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# CHRYSIN AND OTHER LEAF EXUDATE FLAVONOIDS IN THE GENUS *PELARGONIUM*

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**Key Word Index**—*Pelargonium crispum*; *P. quercifolium*; *P. tomentosum*; *P. fulgidum*; *P. exstipulatum*; Geraniaceae; leaf exudates; chrysin; flavone and flavonol methyl ethers.

**Abstract**—In a chemotaxonomic survey of 57 *Pelargonium* species, leaf exudate flavonoids were detected in 35% of the sample, mostly in trace amounts. However, chrysin and a related *C*-methylflavanone were identified as major leaf surface constituents of *P. crispum*, and a mixture of quercetin and kaempferol mono-, and di- and trimethyl ethers of *P. quercifolium*. In two other species, *P. fulgidum* and *P. exstipulatum*, methylated flavones were the only lipophilic flavonoids present. This is the first report of leaf surface flavonoids from the genus *Pelargonium*. © 1997 Elsevier Science Ltd

## INTRODUCTION

More and more examples of plants which produce exudate flavonoids are now being found because workers are routinely testing for these constituents during their chemotaxonomic studies. For example, we have recorded them in the Velloziaceae [1] in the monocots and, in the dicots, they have been reported from 39 families, being most frequent in the Labiatae and Compositae [2]. During the course of a phenolic survey of the genus *Pelargonium* (Geraniaceae) we again looked for lipophilic flavonoids and detected them in various amounts in 35% of the 57 species we tested. Since there has been only one previous report in the Geraniaceae from two species in the closely related genus *Geranium*, we decided to carry out a more detailed analysis of the leaf surface flavonoids in five *Pelargonium* species: *P. crispum*, *P. exstipulatum*, *P. fulgidum*, *P. quercifolium* and *P. tomentosum*, which were rich in these constituents.

## RESULTS

The presence of lipophilic flavonoids in *Pelargonium* species was tested by briefly dipping entire leaves in acetone and running the concentrated extracts on TLC in comparison with standard markers. Although some 35% of the taxa proved positive (Table 1), these constituents were mostly present in only trace amounts. There was no obvious correlation between presence of exudate flavonoids and sectional classification in the genus, because they were found sporadically in all sections except *Isopetalum* (monotypic), *Polyactium* (four of 11 known species were surveyed), *Jenkinsonia* (one of four species), *Hoarea* (three of 15 species) and *Myrrhidium* (two of seven species). However, plants which do produce exudate flavonoids were found to grow in semi-arid or alpine conditions and negative results were obtained from plants growing in sheltered habitats or in lowland areas of unrestricted rainfall, indicating that habitat is the important controlling factor in their distribution.

Eight of the 13 species, which produced surface flavonoids in reasonable amount were further analysed by HPLC; these are indicated in Table 1 by an asterisk. By this means, methylated flavonols were found to be the major exudate flavonoids of *P. quercifolium*. Quercetin 3-methyl ether, kaempferol 3-methyl ether, quercetin 3,7-dimethyl ether and quercetin 3,7,4′- or 3,7,3′-trimethyl ether were identified by co-TLC and co-HPLC with authentic markers. A similar mixture of quercetin and kaempferol 3-mono, 3,7-di and trimethyl ethers was found in *P. fulgidum*, together with three apigenin-based methylated flavones. HPLC analysis of the acetone extract of *P. tomentosum* revealed only two flavonoid constituents, a kaempferol tri and a quercetin tetramethyl ether. By contrast, *P. abrotanifolium*, *P. album* and *P. exstipulatum* produced only lipophilic flavones.

In a more detailed analysis of *P. crispum*, two major exudate constituents were isolated. The first was identified as chrysin, 5,7-dihydroxyflavone, by co-TLC and

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Table 1. *Pelargonium* species found to produce exudate flavonoids

| Species† | Accession Number | Leaves Hairy (H) or Glabrous (G) | Scented or not | Leaf exudate flavonoids Concentration | Constituents identified |
|---|---|---|---|---|---|
| *P. abrotanifolium* (L.f.) Jacq.* | CPG 7036 | H | + | + | Ap-based flavones possibly acylated |
| *P. album* J.J.J. v.d. Walt* | CPG 8230 | H | + | + | Chrysoeriol, acacetin, AptriMe, flavone glycoside |
| *P. alternans* Wendl. | CPG 6171 | H | − | (+) | nd |
| *P. antidysentericum* (Eck. & Zeyh.) Costel | CPG 8183 | H | + | + | nd |
| *P. cortusifolium* L. Herit | CPG 9207 | H | (+) | + | nd |
| *P. crispum* (Berg.) L. Herit 'Whiteknights'* | RNG | H | + | ++ | Chrysin, 6 or 8-C methyl 5,7-dihydroxyflavanone |
| *P. drummondii* Turcz* | CPG 8615 | H | (+) | + | Two unidentified flavanones |
| *P. exstipulatum* (Cav.) L. Herit* | CPG 4422 | H | + | + | Ap-based flavones |
| *P. fragile* (Andr.) Wild | CPG 4214 & 7659 | H | + | (+) | nd |
| *P. fulgidum* (L.) L. Herit* | CPG 8235 | H | − | + | Qu3ME, Km mono ME, Qu3,7-DIME, Km3,7-DIME, KmtriME, QutetraME and three Ap-based flavones |
| *P. grandiflorum* (Andr.) Willd | CPG 6489 | G | − | (+) | nd |
| *P. laxum* (Sweet) G. Don | CPG 5896 | G | − | (+) | nd |
| *P. magenteum* J.J.A. v.d. Walt | CPG 8473 | H | − | (+) | nd |
| *P. mollicomum* Fourc. | Steu 3282 | H | + | (+) | nd |
| *P. multibracteatum* Hochst. | CPG 6868 | H | (+) | (+) | nd |
| *P. ovale* (Burm. f.) L. Herit | CPG 8852 | H | − | + | nd |
| *P. quercifolium* (L.f.) L. Herit* | CPG 8194 | H | + | ++ | Qu 3ME, Km 3ME, Qu 3,7-DIME, Qu 3,7,4′- or 3,7,3′tri ME |
| *P. reniformia* Curt. | CPG 7078 | H | − | (+) | nd |
| *P. rodneyanum* Mitch. ex. Lindl. | CPG 2625 | G | − | (+) | nd |
| *P. tomentosum* Jacq.* | CPG No number | H | + | + | Km triME, QutetraME flavonol glycosides? |
| *P. tricolor* Curt. | CPG 8077 | H | − | (+) | nd |

* HPLC analysis carried out on leaf exudates of these species.

† Species arranged alphabetically not in any taxonomic order.

Key: ++ = present in large amounts, + = present in moderate amounts, (+) = present in trace amounts, Qu = quercetin, Km = kaempferol, Ap = apigenin, ME = methyl ether, CPG = plants growing at Chelsea Physic Garden, RNG = plants growing at the School of Plant Sciences, University of Reading.

comparison of EI mass and UV spectral data with an authentic marker (see Experimental). This is the first report of chrysin in *Pelargonium* or in the Geraniaceae. The second compound proved to be a *C*-methyl derivative of 5,7-dihydroxyflavanone, either strobopinin (the 6-*C*-methyl derivative) or cryptostrobin (the 8-*C*-methyl isomer); identification is described in the Experimental. Lack of material prevented NMR analysis, which would have distinguished between C-6 and C-8 methylation. Both isomers were originally isolated from *Pinus* heartwood and have more recently been detected in leaf surface extracts of *Comptonia peregrina* and *Myrica pensylvanica* (Myrtaceae) by Wollenweber *et al.* [3], where they are also accompanied by chrysin.

## DISCUSSION

Exudate flavonoids are most usually found in plants which grow in arid or semi-arid habitats, and often in association with aromatic terpenoids on the leaf surface, where they are produced from glandular hairs. It has been generally assumed that surface flavonoids serve as a UV-screen and help in the adaptation of plants to alpine, arid or semi-arid conditions [4]. Experimental verification for this assumption that exudate flavonoids do shield the leaf from damaging UV light has been achieved in a recent study of surface flavonoids in two species of *Gnaphalium* (Compositae) [5]. Indeed in the present study, most of the *Pelargonium* species found to produce exudate flavonoids do live in semi-arid or alpine habitats or on exposed cliffs near the sea. Sixteen of these 20 species also have glandular hairs on the leaf surface but only ten produce sweet-smelling essential oils (see Table 1). In some other hairy leaved species, e.g. *P. transvaalsense* and *P. alchemiloides*, neither surface flavonoids nor essential oils were detected. However, in a similar study of North African *Sideritis* species (Labiatae), where all the sample grew in arid sunny habitats, Tomás-Barberán *et al.* [6] found three distinct groups: (a) those species which excreted flavonoids, (b) those which produced surface terpenoids and (c) species which produced no surface constituents but were protected from UV radiation by a dense mat of white hairs.

Within the Geraniales exudate flavonoids have been reported from leaves of two *Geranium* species (Geraniaceae) [7] and *Larrea tridentata* (Zygophyllaceae) [8]. *Geranium macrorrhizum* and *G. lucidum* were found to have very similar external flavonoid profiles, in which some 15 flavonol methyl ethers were identified, *viz*: kaempferol 3-mono-, 4′-mono-, 3,7-di-, 3,4′-di-, 7,4′-di- and 3,7,4′-trimethyl ethers; quercetin 3,7-di, 3,3′-di, 7,3′-di, 3,7,3′-tri, 7,3′,4′-tri and 3,7,3′,4′-tetramethyl ethers and myricetin 7,3′,4′-tri- and 3,7,3′,4′-tetramethyl ethers. The only differences between the species were in the absence of kaempferol 4′-methyl ether in *G. macrorrhizum* and of quercetin 3,7-dimethyl ether in *G. lucidum*. This is a similar flavonol pattern to that found in two *Pelargonium* species, *P. quercifolium* and *P. fulgidum*, in the present study, except that here myricetin methyl ethers were not detected. By contrast, the two flavonoids reported from the leaf resin of *Larrea tridentata* (Zygophyllaceae) [8] were more complex methylated 8-hydroxyflavonols: herbacetin (8-hydroxykaempferol) 3,7,8-trimethyl ether and gossypetin (8-hydroxyquercetin) 3,7,8,3′-tetramethyl ether, compounds not detected in any of the *Pelargonium* species surveyed.

Exudate flavones and flavanones have not been reported previously from the Geraniaceae, although flavanones have been found as surface constituents of two *Cotoneaster* species (Rosaceae, Rosales) in the same class Rosidae as the Geraniales. Similarly, chrysin has been identified in the leaf exudate of the fern, *Cheilanthes kaulfussii* [9], the leaf resin of *Cistus populifolius* [10] and from leaf or aerial parts (probably on the surface but not tested) of three *Scutellaria* species [11–13] in the Labiatae and several other sources [4, 14], but not previously from the Geraniales.

## EXPERIMENTAL

*Plant material.* Leaf material of *Pelargonium* (Berg.) L'Herit Whiteknights was obtained from plants growing in the glasshouses at the School of Plant Sciences, University of Reading, where a voucher specimen has been lodged in the herbarium (RNG) and was verified by Mr R. Rutherford. Leaf samples of the other species were obtained from plants growing at the Chelsea Physic Garden and verified by Dr Mary Gibby of the Natural History Museum, London. Accession numbers are given in Table 1.

*Detection of surface flavonoids.* Whole leaves were dipped briefly in $Me_2CO$ and concd extracts run on silica gel TLC in toluene–HOAc (4:1) against standard flavonol mono, di and trimethyl ether markers. Flavonoid aglycones appear as dark absorbing spots in UV light on silica gel, whereas terpenoid constituents are not visible unless sprayed with a suitable reagent. Extracts which were rich in flavonoid constituents were subjected to further HPLC analysis, again in comparison with standard markers, using a Bondapak phenyl C18 reverse-phase 4 mm I.D. 30 cm column. HPLC conditions: solvent A = 2% HOAc, solvent B = MeOH–HOAc–$H_2O$ (18:1:1) with a gradient of 40%A 60%B → 100%B over 30 min in linear mode at 25°, flow rate 1 ml $min^{-1}$ with diode array detection at 260 and 350 nm.

*Surface flavonoids in* P. quercifolium. Four methylated flavonols were identified in the $Me_2CO$ leaf wash by means of co-TLC on silica gel TLC in toluene–HOAc (4:1) and co-HPLC (conditions as described above) as quercetin and kaempferol 3-methyl ethers, quercetin 3,7-dimethyl ether and quercetin 3,7,4′- or 3,7,3′-trimethyl ether (see Table 2).

*Surface flavonoids in* P. crispum. The two major leaf exudate flavonoids (**1** and **2**) from *P. crispum* were isolated from the concd leaf $Me_2CO$ wash by prep.

Table 2. $R_f$ and HPLC $R_t$s of flavonol methyl ethers identified from leaf surface of *P. quercifolium*

| Flavonol methyl ether | $R_f \times 100$ on silica gel in toluene–HOAc (4:1) | HPLC $R_t$s (min) *P. quercifolium* extract | Extract co-marker | Marker |
|---|---|---|---|---|
| Quercetin 3-methyl ether | 06 | 5.82 | 5.93 | 5.80 |
| Kaempferol 3-methyl ether | 15 | 7.50 | 7.46 | 7.45 |
| Quercetin 3,7-dimethyl ether | 25 | 9.96 | 9.98 | 9.93 |
| Quercetin 3,7,4′- or 3,7,3′-trimethyl ether | 56 | 13.22 | 13.10* | 12.99* |

* The marker used was quercetin 3,7,4′-trimethyl ether. The plant may contain the 3,7,3′-trimethyl ether or a mixture of the two as they do not separate clearly on HPLC.

Table 3. $R_f$ and HPLC $R_t$ data for compounds **1** and **2** from *P. crispum*

| Flavonoid | $R_f \times 100$ on Silica gel TLC Toluene–HOAc, (4:1) | Silica gel TLC 10% MeOH in $CHCl_3$ | Cellulose TLC 30% HOAc | 40% HOAc | 50% HOAc | *n*-BuOH /$NH_3$ | HPLC $R_t$ (min) |
|---|---|---|---|---|---|---|---|
| **1** | 46 | 79 | 40 | 58 | 74 | 75 | 10.81 |
| CO | 46 | 79 | 40 | 58 | 74 | 75 | — |
| Chrysin | 46 | 79 | 40 | 58 | 74 | 75 | 10.79 |
| **2** | 51 | 66 | 31 | 53 | 71 | 84 | 10.67* 12.25† |

* Flavanone.
† Corresponding chalcone in isomeric mixture.

silica gel TLC in toluene–HOAc (4:1) and purified by prep. PC in 30% HOAc on Whatman 3MM paper. Compound **2** was further purified by prep. PC in $H_2O$.

Compound **1** was identified as chrysin by co-TLC and HPLC comparison with an authentic marker (Table 3). UV spectral data for **1** were also consistent with this structure: $\lambda_{max}^{MeOH}$ 269, 315; +NaOAc 270, 325; +$H_3BO_3$ 269, 317, 325 and +NaOH 264, 279, 354. The identity of **1** as chrysin was confirmed by EIMS: *m/z* found 254 (required 254), 226 ([M-CO]$^+$, 12%), 152 ([M-102]$^+$, A-ring 20%), 124 [A-ring-CO]$^+$.

Compound **2** was identified as a *C*-methylflavanone, either 5,7-dihydroxy-6-*C*-methylflavanone (stropopinin) or the 8-*C*-methyl isomer (cryptostrobin). UV spectral data indicated a flavanone structure: $\lambda_{max}^{MeOH}$ 290, with inflection at 330; +NaOAc 293, 326; +NaOH 325, 380 nm. During purification it isomerized to some extent to give the corresponding chalcone with $\lambda_{max}$ 239, 344 nm. $R_f$ values are given in Table 2. The $M_r$ was established as 270 ($C_{16}H_{14}O_4$ requires 270) by MS. *C*-Methylation and 5,7-dihydroxylation in the A-ring were established from the MS fragmentation, which showed intense ions at *m/z* 194 and 167. This was confirmed when attempted demethylation (pyridine chloride) gave unchanged material, indicating the absence of *O*-methylation.

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