“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 Paeania 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 coloring 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 Paeania tenuifolia L., P. delavay Franch., and Spinacea oleracea L. was collected in the Botanic Garden at Münster. The pollen of Paeania daurica Andr., P. lutea Delavay var. ludlowii Stern & Taylor, and P. mlokosewitschi 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. acotosella L. was collected in the area of Münster.

Isolation of flavonoids was performed on pollen of Rumex acetosa, Paeania 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 flavonoid 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 Rf 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 Paeania tenuifolia was readily recognized to be sexangularetin (herbatetin-8-Me) by direct comparison with authentic marker. Its spectral data are identical with those recently described for this compound as isolated.
from the pollen of *Nothofagus antarctica* [2]. The structure elucidation has been described 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 (Rf 0.48, solv. A) of somewhat higher Rf than sexangularetin (Rf 0.36, solv. A), but with the same colour behaviour in UV 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: λmax 380, (355), (270), 257 nm; + AlCl; 440, 363, (305), 268 nm; + NaOEt dec.; + NaOAc 388, 277 nm; + NaOAc + H2BO; 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. Hence it was assumed that it became obvious that it was indeed a flavonol, with free OH-groups at C-3, C-5, C-7 and C-4’ [6].

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 *Flavera brownii* [12], *Tetragonotheca texana* [13] and *Anvillea garcini* [14]. Herbacetin-8-Me (sexangularetin) was known from *Sedum acre var. sexangulare*, *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’-di-Me (spinacetin) had been found for the first time in leaves of *Spinacea oleracea* [15].

In recent years it was reported as an aglycone from *Chrysantherum viscidiflorus* [16] and from *Brickellia california* [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 occurring flavones and flavonols”. As Harborne pointed out later [26], gossypetin (8-hydroxy-kaempferol) 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 quercetagenet (= 6-hydroxy-queretetin) 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 chalcone) 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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Kaempferol</th>
<th>Quercetin</th>
<th>6-Methoxy-kaempferol</th>
<th>Patuletin</th>
<th>Spinacitin</th>
<th>Sexangularatin</th>
<th>Limocitin</th>
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Acknowledgements

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