Pigments in the Blue Pollen and Bee Pollen of *Fuchsia excorticata*

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A comparison of the pigments in *Fuchsia extorticata* pollen with the pigments extracted from the unusually coloured blue-black "fuchsia" bee pollen confirms the New Zealand tree fuchsia as the source of the bee pollen. The pigments were identified as the *p-trans*-coumaroylated derivatives of delphindin-, petunidin and malvidin-3-O-glucosides and delphinidin-3-O-(*p-cis*-coumaroyl-glucoside). The anthocyanins are accompanied by several flavonol glycosides; kaempferol-3-sophoroside, quercetin-3-sophoroside and kaempferol 3-neohesperidoside.

Introduction

Bee pollen is a commercial product of considerable interest owing to its purported health-giving properties. Bees collect pollen selectively and systematically as a protein-rich food source for the hive (Stanley and Linskens, 1974). Bee pollen is composed of many individual pollen grains bound together as a pollen load ca. 1–2 mm in diameter. Colour is the most obvious feature used for differentiating the various pollen types, however morphology of individual pollen grains or chemical analysis of pigments are also employed (Kirk, 1994; Hodges, 1984; Campos *et al.,* 1997). While most bee pollens are yellow through to brown, a particular bee pollen found in hives from certain parts of New Zealand is a very distinctively purple black. It has been generally accepted that this unusual bee pollen is composed of pollen collected from the New Zealand tree fuchsia, *Fuchsia excorticata* (J. R. et G. Forst.) *(Onagraceae). Fuchsia excorticata,* which grows throughout New Zealand, is the world's largest fuchsia, and is commonly seen as a small tree but can grow to 14 m in height. A distinguishing feature is the unusual deep blue pollen colour. This study reports a comparison of the pigments in *Fuchsia extorticata* pollen with the pigments extracted from the "fuchsia" bee pollen in order to confirm the source of the bee pollen.

Materials and Methods

General procedures

NMR experiments were run at 500 MHz or at 300 MHz (75 MHz for ^{13}C). Anthocyanin samples were dissolved in 2% CF_3COOD in CD_3OD. Flavonols were run in DMSO-d_6. RP HPLC analyses were performed as described previously (Bloor, 1997) using a Waters 600 solvent delivery system coupled to a Waters 994 PDA detector. Anthocyanins were detected at 530 nm and flavonols simultaneously at 352 nm.

Purification of anthocyanins and flavonols

A portion of the bee pollen (17 g) was ground in a mortar with 100 ml methanol containing 0.5% TFA. The suspension was centrifuged and the supernatant filtered. The marc was re-extracted for 2 hrs. The combined extracts were reduced to a small volume and chromatographed on a Sephadex G-25 column eluted with a gradient of 1:9 through to 1:1 MeOH: H_2O (with 0.1% TFA). The flavonols were eluted before the anthocyanins. The three major flavonols were purified by RP CC using a Merck Lobar (RP-8) column and an isocratic solvent system (18: 81.9: 0.1 CH_3CN: H_2O: TFA v/v). The two delphinidin anthocyanins, **1** and **2**, were purified using the same system.

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Plant material

Fuchsia excorticata pollen was collected from a tree in Khandallah Park, Wellington in November 1999. The pollen was extracted as above and analysed directly by HPLC.

Source of bee pollen

The Fuchsia bee pollen collected in the 1997/98 season was supplied by Peter Sales, Port Chalmers, Dunedin, NZ.

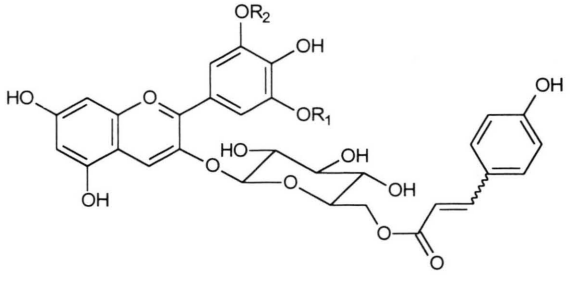
Reference compounds

The mixture of delphinidin-, petunidin- and malvidin-3-O-(*p*-coumaroyl)glucosides used for comparison was prepared from an extract of "Kolor" grapes. The crude grape extract was subjected to CC on polyamide using a solvent gradient beginning with 1% AcOH in H_2O and increasing the proportion of MeOH. The fraction containing these anthocyanins was the final coloured fraction to be eluted from the column and was readily characterised from on-line HPLC spectra.

Results and Discussion

As bees tend to collect pollen in a systematic fashion, concentrating on one species at a time, *Fuchsia* bee pollen is almost completely dark granules which are uncontaminated by pollen from other species. The bee pollen can be collected in large amounts by stripping the loaded bees using a metal mesh (a pollen trap) as they enter the hive. The pigment is readily extracted with acidic methanol and can be analysed by HPLC without prior clean-up. By contrast, only small amounts of blue pollen can be collected by hand from the tree fuchsia flowers in the spring. The pollen from a few flowers was extracted in a similar fashion. The HPLC chromatograms showed the pigments responsible for the pollen's blue colour and the dark colour of the bee pollen are the same. The characteristic uv-vis spectra and behaviour of the pigments confirmed these were anthocyanin pigments. A number of flavonol glycosides were also present in the extracts and these were also identical in both the bee pollen and the pollen from the flowers.

The individual anthocyanins and flavonols were purified from a large-scale extract of the bee pollen. HPLC analysis showed the presence of four anthocyanins, **1**–**4**. The major anthocyanin, **2**, had a UV-Vis spectrum consistent with a trihydroxylated B-ring anthocyanin acylated with a *p*-coumaric acid (Hrazdina and Franzese, 1974). The ^{1}H NMR spectrum showed signals for a 3-O-substituted delphinidin anthocyanidin, a β-D-glucose and a *p-trans*-coumaric acid. The *p*-coumaric acid was clearly located at the C-6 of the glucose as the signals for this were shifted downfield from their usual positions in the free sugar. Thus **2** is delphinidin 3-O-(6-O-*p-trans*-coumaroyl-β-D-glucoside). This anthocyanin has been previously characterised from grapes (*Vitis vinifera*) where it co-occurs with similar derivatives of cyanidin, petunidin, peonidin and malvidin (Hrazdina and Franzese, 1974; Baldi *et al.*, 1995). Indeed, when the *Fuchsia* pollen extract was compared by HPLC with a grape extract fraction, rich in the *p*-coumaroyl anthocyanins, not only was **2** shown to be the same as delphinidin-3-(*p*-coumaroylglucose) but two of the other *Fuchsia* anthocyanins, **3** and **4,** proved to be the petunidin- and malvidin- 3-(*p-trans*-coumaroyl glucosides). The identification of the most polar anthocyanin, **1**, was more difficult. This compound also was a delphinidin-3-glucoside acylated with *p*-coumaric acid (base hydrolysis and subsequent HPLC gave the same products as seen as **2**). Although only a poor ^{1}H NMR spectrum could be recorded, it could be seen that the *p*-coumaroyl double bond had a vicinal coupling constant of 13.8 Hz indicative of a *cis* isomer rather than the *trans* isomer (16.5 Hz) in **2**. The on-line UV-vis spectra of **1** and **2** were also different in the 280–



1 $R_1 = R_2 = H$, *cis-p*-coumaroyl
2 $R_1 = R_2 = H$, *trans-p*-coumaroyl
3 $R_1 = OMe$, $R_2 = H$, *trans-p*-coumaroyl
4 $R_1 = R_2 = OMe$, *trans-p*-coumaroyl

320 nm region. This difference was consistent with *cis* vs. *trans* isomerism and was also seen in the grape extract fraction where significant peaks for the *cis* isomers of petunidin and malvidin-3-(6-*p*-coumaroyl glucose) are seen. Thus **1** must be delphinidin-3-(6-*cis*-*p*-coumaroyl glucose). *Cis/trans* isomerism of cinnamate esters has been observed for a number of flowers (Hosokawa *et al.*, 1995).

The structures of the three flavonols were determined as follows; ^{1}H NMR data suggested two of these compounds were the 3-diglucosides of quercetin and kaempferol and the third the 3-rhamnoglucoside of kaempferol. Confirmation of the sugar linkages as the sophorosides (1–2 linked diglucoside) and neohesperidoside (1–2 linked rhamnoglucoside) was achieved via co-chromatography (HPLC, TLC) with authentic kaempferol-3-sophoroside, quercetin-3-sophoroside and kaempferol 3-neohesperidoside from our laboratory collection.

These results clearly confirm that the source of the dark purple-black bee pollen is indeed the blue *Fuchsia extorticata* pollen and identify the pigments responsible for the pollen colour to be *p*-coumaroylated anthocyanins. Aromatic acylation is often implicated in the generation of blue colour from anthocyanins, especially with regard to flower colour, and a variety of acylated anthocyanin compounds have been described from blue flowers (Goto and Kondo, 1991). These *Fuchsia* pollen compounds are commonly found in grapes but there they only constitute a minor fraction of the total anthocyanins and so have little impact upon the colour. There are few other reports of anthocyanins as pigments in pollen and only one example of analysis of a blue pollen where delphinidin 3-arabinoside is reported to be responsible for the blue colour of *Anemone coronaria* pollen (Tappi and Monzani, 1955).

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