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Peonidin 3-*O*-neohesperidoside and other flavonoids from *Cyclamen persicum* petals

Rosemary F. Webby[a,*], Murray R. Boase[b]

[a]*NZ Institute of Industrial Research and Development, PO Box 31-310, Lower Hutt, New Zealand*
[b]*NZ Institute for Crop and Food Research Ltd., Private Bag 11600, Palmerston North, New Zealand*

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Abstract

The major anthocyanins and flavonols of two cultivars of *Cyclamen persicum* were characterised by ^{1}H- and ^{13}C-NMR spectroscopy. A previously unreported anthocyanin, peonidin 3-*O*-α-L-rhamnopyranosyl-(1-2)-β-D-glucopyranoside was isolated from *C. persicum* cv. Bonfire. The predominant anthocyanins isolated from *C. persicum* cv. Sierra Rose were the 3,5-di-*O*-glucosides of peonidin, cyanidin and malvidin. Quercetin 3-*O*-2^{G}-rhamnosylrutinoside was isolated from both cultivars as the major flavonol.

Keywords: *Cyclamen persicum* cv. Bonfire; *Cyclamen persicum* cv. Sierra Rose; Primulaceae; Cyclamen; Anthocyanin; Flavonol; Peonidin 3-*O*-neohesperidoside; Quercetin 3-*O*-α-L-rhamnopyranosyl-(1-2)-*O*-[α-L-rhamnopyranosyl-(1-6)-β-D-glucopyranoside]

1. Introduction

The anthocyanin and flavonoid pigments of many cyclamen cultivars have been investigated by various groups and reported previously (Miyajima, Doi & Kage, 1990; Miyajima, Maehara, Kage & Fujieda, 1991; Van Bragt, 1962). Van Bragt in particular gives a very comprehensive study of the genus. However, *Cyclamen persicum* Mill. cv. Bonfire (Giganteum series) and *C. persicum* Mill. cv. Sierra Rose (F1 hybrid Sierra series) are cultivars of interest in New Zealand for molecular breeding of novel flower colours by genetic engineering of the flavonoid pigment pathway. The flavonoid chemistry of these cultivars has not been reported previously. A thorough knowledge of this chemistry is one of the important prerequisites to meet before formulating an appropriate molecular breeding strategy. In the present paper, the isolation and identification of the anthocyanin and flavonoid pigments of these cultivars is presented.

2. Results and discussion

In *Cyclamen*, the base (eye) and main part of the petal (slip) are usually coloured differently, with the eye being more intensely coloured than the slip (Van Bragt, 1962). *C. persicum* cv. Bonfire (Bonfire) has a deep red slip with a darker red eye, whilst in *C. persicum* cv. Sierra Rose (Sierra Rose), the slip was pink and the eye purple. The anthocyanin and flavonol pigments of these petals were isolated and identified and while it was shown that the dominant anthocyanins in the two cultivars were different, the same major flavonol was present in both cultivars.

2.1. Anthocyanins

Within a cultivar it was seen that the same anthocyanins were present in both the slip and the eye of

* Corresponding author. Tel.: +64-4-5690-000; fax: +64-4-5690-055.

E-mail address: r.webby@irl.cri.nz (R.F. Webby)

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the petal. However, different anthocyanins predominated in the two areas of the petal. In the slips of Bonfire, **1**, was the major anthocyanin. Low levels of **1** were present in the eye. **1** hydrolysed to give peonidin (co-TLC, HPLC, Vis Spectrum) and Glc:Rha 1:1 (NMR, PC). The absorption spectrum and NMR (in particular the use of HMBC) confirmed a 3-*O*-linkage. The presence of a neohesperidoside was confirmed by ^{13}C NMR (see Section 3) in which the assignments for glucose matched those of the glucose in cyanidin 3-*O*-xylo-(1-2)-glucoside (Johansen, Andersen, Nerdal & Aksnes, 1991). In particular, relative to a 3-linked glucose (Johansen et al., 1991), the Glc-2 moved downfield from 74 to 80 ppm. Glc-6 remained unchanged. Coupling constants of 1.7 and 7.6 Hz confirmed the presence of α-linked rhamnose and β-linked glucose respectively. Thus **1** was identified as peonidin 3-*O*-α-L-rhamnopyranosyl (1-2) β-D-glucopyranoside (peonidin 3-*O*-neohesperidoside).

A trace of the cyanidin neohesperidoside was also observed. Cyanidin, delphinidin and pelargonidin 3-*O*-neohesperidosides have been reported from *Dacrycarpus dacrydioides* and *Podocarpus* species (Andersen, 1988, 1989) but to the author's knowledge, the peonidin glycoside has not been reported previously. Malvidin 3-*O*-glucoside (HPLC, aglycone) was the major anthocyanin present in the eye of the Bonfire flower. Low levels of this glycoside were also present in the slip, whilst low levels of cyanidin and peonidin 3-*O*-glycosides (HPLC, aglycones) were found in both the slip and eye of Bonfire.

The major anthocyanins from Sierra Rose, **2**, **3** and **4**, hydrolysed to give cyanidin, peonidin and malvidin respectively, with glucose as the sugar (PC, HPLC, TLC). **2**, **3** and **4** were shown to be the 3,5-di-*O*-glucosides of cyanidin, peonidin and malvidin respectively, by cochromatography on HPLC and by comparison of their on-line spectra with standards from rose and *Vitis rotundifolia*. These glycosides were present in both the slip and the eye, with the cyanidin and peonidin glycosides predominating in the slip and the malvidin glycoside predominating in the eye. Thus in both cultivars the eye contained a higher proportion of the malvidin glycosides.

2.2. Flavonols

One major flavonol, **5**, was isolated from both cultivars. **5** was very mobile on cellulose (TLC) in 15% HOAc. Partial hydrolysis to quercetin 3-rutinoside and quercetin 3-glucoside (TLC) as well as NMR data (see experimental) showed this compound to be quercetin 2^G-rhamnosylrutinoside. A trace of the kaempferol glycoside was also present. These compounds have been reported previously (Buttery & Buzzell, 1975; Vidal-Ollivier et al., 1989). Traces of quercetin 3-glucoside were isolated from both cultivars, whilst kaempferol 3-glucoside and kaempferol and quercetin 3-rutinosides were isolated from Sierra Rose. These compounds were identified by TLC.

In general the anthocyanins and flavonols isolated from these cultivars are similar to those already reported (Miyajima et al., 1990; Van Bragt, 1962), for example: peonidin, cyanidin and malvidin 3,5 diglucosides and 3 glucosides, kaempferol and quercetin 3 glucosides and quercetin rutinoside. Peonidin 3-*O*-neohesperidoside and quercetin 2^G-rhamnosylrutinoside have not been identified in *Cyclamen* spp. before.

3. Experimental

3.1. Plant material

Flowers of Bonfire and Sierra Rose were collected in 1994 and 1997, respectively, from Levin Research Centre, NZ Institute for Crop and Food Research Ltd., Levin. Voucher specimens have been deposited in the international registered herbarium at Massey University, Palmerston North, New Zealand, that is designated as MPN. Numbers of the voucher specimens are MPN 24585 for cv. Bonfire and MPN 24586 for cv. Sierra Rose. Bonfire was available from Watkins Seeds Ltd., New Plymouth, New Zealand. Sierra Rose was also available from Watkins Seeds Ltd but was produced by Goldsmith Seeds, USA.

3.2. Sample extraction and work-up

Fresh whole petals were ground and then extracted overnight with EtOH–H_2O–HCOOH, 50:43:7. The extract was dried, dissolved in 7% aqueous formic acid and applied to a polyamide column (Macherey-Nagel-CC6). Coloured bands were collected, following which, the solvent was changed to 7% formic acid in MeOH to elute the flavonols and anthocyanidins. An LH-20 column using the same solvents as above was found to be useful in the clean-up of anthocyanins.

3.3. Isolation and identification

Details of the techniques used in the isolation and identification of the flavonols and anthocyanins e.g. HPLC may be found elsewhere (Markham, 1982; Mitchell, Markham & Boase, 1998).

3.3.1. Peonidin 3-O-neohesperidoside

(**1**) R_f (TLC cellulose): 0.80 (TBA), 0.86 (15% HOAc). Acid hydrolysis gave peonidin, Rha:Glc 1:1. ^{1}H NMR (300 MHz, 500 MHz, 3% CF_3COOD in CD_3OD): δ 8.95 (1H, s, H-4), 8.15 (1H, *d, J* = 2.2, H-2′), 8.11 (1H, *dd, J* = 2.7, 10.9, H-6′), 7.02 (1H, *d,*

J = 8.8, H-5′), 6.87 (1H, *d*, J = 0.73, H-8), 6.6 (1H, *br s*, H-6), 5.57 (1H, *d*, J = 7.6, Glc-1), 5.20 (1H, *d*, J = 1.7, Rha-1), 3.96 (3H, *s*, OMe), 0.79 (3H, *d*, J = 6.1 Hz, Rha-Me). ^{13}C NMR sugar region (75 MHz, 3% CF_3COOD in CD_3OD); δ Glc: 101.8 (C-1″), 80.3 (C-2″), 78.9(C-3″), 71.5(C-4″), 78.7(C-5″), 62.3(C-6″); Rha: 102.9 (C-1‴), 72.3 (C-2‴), 72.2 (C-3‴), 74.0 (C-4‴), 70.4 (C-5‴), 17.9 (C-6‴).

3.3.2. Quercetin 3-O-2^{G}rhamnosylrutinoside

(**5**) R_f (TLC on cellulose): 0.55 (TBA), 0.87 (15% HOAc), 0.66 (H_2O). Acid hydrolysis gave quercetin, Rha:Glc 2:1. λ_{max}^{MeOH} nm: 260, 294 (sh), 356; (NaOMe) 274, 328, 400; (NaOAc) 272, 325 (sh), 368; (NaOAc–H_3BO_3) 264, 372; ($AlCl_3$) 276, 305 (sh), 432; ($AlCl_3$–HCl) 272, 302 (sh), 364, 400. ^{1}H NMR (300 MHz, 500 MHz, DMSO d_6): δ 12.65 (1H, *s*, 5.OH), 7.52 (1H, *dd*, J = 1.9, 8.3, H-6′), 7.48 (1H, *d*, J = 1.9, H-2′), 6.82 (1H, *d*, J = 8.3, H-5′), 6.36 (1H, *d*, J = 1.6, H-8), 6.17 (1H, *d*, J = 1.7, H-6), 5.52 (1H, *d*, J = 7.5, Glc-1), 5.05 (1H, *br*. *s*, Rha-1), 4.33 (1H, *br*. *s*, Rha-1), 0.96 (3H, *d*, J = 6.1, Rha-Me), 0.78 (3H, *d*, J = 6.1, Rha-Me). ^{13}C NMR sugar region (75 MHz, DMSO d_6); δ Glc: 98.1 (C-1″), 77.4 (C-2″), 77.4 (C-3″), 70.7[a] (C-4″), 76.0 (C-5″), 67.4 (C-6″); 2^G-Rha: 101.1 (C-1‴), 70.8[a] (C-2‴), 70.6[a] (C-3‴), 72.1[b](C-4‴), 68.5[c] (C-5‴), 18.9 (C-6‴), 6^G-Rha: 102.1 (C-1⁗), 70.8[a] (C-2⁗), 70.6[a] (C-3⁗), 72.1[b] (C-4⁗), 68.5[c] (C-5⁗), 18.5 (C-6⁗). Assignments with the same symbol may be reversed.

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