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# The Homology of Red Flower Colour in *Crassula*, *Cotyledon* and *Tylecodon* (Crassulaceae)

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**Key Word Index**—Crassulaceae; *Cotyledon*; *Crassula*; *Tylecodon*; anthocyanins; chemotaxonomy; generic relationships.

**Abstract**—The chemical basis of red flower pigmentation in species of *Cotyledon, Crassula* and *Tylecodon* is reported. Only six major anthocyanins were found in the 10 species and 22 samples investigated, and each of the genera had a characteristic combination of compounds. *Crassula* flowers invariably have only two of the major pigments, the 3-glucosides of cyanidin and peonidin. In *Cotyledon*, cyanidin-3-sophoroside is the dominant pigment in most of the samples and cyanidin-3-sambubioside only in some of them. This is also the major anthocyanin in orange-coloured *Tylecodon* flowers, but relatively high yields of delphinidin-3-sambubioside are found in dark red flowers. The anthocyanins reflect the close taxonomic relationship between *Cotyledon* and *Tylecodon*, and the more distant affinity of both genera with *Crassula*.

## Introduction

Flower colour is often used as a taxonomic character without considering the homology of the pigments involved. It is known that the same colour in different plants can be due to different pigments, and that different colours can be ascribed to different conditions (e.g. pH of cell sap, intramolecular stacking) acting on one pigment. In this study, we evaluated the homology of similar flower colours in the Crassulaceae, and assessed the chemotaxonomic potential of anthocyanins in red-flowered species of *Cotyledon, Crassula* and *Tylecodon*. *Tylecodon* is a natural group of species with spirally arranged, seasonal leaves, recently segregated from *Cotyledon* (Tölken, 1985). Relationships within the predominantly African genus *Cotyledon* is poorly known, particularly within the *C. orbiculata* complex (Tölken, 1979).

## Materials and Methods

*Plant materials.* Locality data of the material used for extraction are listed in Table 1.

*Procedures.* Petals were extracted in MeOH:acetic acid:$H_2O$ (8:1:1) and chromatographed by analytical HPLC using a diode array detector (Beckman Ultrasphere ODS column, 5 μm particle size, 250 mm × 4.6 mm i.d.; flow rate 1 ml $min^{-1}$; 20 μl sample loop). The solvent system was the same as described by Akavia and Strack (1980) and Strack *et al.* (1980) and comprised a 20–100% linear gradient of A in B. A: 1.5% $H_3PO_4$, 20% acetic acid and 25% MeCN in $H_2O$, B: 1.5% $H_3PO_4$ in $H_2O$. All samples were purified by ODS solid phase extraction prior to analysis. $R_t$ values, UV–VIS spectral data and $R_f$ values were used for comparisons with known standards (Harborne, 1967; Hong and Wrolstad, 1990a,b). Where possible, extracts were co-chromatographed (HPLC and PC) with known standards (Harborne, 1967) to confirm equivalent $R_t$ and $R_f$ values. Samples were subjected to partial hydrolysis (usually 10, 20, 40 and 60 min with 2N HCl @ B.P.) to determine the type of glycoside (Strack *et al.*, 1980). Several freshly collected samples were studied to exclude the possibility of unstable esters.

Isolation of cy-3-sambubioside was achieved after initial enrichment of an extract of *Tylecodon paniculatus* peduncles (see Table 1) with PC, using acetic acid:HCl:$H_2O$ (15:3:82) and eluting the anthocyanin band with 1% HCl in MeOH. The pigment was then isolated by prep. HPLC (Beckman Ultrasphere ODS column, 5 μm particle size, 250 mm × 10 mm i.d.; flow rate 3 ml $min^{-1}$; 1 ml sample loop) using the solvent system given above. For optimal resolution the sample was loaded at 15% A, followed by an increase to 30% after 2 min, and a gradual gradient to 40% over 15 min. Fractions containing the peak of interest were collected for eight separation cycles, concentrated *in vacuo*, and loaded onto an ODS solid phase extraction column (1000 mg).

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TABLE 1. DISTRIBUTION OF ANTHOCYANINS IN RED-FLOWERED SPECIES OF *COTYLEDON, CRASSULA* AND *TYLECODON*. Anthocyanin distributions are given as percentages of total pigments, as estimated from HPLC results, using a diode array detector (see Materials and Methods)

| | Major anthocyanins* | | | | | |
|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** |
| Genus, species and sample | $R_f$†: 16.0 | 16.9 | 18.8 | 21.8 | 22.0 | 27.9 |
| *Crassula alba* Forssk. | | | | | | |
| Darrenwood, Johannesburg | — | — | — | — | 13 | 84 |
| *C. coccinea* L. | | | | | | |
| Kirstenbosch Bot. Gard. | — | — | — | — | 86 | 14 |
| *C. perfoliata* L. | | | | | | |
| ex hort., RAU campus | — | — | — | — | 89 | 11 |
| *Cotyledon adscendens* R. A. Dyer | | | | | | |
| Johannesburg Bot. Gard. | — | 6 | 80 | — | 6 | — |
| *C. barbeyi* Schweinf. ex Bak. | | | | | | |
| ex hort., Vivo | — | 7 | 5 | 75 | — | — |
| ex hort., G. F. Smith | — | 10 | 55 | 20 | — | — |
| *C. orbiculata* L. var. *orbiculata* | | | | | | |
| Olifantskop, E. Cape | — | — | 95 | — | — | — |
| Grootderm, NW Cape | — | tr | tr | 95 | — | — |
| Oudtshoorn, S Cape | — | tr | 96 | — | — | — |
| var. *oblonga* (Haw.) DC. | | | | | | |
| Bushman's River, E Cape | — | tr | 96 | — | tr | — |
| var. *flanaganii* (Schonl. & Bak.f.) Tölken | | | | | | |
| Kei valley, E Cape | — | 8 | 91 | — | — | — |
| *C. papillaris* L.f. | | | | | | |
| Johannesburg Bot. Gard. | 8 | 74 | tr | 17 | — | — |
| *C. velutina* Hook.f. | | | | | | |
| Olifantskop, E. Cape | 23 | 45 | 8 | 23 | — | — |
| Kenton, E Cape | 20 | 65 | — | 10 | — | — |
| *C. woodii* Schonl. & Bak.f. | | | | | | |
| Gouritz river, SW Cape | — | — | 100 | — | — | — |
| *Tylecodon grandiflorus* (Burm.f.) Tölken | | | | | | |
| Kirstenbosch Bot. Gard. | tr | 9 | 5 | 86 | — | — |
| *T. paniculatus* (L.f). Tölken) | | | | | | |
| Meiringspoort, S Cape (orange petals) | — | tr | tr | 82 | — | — |
| Robertson, SW Cape (orange petals) | — | tr | tr | 91 | — | — |
| Robertson, SW Cape (red petals) | — | 6 | tr | 95 | — | — |
| Robertson, SW Cape (dark red peduncle) | — | 14 | tr | 83 | — | — |
| Robertson, SW Cape (purple–red anthers) | — | 16 | tr | 79 | tr | — |
| Johannesburg Bot. Gard. (purple–red petals) | tr | 41 | tr | 53 | — | — |

*Anthocyanins: **1** = delphinidin-3-sophoroside? (sample limitations did not allow complete confirmation of the identity); **2** = delphinidin-3-sambubioside; **3** = cyanidin-3-sophoroside; **4** = cyanidin-3-sambubioside; **5** = cyanidin-3-glucoside; **6** = peonidin-3-glucoside.

†Retention time in min (for HPLC system see Materials and Methods).

The acid was washed out with distilled $H_2O$, and the sample eluted with 0.01% HCl in MeOH. The UV–VIS spectrum was recorded and the absorbance was measured, for samples diluted 20× and 30×. To confirm the identity of the sugar moiety, a hydrolysed sample was purified through Dowex 50 W resin ($H^+$ form) and Dowex 1 resin ($OH^-$ form). Glucose and xylose were detected in the purified hydrolysate by HPLC (Waters Carbohydrate Column; 300 mm × 4 mm i.d.; 85% MeCN in $H_2O$; flow rate 2.5 ml $min^{-1}$; refractive index detection) and TLC on Si-gel 60 (0.25 mm) plates; MeCN:$H_2O$:$CS_2$:formic acid (85:10:5:0.5); detection: *p*-anisidine (1.2 g) and phtalic acid (1.6 g) in 100 ml 95% ethanol (Sturgeon, 1990).

The *Cotyledon* and *Tylecodon* anthocyanins were analysed by PC in four standard solvent systems as described by Harborne (1967). Cyanidin-3-sophoroside, cyanidin-3-sambubioside and delphinidin-3-sambubioside had relative $R_f$-values and $R_f$-values closely equivalent to literature data. Co-HPLC with cy-3-sambubioside from *Saintpaulia ionantha* leaves and cy-3-sophoroside from *Hibiscus rosa-sinensis* petals (Harborne, 1967) confirmed these identities.

## Results

The distribution of anthocyanins in 22 samples from 10 species of Crassulaceae is presented in Table 1. *Cotyledon* flowers predominantly contain cyanidin-3-sophoroside, while in the three species of *Crassula* investigated, the 3-glucosides of cyanidin and peonidin invariably co-occur. In *Tylecodon*, the major compound is cyanidin-3-sambubioside, which co-occurs with minor quantities of delphinidin-3-sambubioside. The two sambubiosides are also major compounds in half of the *Cotyledon* samples investigated, supporting the close affinity between the two genera. Variation in the occurrence of the two cyanidin pigments in *Cotyledon* appears to be independent of species boundaries. This is particularly evident in the polymorphic *C. orbiculata* and in *C. barbeyi*, where either the sophoroside or the sambubioside can be the major constituent.

## Discussion

Each of the genera has a distinctive combination of major anthocyanins. The pattern reflects the relation between *Cotyledon* and *Tylecodon* and also their more distant affinity with *Crassula*. The anthocyanins are remarkably uniform in *Crassula*, less so in *Tylecodon* and quite variable in *Cotyledon*. Red flowers in *Crassula* are marked by the co-occurrence of the 3-glucosides of cyanidin and peonidin and a total absence of the major compounds of *Tylecodon* and *Cotyledon*. The three *Crassula* species are not closely related and belong to three different sections of *Crassula* (Tölken, 1985), so that the anthocyanin pattern is unexpectedly conservative.

The overlap in the occurrence of cyanidin and delphinidin 3-sambubiosides in two species of *Tylecodon* and four species of *Cotyledon* supports the close affinity between the two genera. They are morphologically similar and also have the same chromosome number (Tölken, 1985). An interesting difference is the almost complete absence of cyanidin-3-sophoroside in *Tylecodon*. The flower colour of *Tylecodon paniculatus* varies from orange–yellow to dark purple–red and this variation seems to be related to the concentration of delphinidin-3-sambubioside (see Table 1). In a recent study of flower colour in *Virgilia* and *Podalyria* (Fabaceae), no obvious association could be found between flower colours and anthocyanins (van Wyk and Winter, 1994).

The combination of anthocyanins is almost as variable within the polymorphic *Cotyledon orbiculata* as it is within the genus as a whole. The shape, size and colour of the corolla are important characters in *Cotyledon* (Tölken, 1979) and it may be worthwhile to investigate the complete range of variation in all the species.

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