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# CYANOGENESIS IN THE PROTEACEAE

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**Key Word Index**—Proteaceae; proteacin; dhurrin; cyanogenesis; cyanogenic glycosides.

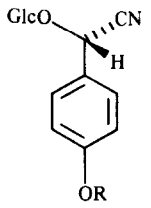
**Abstract**—Living material from 155 species of Proteaceae were tested for cyanogenesis, and 44 species were found to be cyanogenic. The cyanogenic glycosides dhurrin and/or proteacin were identified in eight species.

## INTRODUCTION

The highly diverse plant family Proteaceae is the source of several exotic, horticulturally important species. Species of this family are primarily found in the southern hemisphere; the greatest concentrations, both in terms of generic diversity and of numbers of species, are found in South Africa and in Australia [1]. The 1500 species (75 genera) of this family have been placed in five subfamilies, viz. Proteoideae, Grevilleoideae, Persoonioideae, Sphalmioideae and Carnarvonioideae. The first three subfamilies have been in turn divided into 12 tribes; the two remaining subfamilies are monogeneric with one and two species respectively [1].

Members of the Proteaceae have been known to be cyanogenic since the beginning of this century [2–6], but the compounds responsible have been identified from only one species. The cyanogenic glycoside dhurrin (**1**) was found in the leaves of *Macadamia ternifolia* [7, 8] and the diglucoside proteacin (**2**) was found in the fruit [9]. Both compounds are presumably derived from tyrosine. Proteacin has been reported from only one other plant species, *Thalictrum aquilegifolium* (Ranunculaceae) [10].

Because cyanogenesis appears to be widespread and few of the responsible compounds have been examined, we have initiated a study of cyanogenesis in this family and the cyanogenic glycosides it contains.




**1** R = H
**2** R = Glc

## RESULTS AND DISCUSSION

In this study, reported in greater detail elsewhere [11], we have examined living material from 155 species of Proteaceae and found 44 to be cyanogenic. The species tested represented members of nine tribes distributed in three subfamilies (Proteoideae, Grevilleoideae and Persoonioideae). The cyanogens present in eight of these species were identified as the tyrosine-derived cyanogenic glucosides dhurrin and proteacin. Our discussion of the species examined and their placement in subfamilies and tribes follows Johnson and Briggs [1].

### *Subfamily Proteoideae*

In plants of the subfamily Proteoideae, cyanogenesis has previously been reported in *Petrophile shirleyae* and *Protea cynaroides* [2, 3, 13], *Conospermum tenuifolium* [13] and many species of the genus *Leucadendron* [12]. In the present study species from three tribes and eight genera were examined (Table 1). The data confirm the high incidence of cyanogenesis in the genus *Leucadendron* (tribe Proteeae) previously reported [12]. Species of four other genera of this tribe did not give positive tests for cyanogenesis nor did species of the two other tribes in this subfamily (Table 1). We did not observe cyanogenesis in

Table 1. Distribution of cyanogenesis in the subfamily Proteoideae

| Tribe | Genus | Species* (this study) | Species* (lit.) | References |
|---|---|---|---|---|
| Conospermeae | *Conospermum* | — | 3/1 | [13] |
| | *Petrophile* | 3/0 | 2/1 | [13] |
| | *Isopogon* | 6/0 | 3/0 | [4, 13] |
| | *Stirlingia* | — | 1/0 | [13] |
| Franklandieae | *Adenanthos* | 2/0 | 1/0 | [13] |
| Proteeae | *Leucospermum* | 7/0 | — | — |
| | *Protea* | 27/0 | 2/1 | [13] |
| | *Serruria* | 1/0 | — | — |
| | *Aulax* | 1/0 | — | — |
| | *Leucadendron* | 25/14 | 60/23 | [12] |

*Ratio = number of species tested/number of cyanogenic species.

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the three species of *Petrophile* that we were able to examine, although this genus has previously been reported to be cyanogenic [13].

*Subfamily Grevilleoideae*

Cyanogenesis occurred more widely in the subfamily Grevilleoideae (Table 2). Of the five tribes and seven genera examined, plants of four genera from three different tribes contained cyanogenic compounds [11]. In the tribe Macadamieae, all four species of the genus *Lambertia* tested were cyanogenic. Although the tribe has been reported to have a high incidence of cyanogenesis [2–5, 7, 13–16], the one other genus tested by us, *Gevuina*, was not cyanogenic. Two species of *Telopea* (tribe Embothrieae) were cyanogenic but no other plants in this tribe were available for testing. Other genera, *Lomatia* and *Stenocarpus*, in this tribe are reported to be cyanogenic [4–6, 13–15, 21]. None of the sixteen species of *Banksia* (tribe Banksieae) tested were cyanogenic.

The tribe Grevilleeae is the largest of the subfamily Grevilleoideae and apparently contains the largest number of cyanogenic species. Of 55 species of *Grevillea* and *Hakea* examined, 24 (44%) were cyanogenic. The literature also reports a high degree of cyanogenesis (47%) in these two genera [5, 6, 13–17]. It will be of interest to examine members of the remaining genus in this tribe, *Finschia*, as well as species in the remaining tribe (Oriteae) in this subfamily.

*Other subfamilies*

Four species of *Persoonia*, subfamily Persoonioideae, were examined by us but were not cyanogenic. Earlier workers also failed to observe cyanogenesis in this genus [4, 13, 21]. Members from the other six genera in this subfamily have not been examined, nor were any plants from the two, small, subfamilies Sphalmioideae and Carnarvonioideae.

*Identification of cyanogenic glycosides*

Dhurrin (**1**) and proteacin (**2**) were isolated by prep. HPLC; structure determination was done by $^1$H NMR. In the 360 MHz $^1$H NMR spectrum of proteacin, the chiral proton on C-2 is a sharp singlet at $\delta$ 6.06 which defines the configuration at C-2 as (*S*) (18). Absorptions for glycosidic protons at $\delta$ 5.20 and 4.80 (both $J = 8$ Hz) indicate a $\beta$-linkage for each anomeric proton. The 360 MHz $^1$H NMR spectra of dhurrin isolated from Proteaceae species were identical with a spectrum of dhurrin extracted from *Sorghum bicolor*.

The glycosidic moiety of proteacin and dhurrin was determined to be glucose by the glucose oxidase method. One glucose is attached to the cyanohydrin portion of the molecule in each compound. Proteacin is a bis-glucoside; attachment of the second glucose to the phenolic hydroxyl was established by the lack of a bathochromic (red) shift in alkali [19].

All of the plants extracted contained cyanogenic glucosides derived from the amino acid tyrosine (Table 3). In general, leaf material contained dhurrin while floral tissue contained both dhurrin and proteacin. In accord with this general pattern, leaves of *Hakea* species (subfamily Grevilleoideae) contained dhurrin and the flowers or floral buds contained both dhurrin and proteacin. In contrast, flowers of one species of the closely related genus *Grevillea* contained only proteacin and an unidentified compound, possibly triglochinin. The leaves of this plant, *G. hugellii* were not cyanogenic. The leaves of a species of *Leucadendron*, a genus endemic to South Africa (subfamily Proteoideae), contained only dhurrin. The flowers of this specimen, *L. sessile* were cyanogenic, but the cyanogen was not identified.

Table 2. Distribution of cyanogenesis in the subfamily Grevilleoideae

| Tribe | Genus | Species* (this study) | Species* (lit.) | Reference |
|---|---|---|---|---|
| Macadamieae | *Macadamia* | — | 6/5 | [2–5, 7, 13–16] |
| | *Brabeium* | — | 2/2 | [2, 3, 14] |
| | *Hicksbeachia* | — | 1/1 | [5, 14, 15] |
| | *Roupala* | — | 2/0 | [13] |
| | *Lambertia* | 4/4 | 1/1 | [4, 13–15] |
| | *Gevuina* | 1/0 | — | — |
| Helicieae | *Helicia* | — | 1/1 | [3] |
| | *Xylomelum* | — | 2/2 | [3–5, 14, 15] |
| Grevilleeae | *Grevillea* | 32/9 | 18/3 | [5, 6, 13–15] |
| | *Hakea* | 23/15 | 25/17 | [6, 13–17] |
| Knightieae | *Knightia* | 1/0 | 1/0 | [2] |
| Embothrieae | *Telopea* | 2/2 | 2/2 | [4, 13, 14] |
| | *Lomatia* | — | 13/4 | [4–6, 13–15] |
| | *Buckinghamia* | — | 1/0 | [4, 13, 21] |
| | *Stenocarpus* | — | 3/1 | [13] |
| | *Strangea* | — | 1/0 | [13] |
| Banksieae | *Banksia* | 16/0 | 8/0 | [4, 13, 21] |

*Ratio = number of species tested/number of cyanogenic species.

Table 3. Cyanogenic glycosides found in the Proteaceae

| Species | Plant part | Number of cyanogens | Compound(s) |
|---|---|---|---|
| *Grevillea hugellii* | flowers | 2* | proteacin |
| *Hakea bakerana* | leaves | 1 | — |
| | flowers | 2 | dhurrin, proteacin |
| *Hakea bucculenta* | leaves | 1 | dhurrin |
| *Hakea multilineata* | leaves | 1 | dhurrin |
| *Hakea orthorrhynca* | leaves | 1 | dhurrin |
| *Hakea petiolaris* | flowers | 2 | dhurrin, proteacin |
| *Hakea salcata* | floral buds | 2 | dhurrin, proteacin |
| *Leucadendron sessile* | leaves | 2† | dhurrin |
| *Macadamia ternifolia* | young fruit | 1 | proteacin [9] |
| | leaves | 1 | dhurrin [8] |

*A second cyanogen was tentatively identified as triglochinin.

†A second cyanogenic substance, present in only trace amounts, was not identified.

*Evolutionary implications*

Cyanogensis is found in representatives of the two major subfamilies of the Proteaceae. One of these subfamilies, the Proteoideae, is found both in Australia and in Africa, although in the latter it is represented by the single but very large tribe Proteeae. In this tribe, the genus *Leucadendron* exhibited a large degree of cyanogenesis, both in this study (56%) and in earlier work (38%) [12]; to date other genera examined by us have not been found to be cyanogenic. Only one of the two other tribes of this subfamily, i.e. the non-African Conospermeae, has been reported to have cyanogenic species [13].

By contrast, cyanogenesis occurs more widely in the subfamily Grevilleoideae, a subfamily extensively developed in Australia, elsewhere in the Pacific, and in South and Central America. This subfamily is not found in Africa except for the genus *Brabeium* (tribe Macadamieae) which has been reported to be cyanogenic [2–4].

These results suggest that cyanogenesis must have arisen early, before the present pattern of distribution of the Proteaceae was established. This is further supported by our finding that the cyanogenic glycosides isolated from the Australian genera *Grevillea*, and *Hakea* and the African genus *Leucadendron* are dhurrin and proteacin, compounds having a common biosynthetic origin in the amino acid tyrosine.

## EXPERIMENTAL

*Plant material.* Plant material was collected at the arboreta on the campuses of the University of California at Davis and at Santa Cruz. Voucher specimens were deposited in the herbarium at the University of California, Davis (UCD).

*Extraction and isolation.* All plant material was extracted in a similar manner. Leaf material was ground in boiling 95% EtOH in a Waring blender and floral material was crushed in liquid $N_2$ prior to extraction in boiling EtOH. The filtered solution was concd under vacuum and subsequently extracted with MeOH–$CHCl_3$–$H_2O$ (12:5:3). Additional $H_2O$ and $CHCl_3$ were added as necessary to separate the layers. The aq. layer was treated with 10% PbOAc and then $H_2S$. Open CC was performed on polyamide (Macherey, Nagel and Co.) with $H_2O$ as solvent. Final purification was by HPLC with the *A* monitored at 200 nm. Cyanogen containing fractions from the polyamide column were combined and run on a Whatman C-18 ODS-3 column (9.4 mm × 50 cm) with a solvent of MeCN–$H_2O$ (1:19) at a flow rate of 4 ml/min. Final purification was achieved on an Astec Cyclobond I (10 mm × 50 cm) column using MeOH–$H_2O$ (5:95) at a flow rate of 4 ml/min. Cyanogenic fractions were collected, frozen and lyophilized. The cyanogenic compounds were detected during purification using Feigl–Anger cyanide sensitive paper after hydrolysis of the glycoside with almond emulsin [20].

*Dhurrin.* $^1$H NMR (360 MHz, acetone-$d_6$): aglycone: $\delta$ 6.0 (1H, *s*, H-2); 6.95 (2H, *d*, $J$ = 9 Hz, H-X); 7.45 (2H, *d*, $J$ = 9 Hz, H-Y). glucose: $\delta$ 3.2–3.6 (4H, *m*, H-2–H-5); 3.7 (1H, *dd*, $J_{6\alpha,6\beta}$ = 24 Hz, $J_{6\alpha,5}$ = 6 Hz, H-6$\alpha$); 3.9 (1H, *dd*, $J_{6\beta,6\alpha}$ = 24 Hz, $J_{6\beta,5}$ = 2 Hz, H-6$\beta$); 4.75 (1H, *d*, $J$ = 8 Hz, H-9).

*Proteacin.* $^1$H NMR (360 MHz, $D_2O$): aglycone: $\delta$ 6.06 (1H, *s*, H-2); 7.22 (2H, *d*, $J$ = 9 Hz, H-X, H-Y); 7.61 (2H, $J$ = 9 Hz, H-X, H-Y). glucose: $\delta$ 3.00–4.00 (8H, *m*, H-1′–H-6, and H-1″–H-6″); 4.80 (1H, *d*, $J$ = 8 Hz, H-1′ or H-1″); 5.20 (1H, *d*, $J$ = 8 Hz, H-1′ or H-1″).

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