The Unique Occurrence of the Flavone Aglycone Tricetin in Myrtaceae Pollen

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In pollen, flavonoids are usually found as glycosides and in particular, flavonol 3-O-diglycosides. However, in members of the Myrtaceae, subfamily Leptospermoideae, the rare flavone aglycone tricetin, along with other flavonoid aglycones including 3-*O*-methyl quercetin and luteolin, have been found to comprise a significant portion of the constituent flavonoids.

Introduction

Pollen has been shown to accumulate a species specific selection of flavonoids (Tomás-Lorente *et al.*, 1992; Campos *et al.*, 1997) and by using HPLC, Campos *et al.* (1977) showed that each species studied gave a unique fingerprint of phenolics (i.e. flavonoids plus other phenolics such as hydroxycinnamic acid derivatives).

It is often difficult to obtain enough floral pollen for large scale flavonoid analysis, but Campos *et al.* (1977) and Tomás-Lorente *et al.* (1992) have shown that bee collected pollen from one particular species gives an identical flavonoid profile to that of the source floral pollen, and thus that bee collected pollen can be used for flavonoid analyses.

A wide range of flavonoid types have been isolated from pollen with the most common type by far being the flavonol-3-*O*-diglycosides. However, it would appear from published work that it is very unusual for aglycones to occur naturally in pollen. The few accounts found are those of Wollenweber and Wiermann (1979) who reported the presence of the free chalcone aglycone, isosalipurpol, for the first time in mature pollen and Strohl and Seikel (1965) who found dihydrokaempferol, dihydroquercetin and naringenin in pine pollen. More recently we (Campos *et al.*, 1997) reported that *Eucalyptus globulus* accumulated the unusual aglycones, tricetin and 3-*O*-methylquercetin.

In the present study the flavonoid profiles of several members of Myrtaceae, subfamily Leptospermoideae, from New Zealand are compared with that from *Eucalyptus globulus*. Taxonomically the New Zealand Myrtaceae are grouped with *Eucalyptus* and the Australian tea trees to form a subfamily called Leptospermoideae (Salmon, 1980).

Materials and Methods

Plant material

Floral pollen was collected as follows:

Eucalyptus globulus from Beira Litoral, Portugal in 1980 (from Herbarium specimen); *Kunzea ericoides* (kanuka) from Nelson, New Zealand, 1998 (R. Coers); *Leptospermum scoparium* (manuka) from Nelson, New Zealand, 1998 (R. Coers); *Metrosideros excelsa* (pohutukawa) from Lower Hutt, New Zealand, 1999; *Metrosideros umbellata* (southern rata) from Papatowhai, New Zealand, 2000. The pollen was extracted as detailed below and analysed directly by HPLC.

Bee pollen

Bee pollen was supplied as follows:

Eucalyptus globulus, 1991, 1994 and 1995 collected Maria Campos, Ovar (Beira Litoral), Portugal; *Kunzea ericoides*, 1998, R. Coers, Nelson, New Zealand; *Leptospermum scoparium*, 1995, Comvita New Zealand Ltd; *Metrosideros excelsa*, 1999, Lower Hutt, New Zealand and *Metrosideros umbellata*, 1999, G. Glasson, Westland, New Zealand.

Purification and identification of flavonoids

Bee collected pollens from *Eucalyptus globulus* and *Metrosideros umbellata* were used for the large scale extraction and isolation of flavonoids. These pollens were hand-sorted from bee pollen mixes in which they were the dominant pollens. The floral source of the pollen was confirmed by comparison of the extracts with those of the respective floral pollens using HPLC. Bee collected

pollen from the other sources listed was used only to give HPLC profiles, as with the floral pollens. On-line recorded UV spectra together with retention time data were used to compare the flavonoids in these profiles with those identified from the large scale extractions. Overnight extraction of the pollen with EtOH:H_2O (1:1 v/v) (for example, 2 g bee pollen was extracted with 200 ml EtOH:H_2O (1:1 v/v) or a pollen load weighing 8 mg was extracted with 800 μl EtOH:H_2O (1:1 v/v) (Campos *et al.*, 1997) was aided by ultrasonication (30 min). The extract was then centrifuged and flavonoid cleanup by paper and RP18 column (MeOH) chromatography preceded HPLC analysis. The structures of all compounds were determined using standard techniques such as UV absorption spectroscopy and ^{1}H-NMR (DMSO-d_6, 300 MHz) spectroscopy (Markham, 1982).

Results and Discussion

Flavonoid profiles obtained from pollen extracts are typified by the presence of flavonoid glycosides. However, as part of our study of many floral and bee collected pollens both from Portugal and New Zealand, it was observed that the pollens of the Myrtaceae, subfamily Leptospermoideae, all accumulate the aglycone tricetin. Campos (1997) and Campos *et al.* (1997) have already reported the occurrence of tricetin in *Eucalyptus globulus*. This was the first reported isolation of this compound from pollen and it appears that tricetin aglycone is unknown from any other plant source (Wollenweber, 1994). This makes the finding of this aglycone even more unusual. The pollen sourced tricetin was identified primarily from its ^{1}H-NMR which contains a very distinctive set of signals which match with those detailed by Markham and Geiger (1994).

Further to the presence of tricetin in the pollen of these species, it was observed that other flavonoid aglycones are also accumulated, see Table I. Table I illustrates the aglycones accumulated by the species listed. From Table I it can be seen that luteolin, myricetin and 3-*O*-methylquercetin were identified in pollen from *E. globulus*, while luteolin and 3-*O*-methylquercetin were present in *M. umbellata* pollen. *L. scoparium* pollen accumulated tricetin and luteolin and *K. ericoides* luteolin and myricetin while luteolin was identified in *M. excelsa* pollen. If 3-*O*-methylquercetin is present in the pollen of *K. ericoides*, *L. scoparium* and *M. excelsa*, it is present below the level of detection. None of these flavone aglycones are represented as glycosides in the total flavonoid profile. The only accompanying flavonoid glycosides are the flavonol-3-*O*-diglycosides.

Thus the pollens of the Myrtaceae, subfamily Leptospermoideae, that have been studied all unusually accumulate predominantly flavone aglycones, including the unique flavone tricetin, rather than flavonol glycosides, which is the normal situation. Significantly, the (1–2) linked flavonol diglycosides are also present (at lower levels) which is consistent with the suggestion (Markham and Campos, 1996) that these glycosides may play a universal role in pollen physiology.

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Table I. Flavonoid aglycones found in Myrtaceae, subfamily Leptospermoideae.

	FLAVONOID AGLYCONE			
Species	Tricetin	Luteolin	3-*O*-Methyl quercetin	Myricetin
Eucalyptus globulus	√	√	√	√
Kunzea ericoides	√	√		√
Leptospermum scoparium	√	√		
Metrosideros excelsa	√	√		
Metrosideros umbellata	√	√	√	

Key: √ = present.

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