic nature of the flavonols (azaleatin, quercetin, myricetin 5-methyl ether) present were also studied. TLC on cellulose has been used for isolation and purification.

RESULTS

Anthocyanins

A further study has revealed the presence of sugar residues other than glucose in the anthocyanins. Table 1 lists the different cyanidin pigments that were isolated from the variety Ambrosiana (*Rhododendron simsii* hybrid). The azalea varieties containing peonidin 3,5-diglucoside and the few purple ones containing malvidin 3,5-diglucoside contain these

TABLE 1. SPECTRAL CHARACTERISTICS AND R_f VALUES OF ANTHOCYANINS ISOLATED FROM THE *Rhododendron simsii* HYBRID VARIETY AMBROSIANA

	λ_{max} (nm) in MeOH		R_f			
Pigment	0·01% HCl	+$AlCl_3$	BAW	Bu-HCl	1% HCl	HOAc-HCl
Cyanidin 3-galactoside	526	567	0·23	0·18	0·057	0·23
Cyanidin 3-glucoside	526	558	0·37	0·25	0·048	0·21
Cyanidin 3-arabinoside	526	558	0·37	0·25	0·048	0·21
Cyanidin 3,5-diglucoside	524	559	0·09	0·053	0·11	0·37
Cyanidin 3-galactoside-5-glucoside	526	565	0·16	0·12	0·13	0·40

[1] R. DE LOOSE, *Phytochem.* 8, 253 (1969).

pigments acylated with caffeic acid. This explains the separation of both glucosides into two spots when run in BAW on cellulose-coated glass plates.

Flavonols

The quercetin, azaleatin and myricetin 5-methyl ether glycosides and the two new flavonol glycosides, kaempferol 5-methyl ether 3-galactoside and gossypetin 3-galactoside, recently identified by Harborne[2–4] in other *Rhododendron* species have been found in the plants under investigation. Table 2 gives the properties of the glycosides of azaleatin[5] and myricetin 5-methyl ether,[6] which have not previously been described. These glycosides were isolated from representative varieties from the Belgian hybrids of *Rh. simsii*, *Rh. obtusum* hybrid varieties and also a representative from the subgenus Eurhododendron, i.e. the bluish purple variety Blue Diamond (*Rh. augustinii* × "Intrifast").

DISCUSSION

Anthocyanins

Our present, more detailed, studies show that the earlier reported[1] cyanidin 3-glucosylglucoside and corresponding 3-glucosylglucoside-5-glucoside are, in fact, cyanidin 3-galactoside, cyanidin 3-arabinoside, cyanidin 3-galactoside-5-glucoside accompanied by cyanidin 3-glucoside and cyanidin 3,5-diglucoside. In the *Rhododendron simsii* hybrid variety "Red Wing", Asen and Budin[7] reported an analogous pattern except for cyanidin 3-galactoside-5-glucoside; they found cyanidin 3-arabinoside-5-glucoside instead. This discrepancy may be explained either on a genetic background (Red Wing is an American *Rh. simsii* hybrid) or by the different isolation technique. They used BAW as a solvent after separating the anthocyanins on powdered nylon. We first separated the anthocyanin 3-monoglycosides and 3,5-diglycosides with H_2O–HCOOH–HCl (60:30:3, v/v), followed by BAW and again with H_2O–HCOOH–HCl.

The resolution of both peonidin and malvidin 3,5-diglucosides into two spots when run in BAW on cellulose-coated glass plates is caused by their acylation with caffeic acid.

Flavonols

The flavonol azaleatin occurs as the 3-rhamnoside and 3-galactoside instead of as the 3-rhamnoglucoside and 3-glucoside as previously reported.[1] Azaleatin 3-galactoside is not only found in white varieties. Analogous glycosides (3-rhamnoside and 3-galactoside) of myricetin 5-methyl ether are found in the species and varieties with purple flowers (except the *Rh. simsii* hybrid variety Adrien Steyaert which contains only quercetin).

Next to the principal flavonol, i.e. azaleatin, fluctuating amounts of quercetin (3-rhamnoside and 3-galactoside) are found in the *Rh. simsii* and *Rh. obtusum* hybrids. In the *Rh. simsii* hybrids, only a few varieties are found to completely lack azaleatin and to contain quercetin instead, i.e. Hexe and its sport Mme. Bier, Ernest Thiers and A. Steyaert. Among the blue red *Rh. simsii* hybrids only a few contain azaleatin as the sole flavonol, i.e. Mme. Petrick.

[2] J. B. HARBORNE, *Phytochem.* **4**, 647 (1965).
[3] J. B. HARBORNE, *Phytochem.* **8**, 177 (1969).
[4] J. B. HARBORNE, *Phytochem.* **8**, 419 (1969).
[5] E. WADA, *J. Am. Chem. Soc.* **78**, 4725 (1956).
[6] K. EGGER, *Z. Naturforschung.* **17b**, 489 (1962).
[7] S. ASEN and P. S. BUDIN, *Phytochem.* **5**, 1257 (1966).

TABLE 2. FLAVONOL GLYCOSIDES OF SOME VARIETIES FROM THE *Rhododendron* SUBSERIES OBTUSUM AND FROM THE SUBGENUS EURHODODENDRON

Pigment	λ_{max} (nm) in				R_f*				
	EtOH	EtOH–$AlCl_3$	EtOH–NaOAc	EtOH–NaOAc–H_3BO_3	BAW	60% Isopropanol	H_2O	HOAc 60%	HOAc 15%
Azaleatin (3-galactoside)‡	252, 288, 350	359	+	372	0·69	0·82	0·12	0·71	0·55
Myricetin (5-methyl ether 3-rhamnoside)‖	250, 287, 343	343	268, 314, 360	259, 290 *inf.*, 364	0·64	0·78	0·19	0·70	0·47
Myricetin (5-methyl ether 3-galactoside)§	252, 300, 355	355	†	†	†	†	†	†	†

* R_f determined on purified pigments by descending chromatography on Whatman No. 1 paper.
† Isolated in too small amount to be determined.
‡ Isolated from the *Rh. simsii* hybrid varieties Tempérance and Red Wing, the *Rh. obtusum* hybrid variety Amoena and the bluish purple variety Blue Diamond (*Rh. augustinii* × "Intrifast").
‖ Isolated from the purple varieties Tempérance, Amoena and the bluish purple variety Blue Diamond.
§ Isolated from the purple variety Beethoven (*Rh. obtusum* hybrid).

TABLE 3. FLAVONOL TYPES IN SOME *Rhododendron* SPECIES AND VARIETIES

Hybrid group	Quercetin type* (without 5-*O*-methylation)	Azaleatin type† (with 5-*O*-methylation)	Quercetin + azaleatin type‡
Rh. Simsii hybrids	a. Blue-red and red (cyanidin): var. Hexe Mme. Bier (sport from Hexe) Ernest Thiers	Blue-red, red and white: var. Mme. Petrick	a. Blue-red, red (cyanidin) and white: var. Ambrosius, Mme. De Waele
	b. Purple (malvidin): var. A. Steyaert		b. Purple (malvidin): var. Violacea
Rh. obtusum hybrids	Blue-red and red (cyanidin): *Rh. kaempferi* *Rh. indicum* Kaempferi × Malvatica Kurume	Not found	a. Blue-red, red (cyanidin) and white: var. Toreador b. Purple (malvidin): *Rh. yedoense poukhanense*, *Rh. mucronatum ripense*
Subgenus Eurhododendron	Yellow: *Rh. campylocarpum* var. *elatum* × *Rh. discolor* var. Lady Bessborough cultivar Montreal	Not found	a. Yellow: 1. *Rh. fortunei* × Mrs. W. C. Slocock var. New Moon. 2. *Rh. campylocarpum* × Mrs. W. C. Slocock. var. Dairy Maid b. Bluish purple: *Rh. augustinii* × "Intrifast" var. Blue Diamond‖

*Quercetin 3-rhamnoside, quercetin 3-galactoside for the first two hybrid groups, accompanied by gossypetin 3-galactoside for the last group.

† Azaleatin 3-rhamnoside, azaleatin 3-galactoside, kaempferol 5-methyl ether 3-galactoside.

‡ Quercetin and azaleatin 3-rhamnoside and 3-galactoside, kaempferol 5-methyl ether 3-galactoside for the first two hybrid groups sub. a, accompanied by myricetin 5-methyl ether 3-rhamnoside and 3-galactoside within sub. b. Moreover within group 3a: + gossypetin 3-galactoside, myricetin 3-glycoside.

‖ Analogous to the two foregoing yellow Rhododendrons but quercetin 3-galactoside, myricetin 3-glycoside and gossypetin 3-galactoside are absent.

Most of the other blue-red forms, as previously stated,[1] contain azaleatin and quercetin, whilst those with low flavonol content only show traces of azaleatin.

In the *Rh. obtusum* subseries, *Rh. kaempferi* and *Rh. indicum* contain quercetin instead of azaleatin. This is also the case with most Kaempferi–Malvatica and Kurume hybrids (in the last series also the two flavonols may occur). In all other species and hybrids from the same *Rh. obtusum* subseries quercetin occurs next to azaleatin and in the purple-flowered types (except the variety A. Steyaert) with azaleatin and myricetin 5-methyl ether. This implies that in Table 3 of the previous paper[1] the values in the second last column for quercetin should be changed from – to ++. Kaempferol 5-methyl ether was identified for the first time in *Rhododendron* by Harborne.[4] We found it in measurable quantities in the bluish purple *Rhododendron* variety Blue Diamond (*Rh. augustinii* × "Intrifast"), a representative of the subgenus Eurhododendron. In all other varieties and species from the subseries obtusum it only occurs in traces (not present in quercetin types, i.e. Hexe, *Rh. kaempferi*, *Rh. indicum*). The yellow gossypetin 3-galactoside isolated from the yellow-flowered Eurhododendron variety Lady Bessborough cultivar Montreal (*Rh. campylocarpum* var. *elatum* × *Rh. discolor*) has the same absorption and R_f as described by Harborne.[2,3] The aglycone in solution shows the blue colour in the test with EtOH–NaOAc; its λ_{max} (380 nm) is somewhat different from the value reported by Harborne (338, 386 nm);[3] this may be due to its oxidation during hydrolysis. A caffeic acid galactose ester is present in appreciable quantities in the purple *Rh. simsii* hybrid varieties (A. Steyaert, Tempérance, Violacea, Concinna) and also in the quercetin types (Hexe and its sport Mme. Bier). In the *Rh. obtusum* series also it occurs in quercetin types (*Rh. kaempferi*, *Rh. indicum* and the Kaempferi × Malvatica and Kurume hybrids, i.e. the varieties Brazier, Wuyts, Betty, Royal Pink, Alice, Blauw's Pink, Kirin, Hinomayo). Table 3 summarizes the distribution of flavonol glycosides in *Rhododendron* species and varieties studied.

EXPERIMENTAL

Flavonoids were separated and identified by standard procedures.[8–11] TLC was carried out on cellulose MN 300. The solvent used for primary separation of anthocyanins was H_2O–HCOOH–HCl (60:30:3, v/v), followed by BAW (4:1:5, v/v) and again the first solvent. Sugars were identified by paper chromatography[12] on Whatman No. 1 with the EtoAc–pyridine–H_2O, (5:2:5 and 8:2:1, v/v);[7] the second mixture gives the best ΔR_f between galactose and glucose. Misidentification of the sugars in the previous work[1] was probably due to insufficient resolution obtained with the ratio (2:1:2) then used. The peonidin and malvidin 3,5-diglucosides isolated with the solvent H_2O–HCOOH–HCl (60:30:3) separate into two spots when run in BAW. Alkaline hydrolysis of the spot with highest R_f and extraction with ether gives caffeic acid (λ_{max} 278, 328 nm), identified by cochromatography with authentic material. Caffeoylgalactose has λ_{max}^{EtOH} 334, 298, 242 inf., 232 inf., nm. Its R_fs are 0·60 (BAW), 0·61 and 0·72 (60% HOAc), 0·67 (Forestal), 0·75 (H_2O). Alkaline hydrolysis of the ester gives caffeic acid and galactose. For the known flavonols, R_f and spectral data agreed closely with published results.

Acknowledgements—The author thanks the Belgian Institute for the Encouragement of Scientific Research in Industry and Agriculture (I.W.O.N.L.–I.R.S.I.A.) for financial help. He also thanks Director J. van Onsem from the Government Ornamental Plant Culture Station for facilities and Ir. J. Heursel for his sustained interest and kindness in putting the many varieties and species at his disposal.

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