On the Pigmentation of the Pollen of *Nothofagus antarctica* (Forst.) Oerst. (Fagaceae)

Eckhard Wollenweber

Institut für Botanik der Technischen Hochschule Darmstadt, Schnittspahnstr. 3, D-6100 Darmstadt

Rolf Wiermann

Botanisches Institut der Universität Münster/Westf.

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Extraction of pollen of *Nothofagus antarctica* with acid hydrolysis yields kaempferol, quercetin, the rare flavonol sexangularetin, and naringenin. The tetrahydroxy-chalcone isosalipurpol is shown for the first time to occur in such high amount as free aglycone in mature pollen; it yields naringenin in usual extraction procedures. The bright yellow coloration of the pollen is due to the presence of isosalipurpol and sexangularetin.

The intense yellow colour abundant in pollen is caused in very many cases by the accumulation of carotenoids (see [1] for a survey). Chalcones can also bring about yellow pigmentation to an as yet unknown extent [2, 3; for antheres: 4]. In the following we will show that in addition rare "yellow flavonols" [5] may contribute to such coloration.

Materials and Methods

Pollen of *Nothofagus antarctica* was collected in spring 1975 and 1976 from a tree grown in the Botanical Garden at Münster. Samples were stored deep-frozen. The material was extracted and hydrolyzed by stirring it in boiling methanol/water with some diluted sulphuric acid for about 1 hour. The solution after cooling was treated with diethyl ether and ethyl acetate and the upper layer was taken to dryness. The flavonoid aglycones thus obtained could be separated on thick layers (polyamide DC-11; solvent I: toluene/methylethyl ketone/methanol 60 : 25 : 15). Four bands were scraped off and eluted with acetone and ethanol. Hereby a remarkable amount of the adsorbent is dissolved. The unknown compound in band 2 was therefore further purified by preparative TLC on silica. The sample was sufficient for running UV-spectra and MS. In addition demethylation with pyridinium hydrobromide [6] could be performed. The reaction product, as well as the flavonoids of bands 1, 3, and 4, was compared directly with authentic markers. A second solvent was used for the more polar region (polyamide, solvent II: toluene/methanol/methylethylketone/acetyl acetone 40 : 30 : 20 : 10). For TLC on silica we used solvent III (toluene/acetone 9 : 1). – A small sample of the pollen material was extracted with some ethylene glycol monomethyl ether (*i. e.* without hydrolysis) at room temperature and the yellow solution was chromatographed on cellulose (solvent IV: BAW; solvent V: isobutyric acid/ammonia/water 66 : 1 : 30; solvent VI: 30% acetic acid; solvent VII: 30% ethanol). For polyamide both solvents I and II can be used.– Herbacetin 7-Omethyl ether was obtained by saponification of its butyryl ester, isolated from *Nothofagus aschenborniana* [7], 6-Methoxy kaempferol comes from the bud excretion of sweet cherry tree [8]. A sample of isosalipurpol was kindly supplied by Prof. H. Grisebach (Freiburg); herbacetin was a gift of Prof. H. Wagner (Munich).

Reprint requests to Doz. Dr. E. Wollenweber.
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Results and Discussion

Extraction and hydrolysis of the pollen of the antarctic beech, *Nothofagus antarctica,* yields four identifiable flavonoids, among which there is one rare flavonol. Kaempferol obviously is the major constituent, followed by naringenin, an unknown flavonol (called "Nothof. Substanz" in [3]), and traces of quercetin. Naringenin, kaempferol and quercetine could be readily identified by direct comparison on polyamide and on silica with authentic substances, with which they were identical in R_F and in colour properties in UV_{366} before and after spraying with "Naturstoffreagenz A" (β-amino-ethyl ester of diphenyl boric acid). The unknown flavonol exhibits the following spectral data. UV: λ_{max}^{EtOH} 376, 327, 272 nm; + $AlCl_3$ 438, 360, (310), 275, 265 nm, unchanged on addition of HCl; + NaOEt dec.; + NaOAc 402, 313, 284 nm; + NaOAc + H_3BO_3 385, 313, 275 nm. MS: *m/e* (rel. int.) 316 (57, M^+), 302 (16), 301 (100, $M^+ - CH_3$), 287 (6), 273 (27), 181 (10), 167 (13), 153 (10), 149 (26), 139 (24), 121 (46). The product appears on polyamide as a brownish-yellow spot (h R_F in solvent I: 42; naringenin 63, kaempferol 28, quercetin 17). After spraying with "Naturstoffreagenz A" the spot turns greenish-brownish. This behaviour is observed *e.g.* with derivatives of 6-hydro-

xy kaempferol; it points to a flavonol with 6- or 8-substitution. The molecular weight 316 shows that it must be a flavonol with 4 OH groups and 1 OCH_3 group, which is confirmed by the base-peak at *m/e* 301 ($M^+ - 15$). Hence it should be a monomethyl ether either of 6-hydroxy kaempferol (galetin) or of 8-hydroxy kaempferol (herbacetin). 6-methoxy kaempferol shows the same R_F and very similar, but not identical colour behaviour on polyamide thin-layer. The position of band I in the UV-spectrum beyond 372 nm as well as the small peak at 327 point to substitution at C-8 [9]. On addition of $AlCl_3$ an absorption spectrum with 4 maxima is observed, which strongly recalls the flavonols reported recently to occur as esters [7]. As a matter of fact demethylation of the substance with pyridinium hydrobromide yields herbacetin, which can be identified by comparison with an authentic sample. It is characteristic for flavonols with 8-OH substitution that the spots after spraying with the reagent appear bluish in daylight (see [7]). Thus we now know that the substance under investigation is a monomethyl ether of herbacetin. The position of the methoxy group can be determined according to the rules of classical UV spectroscopy. The reaction with $AlCl_3$ ($\Delta\,\lambda_{max\,I}$ 62 nm) indicates free OH groups at C-3 and C-5; because of the reaction with NaOAc ($\Delta\,\lambda_{max\,II}$ 12 nm) there must also be a free OH at C-7, and the decomposition with NaOEt demonstrates a free OH at C-4′. Hence our substance must be herbacetin 8-Omethyl ether. This is corroborated finally by comparison with literature data [10]. In addition direct comparison with the isomer 7-Omethyl ether (pollenitin) shows that it is not identical with this compound.

It has been mentioned several times in the literature that free, *i. e.* non-glycosylated, chalcones may occur in pollen or in anthers [4, 5, 11, 12]. We wanted to examine whether such compounds are accumulated in the pollen of *Nothofagus*. Extraction with ethylene glycol monomethyl ether indeed yields free 2′,4′,6′,4-tetrahydroxy chalcone. Its identity is proven by direct comparison with an authentic sample in various TLC and PC systems and by its UV spectral properties.

Glycosides of kaempferol and quercetin are fairly widely distributed in pollen [3]. The occurrence of both flavonols in hydrolysed extract of *Nothofagus* pollen thus could be expected. The accumulation of the tetrahydroxy chalcone as aglycone, which tends readily to isomerize, however, is a phenomenon which has been reported as yet only for the system "pollen". It cannot yet be decided whether the chalcone observed here is perhaps a non-metabolized residue of the chalcone pool that exists during certain stages of the pollen development (described for the contents of antheres = pollen + tapetum) of *Tulipa* and *Lilium* in [11] and [12]). Among the phenylpropanoid compounds occuring in pollen naringenin is encountered often, though mostly in trace amounts [13, 3]. In many cases, perhaps in most, this flavanone may have been built by cyclization of the chalcone during extraction procedures as an artefact.

This is the first report on the occurence of isosalipurpol as a free aglycone in such high amount in mature pollen. Although its 4′,4-dimethyl ether is abundant as a farina constituent of goldback ferns [14], the tetrahydroxy compound itself has not been detected as yet. Similarly, the 4′-monomethyl ether of 2′,4′,6′-trihydroxy chalcone is produced by these plants in abundance, whereas the free trihydroxy compound has been found only twice in nature [15].

8-Omethyl herbacetin (sexangularetin) had been found previously only three times in the plant kingdom: in *Sedum acre* var. *sexangularis* [16], in *Lotus corniculatus* [17], and in *Dorycnium suffruticosum* [18], always as a glycoside in leaves. Like other 6- and 8-substituted flavonols, too, this compound is a "yellow flavonol". Together with the deep yellow chalcone it is responsible for the bright yellow colour of the *Nothofagus* pollen. Carotenoids that might contribute to the pigmentation could not be found in this pollen.

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