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CYANOGENESIS IN *ACACIA PACHYPHLOIA*

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Abstract—The cyanogenic glycoside, proacacipetalin, is reported from *Acacia pachyphloia* (*Acacia* subgenus *Acacia*) This represents the first record of a glycoside with an aliphatic aglycone from a species of *Acacia* indigenous to Australia This finding reinforces the taxonomic distinctions between subgenus *Acacia* and subgenus *Phyllodineae*

INTRODUCTION

The genus *Acacia* comprises about 1100–1200 species distributed in all continents, except Europe and Antarctica, principally in regions where the rainfall is markedly seasonal or low A review of *Acacia* containing chapters on classification, phylogeny, ecology, uses and biological inter-relationships was published recently by New [1]

As presently recognized, *Acacia* comprises three subgenera, viz *Acacia*, *Aculeiferum* and *Phyllodineae* In recent years there have been suggestions that these subgenera may be treated as two [2] or three [3, 4] distinct genera but, as yet, the new generic groupings have not been formally published In the past, a number of different infrageneric classifications have been proposed for *Acacia* and Seigler and Conn [5] have summarized the most important of these The most recent classification is that of Pedley [6] and is the one we have adopted here It should be noted that the subgeneric name *Heterophyllum* which was used by Pedley [6] and Seigler and Conn [5] must be replaced by the earlier name *Phyllodineae* [7, 8]

Acacia pachyphloia W V Fitzg occurs in subgenus *Acacia*, which is a group of world-wide distribution (Fig 1 of ref [7]) In Australia the subgenus is represented by about nine species * [9] and these occur in the northern tropical/subtropical parts of the continent (Fig 14 of ref [7]) *Acacia pachyphloia* occurs in Western Australia and Northern Territory [10] It grows to a tree about 10 m tall and has pendulous branchlets and a straight trunk with a thick, corky bark Its bipinnate leaves are 2–5-jugate and they sometimes have at their base a pair of short (to 5 mm long) spinescent stipules The flower-heads are globular and white (but on aging, yellow) and the seeds uniseriate in the legumes

Until now, cyanogenesis has been reported in 14 species from subgenus *Acacia* [5] These species, which occur in both Africa and America, synthesize glycosides derived (or may be presumed to be derived) from the aliphatic amino acids L-leucine (proacacipetalin and related compounds), L-valine (linamarin) and L-isoleucine (lotaustralin) According to Seigler and Conn [5], there does not appear to be a strong correlation between the taxonomic groups within the subgenus and the cyanogens present This is not surprising because, as noted by Ross [7], there is no satisfactory classification of the subgenus The cyanogens of subgenus *Acacia* differ dramatically from those of subgenus *Phyllodineae*, a group in which nearly all of the Australian acacias are placed In the latter subgenus, the cyanogens are derived from L-phenylalanine ([11], Maslin, B R and Conn, E E, in preparation)

RESULTS AND DISCUSSION

Chemical identification

Proacacipetalin, identified by NMR, was isolated from *A pachyphloia* and purified as described in the Experimental

Taxonomic interpretation

Our detection of proacacipetalin in *A pachyphloia* represents the first record of a cyanogenic glycoside from an indigenous Australian member of subgenus *Acacia* Proacacipetalin is the most commonly encountered cyanogenic glycoside in this subgenus and has previously been recorded in both African and American species [5] This glycoside is not known from either of the other two subgenera of *Acacia* nor is it known to occur elsewhere in the plant kingdom [12]

Because the classification of subgenus *Acacia* is so inadequate and because cyanogenic glycosides have been characterized for only *ca* 10% of the species within this group, there are constraints on our using the present biochemical data to ascertain the taxonomic position of *A pachyphloia* within the subgenus Nevertheless, it is noted that of the 14 subgenus *Acacia* species in which cyanogenesis occurs proacacipetalin has been reported in eight species from America, viz *A atramentaria*, *A chiapensis*, *A cochliacantha*, *A constricta*, *A hindsii*, *A macrocantha*, *A schaffneri* (var *schaffneri* and var *bravoensis*) and *A*

**Acacia farnesiana* is not included in this total, it almost certainly being an early introduction into Australia [6]

tortuosa [5], and three from Africa, viz *A giraffae* (this record is perhaps an error for *A erioloba*), *A hebeclada* and *A sieberiana* var *woodii* [5] These data show that *A pachyphloia* is related, at least biochemically, to both the African and American members of subgenus *Acacia*

Results given in the present work lend partial support to previous subgeneric classifications of *Acacia* A number of recent studies have shown subgenus *Acacia* to be taxonomically distinct from both subgenus *Phyllodineae* and subgenus *Aculeiferum* [2, 7, 13, 14] While there is no information on the cyanogenic glycosides of species from subgenus *Aculeiferum**, these compounds reveal basic differences between subgenus *Acacia* and subgenus *Phyllodineae* As shown by Secor *et al* [11] and Conn and Maslin (in preparation), the Australian members of subgenus *Phyllodineae* contain cyanogenic glycosides presumed to be derived from the aromatic amino acid L-phenylalanine In subgenus *Acacia*, on the other hand, our present results for *A pachyphloia*, as well as those of numerous previous studies [11, 15–25], show that these species contain glycosides derived from the aliphatic amino acids leucine, valine and isoleucine While these biochemical pathways are fundamentally different, their use as a taxonomic tool is somewhat limited because of the relatively low number of species in each subgenus which contain cyanogenic glycosides [subgenus *Acacia* 15 cyanogenic species recorded (ref [5] excluding *A pachyphloia*) from a total of between 150 and 200 species, subgenus *Phyllodineae* 40 cyanogenic species recorded [26] from a total of *ca* 900 species] Nevertheless, cyanogenic glycosides do provide further evidence to reinforce differences between subgenus *Acacia* and subgenus *Phyllodineae* The judgment as to whether these differences warrant generic or subgeneric recognition for the taxa concerned is a taxonomic decision beyond the scope of this paper

EXPERIMENTAL

Plant material Leaf material of *A pachyphloia* was collected at Kimbolton Station, Yampi Peninsula, North of Broome, Western Australia and air-dried before shipment by air to California for extraction and purification A voucher (T Willing, 74) of the specimen has been lodged at the Western Australian Herbarium (Perth)

Procedures Leaf material (145 g) was extracted with 500 ml boiling 95% EtOH for 2 min, the EtOH was removed by filtration through cheesecloth, and the residue was re-extracted with another 500 ml boiling 95% EtOH The combined extract was reduced to a thick syrup under vacuum (temp not exceeding 40°) and redissolved in 60 ml MeOH–$CHCl_3$–H_2O (12 5 3) Additional $CHCl_3$ (15 ml) and H_2O (21 ml) and shaking produced two phases, the $CHCl_3$ phase was removed and discarded The aq phase was taken to dryness at room temp and redissolved in sufficient H_2O to give a thin syrup Flavonoids were removed by the addition of 10% lead acetate until no more ppt formed The ppt was removed by centrifugation at 10000 *g* for 10 min and excess lead acetate removed by bubbling H_2S through the soln The PbS was removed by centrifugation at 10000 *g* for 10 min The supernatant was taken to dryness under vacuum at temps not exceeding 40°, redissolved in a minimum vol of H_2O, and placed on the top of a polyamide column (2 5 × 90 cm) The column was eluted with H_2O and the cyanogenic fractions were identified by incubation of an aliquot with enzyme (a mixture of almond emulsin and linamarase) in a closed vial with picrate paper All cyanogenic fractions were combined, reduced in vol under vacuum, and chromatographed on a microcrystalline cellulose column (2 5 × 90 cm) Elution was with 2-butanone–Me_2CO–H_2O The cyanogenic fractions were identified as before, combined, and reduced in vol Aliquots of the sample were purified further by HPLC on a C-18 column with 15% acetonitrile The effluent was monitored at 190 nm and cyanogenic peaks were identified with picrate paper as before Cyanogenic peaks from multiple HPLC runs were combined, taken to dryness by lyophilization, and redissolved in Me_2CO-d_6 for NMR

The cyanogenic glycoside was identified as proacacipetalin by comparison of its NMR spectrum with authentic proacacipetalin isolated from *A tortuosa* [27] The relevant features of the ^1H NMR spectrum which are attributable to the hydrogens of the aglycone moiety are ^1H NMR (360 MHz, Me_2CO-d_6) methyl, δ1 90 (3H, *s*), cyanohydrin 5 18 (1H, *s*), and vinyl 5 38 (1H, *s*) and 5 42 (1H, *s*)

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*Cyanogenesis has been reported in subgenus *Aculeiferum* but, as yet, no compounds have been isolated or characterized [5]

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