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ANTHOCYANINS IN THE EPACRIDACEAE

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Key Word Index—Epacridaceae; anthocyanin pigments; distribution among species; chemotaxonomy.

Abstract—An examination of 73 species of the family Epacridaceae resulted in the identification of the following anthocyanins: cyanidin 3-galactoside, cyanidin 3-glucoside, cyanidin 3-arabinoside, cyanidin 3-rhamnoside, cyanidin 3-rhamnosylgalactoside, cyanidin 3-rhamnosylglucoside, cyanidin 3-xylosylgalactoside, cyanidin 3-xylosylarabinoside, delphinidin 3-galactoside, delphinidin 3-arabinoside, delphinidin 3-rhamnosylgalactoside, delphinidin 3-rhamnosylglucoside and pelargonidin 3-rhamnosylglucoside. No acylated or 5-substituted anthocyanins were detected in any of the species examined. Evidence of methylated anthocyanidin was found only in one species, *Woollsia pungens*. The occurrence of cyanidin 3-galactoside and cyanidin 3-arabinoside forms a chemical link between this family and the related Ericaceae.

INTRODUCTION

WITH FEW exceptions, species of the Epacridaceae are confined to the Southern Hemisphere where their distribution is centred in Australia and nearby islands. The family divides naturally into two subfamilies on the basis of fruit and gynoecium characters. Thirty-one genera and *ca.* 400 species are included in the family. The only genus to attain any size is *Leucopogon* (about 145 spr.) of which at least 94 species are isolated in Western Australia. The remaining genera, including 9 which are monotypic, contain fewer than 40 species each.

Sixteen genera and 74 species, including 45 which are endemic, have been recorded in the island state of Tasmania.[1] The family has no economic value, but because of its widespread and frequent occurrence in the state, it plays an important part in the floristics of the plant communities. To date only limited phytochemical information is available for the Epacridaceae.[2–5] This present paper reports on anthocyanins in 73 representatives of the family.

RESULTS

Twenty anthocyanins have been detected in the Epacridaceae, and the identity of 13 has been determined. Three pigments, cyanidin and delphinidin 3-rhamnosylgalactoside and cyanidin 3-xylosylarabinoside, are novel anthocyanins which have not yet been reported from any other plant family.[5] The identification of 7 minor pigments is tentative only. From their R_f data, colour and co-occurrence with known anthocyanins, these pigments are probably pelargonidin 3-galactoside, pelargonidin 3-arabinoside, pelargonidin

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[1] CURTIS, W. M. (1963) *The Student's Flora of Tasmania*, Part 2, Govt. Printer, Hobart, Tasmania.

[2] GASCOIGNE, R. M., RITCHIE, E. and WHITE, D. E. (1948) *J. Proc. Roy. Soc. N.S.W.* **82,** 44.

[3] HEGNAUER, R. (1966) *Chemotaxonomie der Pflanzen*, Vol. IV, Birkhauser, Basel.

[4] JARMAN, S. J. and CROWDEN, R. K. (1971) *Phytochemistry* **10,** 2235.

[5] JARMAN, S. J. and CROWDEN, R. K. (1973) *Phytochemistry* **12,** 171.

3-rhamnosylgalactoside, delphinidin 3-glucoside, delphinidin 3-xylosylgalactoside, malvidin 3-arabinoside and malvidin 3-galactoside.

The distribution of the 20 anthocyanins within the family is shown in Table 1. Pigments listed in the final column of the table were trace constituents, only detected at the scale of isolation procedures. Even where their identity is known, they are not placed in the main section of the table if they were too weak to detect on 2-D chromatograms. This procedure was adopted in order to standardize the comparison between species.

TABLE 1. ANTHOCYANIN DISTRIBUTION IN THE EPACRIDACEAE

Species	C3Gal	C3A	C3G	C3R	C3RGal	C3RG	C3XA	C3XGal	D3Gal	D3A	D3G	D3RGal	D3RG	Minor constituents
Subfamily Epacrideae														
Archeria comberi Melville														
Leaves*	⊙	●												
Flower galls	⊙	●												
A. eriocarpa Hook f.														
Petals*	○	●			○									1 u.
Capsules	⊙	●												
A. hirtella (Hook. f.) Hook. f.														
Petals	⊙	●												
Leaves	⊙	●												
Capsule*	⊙	●												C3RGal?
Bark*	⊙	●												C3RGal?
A. serpyllifolia Hook. f.														
Anthers	●	⊙												
Inflorescence	●	⊙							○	+				
Leaves	⊙	●							+	○				
Bark	⊙	●							+	○				
Dracophyllum milliganii Hook. f.														
Anthers	●	⊙												
Inflorescence bracts	●	⊙												
Leaves	●	⊙												
D. secundum R. Br.														
Inflorescence	●	⊙												
Leaves	●	⊙												
Epacris acuminata Benth.														
Anthers	⊙	●												
E. breviflora Stapf.														
Leaves	⊙	●							○	○				
Bark	+	○							○	●				
E. corymbiflora Hook. f.														
Bark	⊙	●												
E. gunnii Hook. f.														
Inflorescence*	⊙	●			○									
Leaves*	⊙	●							+	○				
Bark*	⊙	○							○	●				
E. heteronema Labill.														
Anthers	⊙	●												
Sepals	⊙	●												
E. impressa Labill.														
Petals*	⊙	●												P3A?+1u.
Leaves*	⊙	●								○				
Bark*	○	●							+	⊙				
E. lanuginosa Labill.														
Inflorescence*	⊙	●												
Leaves	⊙	●								○				
Bark	⊙	●								○				

TABLE 1. *Continued*

Species	C3Gal	C3A	C3G	C3R	C3RGal	C3RG	C3XA	C3XGal	D3Gal	D3A	D3G	D3RGal	D3RG	Minor constituents
E. longiflora Cav.														
Petals*	⊙	●												
E. marginata Melville.														
Anthers	⊙	●												
Bark	⊙	●												
E. microphylla R. Br.														
Petals	⊙	●												
Leaves	⊙	●												
E. myrtifolia Labill.														
Inflorescence*	⊙	●												D3A + 1u.
Leaves*	⊙	●												
Bark	⊙	●												
E. obtusifolia Sm.														
Inflorescence*	⊙	●												
Leaves*	●	⊙												1 u.
Bark	⊙	●												
E. paludosa R. Br.														
Leaves	⊙	●							+	○				
Bark	○	⊙							+	●				
E. petrophila Hook. f.														
Leaves*	⊙	●							○	○				
Bark*	⊙	●							⊙	●				
E. pulchella Cav.														
Petals	⊙	●												
E. reclinata A. Cunn.														
Petals	⊙	●												
E. serpyllifolia R. Br.														
Anthers	⊙	●												
Inflorescence	○	●							+	⊙				
Leaves*	⊙	●							+	○				
Bark*	○	●							○	⊙				
E. tasmanica W. M. Curtis														
Inflorescence*	⊙	●												
Leaves*	⊙	●												
Prionotes cerinthoides (Labill.) R.Br.														
Petals*	●	●												C3XGal? + 1 u.
Leaves + stems	●	●												
Capsules	●	●												
Richea acerosa (Lindl.) F. Muell.														
Leaves*	●	⊙												D3Gal? + 1 u.
R. continentis B. L. Burtt.														
Leaves	●	⊙												
R. dracophylla R. Br.														
Leaves*	●	⊙												
Capsules	●	⊙												
R. gunnii Hook. f.														
Operculum	●	⊙												
Leaves*	●	⊙												
R. milliganii (Hook. f.) F. Muell.														
Leaves	●	⊙												
R. pandanifolia Hook. f.														
Operculum*	●	⊙						○						C3XA?
Capsules*	●	⊙						○						1 u.
R. procera (F. Muell.) F. Muell.														
Operculum*	●	⊙												

Table I. *Continued*

Species	C3Gal	C3A	C3G	C3R	C3RGal	C3RG	C3XA	C3XGal	D3Gal	D3A	D3G	D3RGal	D3RG	Minor constituents
R. scoparia Hook. f.														
Operculum*	●	⊙			○									
Leaves	●	⊙												
Capsules	●	⊙												
R. sprengelioides (R. Br.) F. Muell.														
Operculum	●	○												
Leaves	●	○												
Sprengelia incarnata Sm.														
Inflorescence*	⊙	●												
Leaves*	⊙	●												
Woollsia pungens F. Muell.														
Petals*	○	●							+	⊙				C3R + M3A? + M3Gal?
Sepals + style*	○	●							+	⊙				C3R + M3A? + M3Gal?
Sub-family Styphelieae														
Acrotriche serrulata (Labill.) R. Br.														
Inflorescence*	⊙	●			+				t	○				D3RGal? + P3A?
Bark*	○	●							+	⊙				C3RGal?
Astroloma humifusum (Cav.) R. Br.														
Petals*	⊙	●							○					
Brachyloma daphnoides (Sm.) Benth.														
Leaf bracts	●	●												
Cyathodes abietina R. Br.														
Sepals	●													
Fruit*	●	○			⊙									1 u.
C. dealbata R. Br.														
Inflorescence*	●	⊙												
Fruit*	●	○						+	⊙	○				C3R + D3XG?
C. divaricata Hook. f.														
Leaves*	●	⊙												
Fruit	●	⊙												
Bark*	●	⊙												D3Gal?
C. glauca Labill.														
Leaves*	●	○			⊙				+			t		
Fruit*	+	+			⊙				○			●		D3A + 2 u.
Bark	●	○			+				⊙			t		
C. juniperina (Forst.) Druce														
Anthers	●				⊙									
Leaves*	●	⊙			○									
Fruit*	⊙	○			●									D3Gal? + C3R? + 1 u.
Bark*	●	⊙			○									D3Gal?
C. nitida S. J. Jarman[11]														
Fruit*	●	+			○				⊙					P3Gal?
Bark	●								⊙					
C. parvifolia R. Br.														
Inflorescence*	●	⊙			○									
Leaves*	●	⊙												C3RGal?
Leaves + stems	●	⊙												
Fruit*	●	⊙		+	○									D3Gal? + 1 u.
C. petiolaris (DC.) Druce														
Anthers	⊙	●												
Leaves	⊙	●												
Fruit*	⊙	●			○					+				D3Gal? + P3A? + 1 u.

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[11] Jarman, S. J. (1973) *Pap. and Proc. Roy. Soc. Tas.* **107**, in press.

TABLE 1. *Continued*

Species	C3Gal	C3A	C3G	C3R	C3RGal	C3RG	C3XA	C3XGal	D3Gal	D3A	D3G	D3RGal	D3RG	Minor constituents
C. straminea R. Br.														
Fruit*	○	⊙			●				+			t		C3R + 1 u.
Leucopogon australis R. Br.														
Inflorescence*	●	⊙			○									
L. collinus (Labill.) R. Br.														
Inflorescence*	⊙	●												1 u.
Leaves	⊙	●												
Bark	⊙	●												
L. ericoides (Sm.) R. Br.														
Inflorescence*	⊙	●												D3Gal? + D3A?
Leaves	⊙	●												
Bark*	○	●							+	⊙				
L. hookeri Sond.														
Inflorescence*	●	⊙												1 u.
Leaves*	●	⊙												
Fruit	●	⊙							○					
L. lanceolatus (Sm.) R. Br.														
Inflorescence	●	⊙												
L. microphyllus Spreng.														
Inflorescence	⊙	●												
L. milliganii (F. Muell.) Rodw.														
Fruit	●	⊙			⊙									
L. parviflorus (Andr.) Lindl.														
Inflorescence*	●	⊙												
Bark	●	⊙			○									
L. setiger Spreng.														
Anthers	⊙	●												
Inflorescence	⊙	●												
L. stuartii F. Muell.														
Inflorescence*	⊙	●												1 u.
L. virgatus (*Labill.*) R. Br.														
Inflorescence*	⊙	●						○						
Leaves	⊙	●												
Bark	⊙	●												
Lissanthe montana R. Br.														
Anthers	●	⊙												
Inflorescence*	●	⊙												1 u.
Fruit*	●	⊙												1 u.
L. strigosa (Sm.) R. Br.														
Inflorescence*	●	⊙												P3Gal? + P3RGal?
Fruit	●													
Monotoca empetrifolia R. Br.														
Inflorescence*	●	⊙												D3Gal? + 3RGal?
Leaves + stems*	●	⊙												D3Gal?
M. glauca (Labill.) Druce														
Leaves*	●	⊙												
Bark	●	●							⊙	⊙				
M. linifolia (Rodw.) W. M. Curtis														
Fruit*	⊙	+			⊙				●	○		●		
Pentachondra ericaefolia Hook. f.														
Leaves + stems*	⊙	●							○	⊙				1 u.
P. involucrata R. Br.														
Petals	○	●			⊙									
Leaves + stems*	●	●			⊙				○	○				
P. pumila (Forst.) R. Br.														
Fruit*	⊙	●			○									

TABLE 1. *Continued*

Species	C3Gal	C3A	C3G	C3R	C3RGal	C3RG	C3XA	C3XGal	D3Gal	D3A	D3G	D3RGal	D3RG	Minor constituents
Styphelia adscendens R. Br.														
Sepals*	⊙	●												C3RGal?
S. tubiflora Sm.														
Petals*	⊙	●												
Leaves	⊙	●												
Trochocarpa cunninghamii (DC.) W. M. Curtis														
Petals	●	○			⊙									
Inflorescence*	●	○			⊙									
Leaves*	●	○			⊙				⊙					
Fruit*	○				⊙				+			●		C3A + 1 u.
T. disticha (R. Br.) Spreng.														
Petals			⊙			●								
Leaves			●			⊙								
Fruit*			+			⊙					○		●	2 u.
T. gunnii (Hook. f.) Benth.														
Inflorescence			⊙			●								
Leaves			●			●								
Fruit*			○			⊙					+		●	P3RGluc + 2 u.
Bark			⊙			●					+		○	
T. thymifolia (R. Br.) Spreng.														
Inflorescence*	●	+			⊙				○					
Leaves	●	○							⊙					
Fruit*					⊙							●		(C3A + C3Gal?+
Bark	●	○							⊙					(D3Gal? + 1 u.

Relative concentrations: ● > ⊙ > ○ > + > t.
Key: C—Cyanidin; D—Delphinidin; P—Pelargonidin; M—Malvidin; Gal—galactose; A—arabinose; G—glucose; R—rhamnose; X—xylose; u—unidentified.
* Pigments isolated and identified in full.

Intraspecific differences in pigment patterns, when present, were usually associated with minor compounds and were probably quantitative in nature. However, when major pigments were involved, e.g. twig bark and young leaves, the differences are likely to have been qualitative, resulting from physiological changes within the plant associated with seasonal variation.

The presence of delphinidin 3-galactoside and 3-arabinoside in leaves and flowers, with the exceptions of *Acrotriche serrulata* and *Woollsia pungens*, was probably due to contamination by traces of highly coloured bark tissue which remained attached to pedicels and petioles. In white-flowered species, anthocyanins were derived from red anthers, or from sepals and bracts which were too small to be removed conveniently. Anthocyanins were not detected (or were present at concentrations too low for chromatographic analysis) in the fruits of 14 species from the subfamily Styphelieae. These species were *Acrotriche serrulata*, *Astroloma humifusum*, *A. pinifolium*, *Leucopogon australis*, *L. collinus*, *L. ericoides*, *L. parviflorus*, *L. stuartii*, *L. virgatus*, *Monotoca elliptica*, *M. empetrifolia*, *M. glauca*, *M. scoparia* and *Styphelia adscendens*. Pigmentation of fruits in these species was caused by other flavonoids, carotenoids or chlorophylls.

Although the majority of plants were collected in Tasmania, 19 species were collected from the Australian mainland. Of these species, 7 are common to both Tasmania and the mainland. Twelve species, *Dracophyllum secundum*, *Epacris brevifolia*, *E. longiflora*, *E. mic-*

rophylla, *E. paludosa*, *E. pulchella*, *E. reclinata*, *Richea continentis*, *Woollsia pungens*, *Leucopogon microphyllus*, *L. setiger* and *Styphelia tubiflora* are not found in Tasmania. The 19 mainland accessions exhibited the same general patterns as the Tasmanian species.

DISCUSSION

The general lack of species-specific compounds and the scarcity of unique chemical patterns precludes the identification of individual species using anthocyanin chemistry only. A few species (*Cyathodes dealbata*, *Leucopogon virgatus*, *Richea pandanifolia*) produce anthocyanins which may be considered species-specific if individual organs are compared. Unfortunately, the relevant anthocyanins are minor compounds whose presence show intra-specific variation. For identification purposes, anthocyanin characters offer no advantage over the more conventional morphological characters. The widespread occurrence of cyanidin 3-galactoside and 3-arabinoside serves to unite species within the family.

Two species, *Trochocarpa gunnii* and *T. disticha*, are unusual in producing anthocyanins containing glucose rather than galactose. One of these, *T. disticha*, is morphologically very similar to *T. cunninghamii*, and has been placed as a variety of this species by some taxonomists.[6,7] *T. cunninghamii* and *T. disticha* differ chemically by their incorporation of either glucose (*T. disticha*) or galactose (*T. cunninghamii*), but not both into their anthocyanins. Unfortunately, it is not possible to use this knowledge for routine identification purposes because R_fs of the relevant anthocyanins are so similar that they cannot be differentiated easily on 2-D paper chromatograms.

Examination of the quantitative relationship between the two major glycosidic types, galactosides and arabinosides, may have limited taxonomic application. The accumulation of galactosides in excess of arabinosides or vice versa is consistent in all species and, with few exceptions, for all genera. Of those genera examined here, *Archeria*, *Epacris*, *Sprengelia*, *Acrotriche*, *Astroloma*, *Pentachondra* and *Styphelia* all accumulate more arabinoside than galactoside while the converse is true for *Dracophyllum*, *Richea*, *Cyathodes* (with one exception), *Lissanthe*, *Monotoca* and *Trochocarpa*. Both situations are found in *Leucopogon* but it is not possible to ascertain the significance of this because of the low number of species available for study. In *Prionotes* and *Brachyloma* it is difficult to estimate which glycosidic type is dominant because both appear to occur in equal amounts.

Three species are not consistent with the patterns described above. *Cyathodes petiolaris* accumulates more arabinosides than galactosides, in contrast to other species in the genus. However, this species is very closely allied to *Leucopogon* and has several morphological characteristics which are typical of that genus. It appears that the chemistry is simply reflecting an anomaly already apparent from morphology. *Archeria serphyllifolia* and *Epacris obtusifolia* are unusual in having different glycosidic relationships in the leaves and the flowers.

At the subfamily level, an examination of anthocyanin differences relates to the proposed evolutionary status of the two groups. Species of the subfamily Stypheliеae show greater anthocyanin diversity than species of the subfamily Epacrideae. This is apparent, not only in the number and structure of pigments but also in anthocyanin patterns for individual species. A comparison of the two subfamilies reveals nine anthocyanins in the Epacrideae and eighteen in the Stypheliеae. Only four (all monosides) were present in significant quantities in the Epacrideae, and two of these, the delphinidin derivatives, were

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[6] Rodway, L. (1903) *The Tasmanian Flora*, Govt. Printer, Hobart, Tasmania.

[7] Bentham, G. (1869) *Flora Australiensis*, Vol. 4, Reeve, London.

major pigments in the bark only. The five minor constituents included two cyanidin biosides, one pelargonidin and two malvidin monosides. The pelargonidin and malvidin glycosides were each found in only one species. Delphinidin and pelargonidin biosides were not detected in the Epacrideae. In the Styphelieae, ten anthocyanins were present in significant quantities, and of these, biosides and delphinidin derivatives were common. Although pelargonidin derivatives were minor compounds, they were more widespread (6 species) and showed greater structural variation than was apparent in the Epacrideae. Assuming that structural diversity and complexity of anthocyanins is indicative of evolutionary advancement, the Styphelieae may be considered more advanced than the Epacrideae. A similar conclusion has been derived from cytological[8] and morphological studies.[9]

At the family level, the Epacridaceae is closely related to the Ericaceae and is regarded by some taxonomists as an "advanced" branch of the Ericaceae. The two families are similar in many morphological characters and they occupy a similar ecological niche but on different continents. Although cyanidin 3-galactoside and 3-arabinoside are present in both families the occurrence of other anthocyanins is markedly different. In the Ericaceae all common anthocyanidins have been reported with the exception of pelargonidin.[10] In the Epacridaceae, pelargonidin derivatives are present (as minor constituents) but only one methylated anthocyanidin (in one species) was detected. We have examined *Styphelia tubiflora* and failed to confirm the earlier claim[2] that "partially methylated delphinidin glycosido-acylated derivatives" are present in this plant.

Other differences between the Ericaceae and Epacridaceae include the presence of xylose, rhamnose, galactose, arabinose and glucose in Epacrid anthocyanins, whilst only the latter 3 sugars are reported from the Ericaceae. In addition, 3-biosides occur commonly in the Epacridaceae, whereas these have been reported infrequently and only in minor quantities in the Ericaceae.[10] Finally, 5-substituted anthocyanins, commonly found in the Ericaceae, and acylated anthocyanins have not been found in the Epacridaceae.

From these observations it is apparent that evolution of anthocyanin pigments has progressed in two separate directions in the two families. The Ericaceae has evolved more complex aglycones but has retained a simple pattern of monoglycosidic substitution at position 3, or positions 3 and 5. In contrast, less complex aglycones (non-methylated) have persisted in the Epacridaceae and glycosidic patterns have evolved in which 5-substitution appears to play no part but the range of sugars involved in 3-glycosylation has increased and includes more complex sugars (biosides).

EXPERIMENTAL

Materials. Fresh material was used for all extractions, and wherever possible, flowers, leaves, bark and fruit were examined from each species. Most material was collected in Tasmania but a few species were collected from the mainland states, Victoria and New South Wales. However, with most mainland species only limited examination could be undertaken because of the small quantities of material available. Voucher specimens of all species studied were placed in the University of Tasmania herbarium.

Extraction, isolation and identification. Anthocyanins were extracted, purified and identified according to the procedures described previously.[4,5] Minor pigments were frequently identified by co-chromatography against known reference compounds. Pelargonidin 3-rutinoside, used as a reference, was obtained from *Anthurium* species.

Acknowledgements—We gratefully acknowledge assistance from Mrs. E. Reilly, in the collection and identification of N.S.W. species. We are also grateful to the National Parks Trust of Victoria for permission to collect plants from Wilson's Promontory.

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[8] PATERSON, B. (1961) *Bot. Gaz.* **122**, 259.

[9] SMITH-WHITE, S. (1948) *Proc. Linn. Soc. N.S.W.* **73**, 37.

[10] HARBORNE, J. B. (1966) *Comparative Biochemistry of Flavonoids*, Academic Press, New York.