"Yellow Flavonols" as Components of Pollen Pigmentation

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Yellow coloration of pollen in some exceptional cases can be caused by the presence of intensely coloured flavonoids. Derivatives of herbacetin (sexangularetin) and of gossypetin (limocitrin) have been found as "yellow flavonols" in the pollen of some species of *Paeonia* and of *Rumex*. The pollen of *Spinacea oleracea* contains the 6-methyl ether of galetin and the quercetagetin derivatives patuletin and spinacetin.

The pollen of higher plants can be very variable in colour: as a rule it is yellow, but it can also appear white or not infrequently red or blue. Yellow and red pigmentation is essentially related to the accumulation of carotenoids. Blue tint can be due to anthocyanins (*cf.* summary in [1]). Chalcones as intensely coloured flavonoids can occasionally cause yellow pollen pigmentation (*cf.* [2]). Flavonols in glycosidic combinations are very widespread in pollen [3], but they do not normally contribute to the pigmentation. Recently we described a "yellow flavonol" contributing to the coloration of the pollen of *Nothofagus antarctica* (Forst.) Oerst [2]. A screening for such flavonols done on the pollen of more than 140 species shows that the occurrence of "yellow flavonols" is a very rare feature.

Materials and Methods

Pollen of *Paeonia tenuifolia* L., *P. delavay* Franch., and *Spinacea oleracea* L. was collected in the Botanic Garden at Münster. The pollen of *Paeonia daurica* Andr., *P. lutea* Delavay var. *ludlowii* Stern & Taylor, and *P. mlokesewitschi* Lomak. came from plants cultivated in the Botanic Garden at Munich; it was kindly collected and made available by Prof. K. Kubitzki. The pollen of *Rumex acetosa* L. and *R. acetosella* L. was collected in the area of Münster.

Isolation of flavonoids was performed on pollen of *Rumex acetosa, Paeonia tenuifolia,* and *Spinacea oleracea.* Small portions of the pollen material were suspended in methanol/water, dilute sulphuric acid was added and the mixture was kept boiling for 1–2 h. After cooling, the solution was filtered and treated with diethyl ether and ethyl acetate to extract the flavonid aglycones. These were subjected to column chromatography on polyamide SC-6 (Macherey-Nagel). Elution was done with toluene and increasing quantities of methylethyl ketone and methanol. Some fractions had to undergo further separation and purification by preparative TLC on polyamide DC-11 (Macherey-Nagel). The flavonoid aglycones thus isolated could be identified by their spectral properties and by direct comparison with markers.

The solvents used for TLC on polyamide were A) toluene/methylethyl ketone/methanol 60:25:15; B) toluene/dioxane/methanol 80:10:10. For silica solvent C) toluene/methylethyl ketone 9:1 was used. – Samples of sexangularetin and of limocitrin were supplied by M. Jay. 6-Methoxy-kaempferol comes from the bud excretion of the sweet cherry tree [4]. Patuletin was a gift of H. Combier. Spinacetin was obtained by partial demethylation of jaceidin (from sweet cherry tree, [4]) with anilin-HCl [5]. This procedure unexpectedly yielded a mixture of at least 5 compounds. The flavonoid assumed to be spinacetin by R_f and spot colour was isolated by prep. TLC and its identity was confirmed by its spectral data (UV, MS).

Results and Discussion

The major flavonoid aglycone isolated from the pollen of *Rumex acetosa* as well as *Paeonia tenuifolia* was readily recognized to be sexangularetin (herbacetin-8-Me) by direct comparison with authentic marker. Its spectral data are identical with those recently described for this compound as isolated

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from the pollen of *Nothofagus antarctica* [2]. The structure elucidation has been described there in detail. In both cases minor amounts of kaempferol were detected as well. Pollen of *Rumex acetosa* contains traces of a further flavonoid, possibly a methyl derivative of quercetagetin. This could not be identified, however, because of lack of material. By comparative TLC it could be clearly demonstrated that the pollen of *Rumex acetosella* exhibited the same compounds.

The pollen of *Paenonia* showed one further flavonoid component, appearing on polyamide as a spot (R_f 0.48, solv. A) of somewhat higher R_f than sexangularetin (R_f 0.36, solv. A), but with the same colour behaviour in UV_{366} before and after spraying with "Naturstoffreagenz A" (β-aminoethyl ester of diphenyl boric acid). Hence it was assumed that it was likewise a methyl derivative of herbacetin, or a methyl derivative of gossypetin. The substance was isolated in minute amount by preparative TLC and after purification showed the following UV spectra: λ_{max}^{EtOH} 380, (355), (270), 257 nm; + $AlCl_3$ 440, 363, (305), 268 nm; + NaOEt dec.; + NaOAc 388, 277 nm; + NaOAc + H_3BO_3 383, 275 nm. From these data it became obvious that it was indeed a flavonol, with free OH-groups at C-3, C-5, C-7 and C-4′ [6], substituted at C-8 [7]. M^+ 346 indicated a flavonol with 4 OH-groups and 2 OCH_3-groups [8], $M-15 > M^+$ pointing further to a OCH_3 located at C-8 [9]. The existence of "normal" substitution presumed, the OCH_3-groups should be situated at C-8 and C-3′, and the product under investigation would thus be the 8.3′-dimethyl ether of gossypetin. This result was corroborated by direct TLC comparison with an authentic sample of limocitrin as well as by comparison of the UV- and MS-data with those reported for this flavonol in the literature [10]. The major fragments in the MS are 346 (M^+, 60%), 331 (M^+-15, 100%), 303 (17%), 151 (21%). According to the results of comparative TLC the accumulation of the compounds described for *Paeonia tenuifolia* is found in other species of *Paeonia* as well.

Polyamide TLC of the flavonoid aglycones of pollen of *Spinacea oleracea* revealed three spots of orange-brown colour in UV_{366}. One had the same R_f as limocitrin but gave a different colour reaction on spraying with "Naturstoffreagenz A". A similar difference in colour reaction was observed for the second spot, which had the same R_f as sexangularetin. The third spot appeared at R_f 0.22. Obviously they were all methyl derivatives of 6- or 8- substituted flavonols. By direct comparison with reference compounds they were recognized as quercetagetin-6.3′-dimethyl ether (spinacetin, at 0.48), 6-methoxy-kaempferol (at 0.36) and quercetagetin-6-methyl ether (patuletin, at 0.22). Possible identity of the upper spot with quercetagetin-7.3′-di-Me (same R_f) was excluded by direct comparison (different colour reaction). The identification of spinacetin was further confirmed by the UV-spectra measured after isolation and purification by prep. TLC. λ_{max}^{EtOH} 371 m 257 nm; + $AlCl_3$ 430, 368, 265 nm; + NaOEt 323, 289 nm (dec.); + NaOAc 389, 261 nm.

6-Methoxy-kaempferol had been isolated for the first time as an aglycone from the bud excretion of the sweet cherry tree [4]. In the free state it was also found in *Flourensia illicifolia* [11]. In glycosidic combination it occurs in *Flaveria brownii* [12], *Tetragonotheca texana* [13] and *Anvillea garcini* [14]. Herbacetin-8-Me (sexangularetin) was known from *Sedum acre* var. *sexangularis*, *Lotus corniculatus* and *Dorycnium suffruticosum* and was found recently in pollen of *Nothofagus antarctica* (*cf.* [2] and references therein). Quercetagetin-6-Me (patuletin) is rather widespread in glycosidic combination. Quercetagetin-6.3′-diMe (spinacetin) had been found for the first time in leaves of *Spinacea oleracea* [15]. In recent years it was reported as an aglycone from *Chrysanthemum viscidiflorus* [16] and from *Brickellia californa* [17], and as a glycoside from *Lepidophyllum repandum* [18], *Tetragonotheca ludoviciana* [13] and *Anvillea garcini* [14]. The isomeric gossypetin-8.3′-di-Me (limocitrin) has been found only twice before: first in peel of *Citrus limon* fruit [19], then in flowers of *Lotus corniculatus* [10].

In higher plants the pollen represents a ubiquitously widespread accumulation area for secondary plant products. A screening of over 140 species showed that mainly flavonols are accumulated [3; see also 20–24]. Relatively high amounts of quercetin, kaempferol and other flavonoids encountered in pollen have been assumed by several authors to cause the yellow coloration (*cf.* [1]). Harborne in 1965 introduced the term "yellow flavonols" [25]. He used it "to distinguish those flavonols which definitely appear yellow in visible light from those more commonly accurring flavones and flavonols". As Harborne pointed out later [26], gossypetin (8-hydroxy-quercetin) and herbacetin (8-hydroxy-kaempferol) and their methyl derivatives are the

compounds meeting the properties of "yellow flavonols". As a matter of fact, the introduction of a OH-group or a OMe-group at C-8 in flavonols produces a bathochromic shift of 13–18 nm on Band I in the UV-spectrum and an additional peak at 330 nm. Introduction of a OH-group at C-6 on the other hand produces a hypsochromic shift [7]. The relatively high wavelength of the absorption maxima of 8-oxygenated flavonols is the reason for these compounds' yellow appearance in daylight [*cf.* 27]. Harborne found a very good example of "yellow flavonols" in flowers of *Coronilla valentina*, where they occur only in the wings [28]. In pollen "yellow flavonols" are encountered very seldom. The above-mentioned screening of a great number of plants revealed them in a few species only. The result is presented in Table I. The derivatives of galetin (= 6-hydroxy-kaempferol) and of quercetagetin (= 6-hydroxy-quercetin) in pollen of spinach are no "yellow flavonols" according to the definition. The pollen of *Spinacea oleracea* remains yellow after extraction with hydrolysis. The unknown pigment(s) cannot be extracted with any organic solvent. It seems to be strongly bound to the cell wall material. Kaempferol, quercetin and isosalipurpol (2′.4′.6′.4-tetra-OH chalkone) are included in the Table to give a complete survey of the flavonoids identified in the pollen material investigated.

Table I. The occurrence of flavonols and "yellow flavonols" in various pollen.

	Kaempferol	Quercetin	6-Methoxy-kaempferol	Patuletin	Spinacetin	Sexangularetin	Limocitrin	Isosalipurpol
Paeonia daurica	+	+				+	+	
Paeonia delavay	+	+				+	+	
Paeonia lutea var. *ludlowii*	+	+				+	+	
Paeonia mlokosewitschii	(+)	+				+	+	
Paeonia tenuifolia		+				+	+	
Rumex acetosa	+	+				+		
Rumex acetosella	+	+				+		
Nothofagus antarctica [2]	+	+				+		+
Spinacea oleracea			+	+	+			

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