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CYANOGENESIS IN AUSTRALIAN SPECIES OF *ACACIA*

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Key Word Index—*Acacia*; Leguminosae; cyanogenesis; cyanogenic glycosides; prunasin, sambunigrin; proacacipetalin; chemotaxomy.

Abstract—In a survey in which approximately 96% of the Australian species of *Acacia* were examined, 45 species were shown to be cyanogenic. Forty-three of the 45 cyanogenic species occurred in subgenus *Phyllodineae* and two in subgenus *Acacia*. Within subgenus *Phyllodineae*, 37 cyanogenic species occurred in section *Juliflorae*, five in section *Botrycephalae*, and one in section *Pulchellae*. Cyanogenesis was not observed in the four other sections of the subgenus. The cyanogenic glycosides in subgenus *Phyllodineae* are prunasin and/or sambunigrin, derived from phenylalanine. The two species in subgenus *Acacia* contain proacacipetalin, derived from leucine. These biochemical studies are discussed in the context of the existing taxonomy of the genus.

INTRODUCTION

The ability of many plants to produce hydrogen cyanide (HCN) is well established [1]. This process, cyanogenesis, occurs when the tissues of a cyanogenic plant are crushed and cyanogenic substrates (usually glycosides, but in a few species, lipids) are brought into contact with endogenous enzymes which catalyse their hydrolysis. In such species as *Sorghum bicolor*, *Manihot esculenta*, *Prunus laurocerasus*, *Acacia binervia* (syn. *A. glaucescens*) and *Eucalyptus cladocalyx*, the amount of HCN produced can prove lethal to animals consuming the cyanogenic plants [2, 3].

The genus *Acacia* which contains about 1200 species [4] belongs to subfamily Mimosoideae within the Leguminosae. Discussions on the classification of *Acacia* are numerous, but some of the more recent ones are refs [5–11]. As currently recognized *Acacia* is usually regarded as comprising three subgenera, namely, *Acacia*, *Aculeiferum* and *Phyllodineae* (syn. *Heterophyllum*) [4]. Pedley [11] considered that these subgenera warranted generic status and accordingly applied the names *Acacia*, *Senegalia* and *Racosperma* respectively to them. Throughout the present work we regard *Acacia* as one genus comprising three subgenera. Subgenus *Acacia* and subgenus *Aculeiferum*, have world-wide, pan-tropical distributions [8], and contain species with bipinnate foliage. These two subgenera are represented in Australia by only six [throughout this work *A. farnesiana* is excluded, it most probably being an early introduction to Australia [12] and one species respectively and are confined to the north of the continent [8, 13]. Subgenus *Phyllodineae* on the other hand is almost entirely restricted to Australia [14], where it has undergone remarkable speciation. Within Australia this subgenus comprises about 830 species (including both described and undescribed taxa) which are contained in seven sections as defined by Pedley [7]. Two of these sections (*Botrycephalae* and *Pulchellae*) contain bipinnate-leaved species, but these species are not closely related to those of subgenus *Acacia* and *Aculeiferum* [5, 11, 15]. The remaining five sections (*Alatae*, *Juliflorae*, *Lycopodiifoliae*, *Phyllodineae* and *Plurinerves*) contain species with phyllodinous foliage and these comprise the majority of the Australian *Acacia* flora. An outline of the classification of the Australian acacias is given in Table 1 below. This scheme, which follows Pedley's classification [7], is used throughout the present work.

Several members of the Mimosoideae have been reported as cyanogenic, but the cyanogenic glycosides have been characterized in only a few species of *Acacia* [9]. *Acacia* is of special interest because it is one of only three or four genera in which cyanogenic compounds are biosynthesized from more than one precursor amino acid. Although cyanogenesis has been observed in all three subgenera of *Acacia*, the cyanogenic compound(s) have not yet been characterized for subgenus *Aculeiferum*. It has been shown that a number of African, American and Australian species from subgenus *Acacia* synthesize glycosides which are derived (or may be presumed to be derived) from the aliphatic amino acids leucine, isoleucine and valine; the cyanogenic species of the Australian subgenus *Phyllodineae*, on the other hand, contain glycosides derived (or may be presumed to be derived) from the aromatic amino acid phenylalanine [9, 16]. Furthermore, many members of subgenus *Phyllodineae* are almost unique in that they contain within the same species the two closely related epimeric cyanogenic glycosides of mandelonitrile, prunasin and sambunigrin. Finnemore and Cox [17] identified sambunigrin, the β-glucoside of (*S*)-mandelonitrile, in two members of section *Juliflorae*, namely, *A. glaucescens* (= *A. binervia*) and *A. cheelii*. Secor *et al.* [18], on the other hand, found that *A. deanei* subsp. *paucijuga* (section *Botrycephalae*) contained prunasin, the β-glucoside of (*R*)-mandelonitrile. An outline of the history of previous studies which have led to the present work is given in refs [19, 20].

The aims of the present study are to document the occurrence of cyanogenesis in Australian *Acacia* species,

Table 1. Cyanogenic Australian species of

Subgenus	Section (Pedley 1978)	Cyanogenic taxon
Acacia (6 species)	Acacia	*A. pachyphloia* W. Fitzg. ex Maiden
		A. sutherlandii (F. Muell.) F. Muell.
Aculeiferum (1 species)	Spiciflorae	None detected
Phyllodineae	Alatae (10 species)	None detected
(833 species)	Lycopodiifoliae (11 species)	None detected
	Phyllodineae (352 species)	None detected
	Plurinerves (178 species)	None detected
	Botrycephalae (36 species)	*A. deanei* (R. Baker) Welch *et al.* subsp. *deanei**
		A. deanei subsp. *paucijuca* (F. Muell. ex Wakef.) Tind.
		A. irrorata Sieber ex Spreng. subsp. *irrorata*
		A. parramattensis Tind.
		A. polybotrya Benth.
		A. ? schinoides Benth.‡
		A. ? schinoides Benth.‡
	Pulchellae (27 species)	*A. pulchella* var. *glaberrima* Meisn.
		—typical variant
		—Fitzgerald River variant
		—Wannamal variant
		A. pulchella var. *goadbyi* (Domin) Maslin
		—typical variant
		—? var. *glaberrima* Meisn. x var. *goadbyi* (Domin) Maslin
		A. pulchella R. Br. var. *pulchella*
		A. pulchella var. *reflexa* Maslin
		A. pulchella var. *subsessilis* Maslin
		A. pulchella R. Br. var.?
	Juliflorae (219 species)	*A. adsurgens* Maiden
		A. atkinsiana Maiden
		A. beauverdiana Ewart and Sharman
		A. binervia (H. L. Wendl.) Macbr.
		A. blakei Pedley
		A. burrowii Maiden
		A. caroleae Pedley
		A. cheelii Blakeley
		A. conniana Maslin
		A. curvinervia Maiden
		A. diphylla Tind.
		A. doratoxylon Cunn.
		A. exilis Maslin
		A. gonocarpa F. Muell.
		A. gracillima Tind.
		A. gracillima Tind.
		A. granitica Maiden
		A. julifera Benth. subsp. *julifera*
		A. kempeana F. Muell.
		A. lasiocalyx C. Andrews
		A. longiphyllodinea Maiden
		A. lysiphloia F. Muell.
		A. olgana Maconochie
		A. pubifolia Pedley
		A. pycnostachya F. Muell.
		A. resinomarginea W. Fitzg.
		A. rhodophloia Maslin
		A. sibina Maslin
		A. signata F. Muell.‖
		A. signata F. Muell.‖
		A. sparsiflora Maiden
		A. stowardii Maiden
		A. trachycarpa E. Pritzel
		A. trachycarpa E. Pritzel
		A. yorkrakinensis C. Gardner
		A. aff. *atkinsiana* Maslin

Acacia

Major cyanogen present	S:P ratio (NMR)	Voucher specimen
Proacacipetalin	N/A	T. Willing 74 (PERTH)
Proacacipetalin	N/A	N. Hall H84/27 (BRI, PERTH)
Unknown		
Prunasin	4:96	N. Hall H85/10 (PERTH)
Unknown		
Prunasin	0:100†	Unvouchered (cf. Secor *et al.* 1976)
Prunasin	0:100	N. Hall H85/6 (PERTH)
Prunasin	0:100	CBG 8317443 (CBG)
Prunasin	4:96	CBG 8500770 (CBG)
Unknown		
Unknown		
Sambunigrin	100:0	B. R. Maslin 5503 (PERTH)
Sambunigrin	90:10	B. Grivell s.n., 1968 (AD, PERTH, UCD)
Unknown		
Unknown		
Heterodendrin	N/A	B. R. Maslin 5758 (PERTH)
Unknown		
Unknown		
Sambunigrin	62:38	B. R. Maslin 5280 (PERTH)
Sambunigrin	77:23	K. Atkins 1263 (PERTH)
Sambunigrin	90:10	E. E. Conn 15-82 (PERTH)
Sambunigrin	83:17	E. E. Conn s.n., 14 Mar. 1982 (AD)
Sambunigrin	95:5	H. Dillewaard 907 (BRI)
Unknown		
Prunasin	3:97	N. Hall H83/76 (PERTH)
Sambunigrin	94:6	CBG 8500774 (CBG)
Sambunigrin	81:19	S. Hopper s.n. (PERTH 00582131)
Sambunigrin	83:17	R. E. Cottam s.n. (BRI)
Sambunigrin	85:15	N. Hall H84/5 (PERTH)
Sambunigrin	94:6	N. Hall H84/58 (PERTH)
Sambunigrin§	89:11	B. R. Maslin 4666 (PERTH)
Unknown		
Prunasin	44:56	T. Willing 88 (PERTH)
Sambunigrin	68:32	T. Willing 75 (PERTH)
Sambunigrin	90:10	N. Hall H83/20 (PERTH)
Sambunigrin	89:11	N. Hall H83/39 (PERTH)
Unknown		
Sambunigrin	80:20	E. E. Conn 41-82 (PERTH)
Sambunigrin	82:18	B. R. Maslin 5316 (PERTH)
Unknown		
Prunasin	22:78	D. V. Matthews 441 (NT)
Sambunigrin	92:8	N. Hall H83/17 (PERTH)
Sambunigrin	85:15	N. Hall H84/51 (PERTH)
Sambunigrin	83:17	E. E. Conn 20–82 (PERTH)
Unknown		
Prunasin	11:89	B. R. Maslin 5314 (PERTH)
Sambunigrin	81:19*	B. R. Maslin 5158 (PERTH)
Sambunigrin	80:20	E. E. Conn 18–82 (PERTH)
Sambunigrin	79:21	N. Hall H83/70 (PERTH)
Sambunigrin	92:8	B. R. Maslin 5571C (PERTH)
Prunasin/Sambunigrin	50:50	B. R. Maslin 5356+5357 (PERTH)
Prunasin	43:57¶	B. R. Maslin 5357 (PERTH)
Sambunigrin	80:20	E. E. Conn 2–82 (PERTH)
Unknown		B. R. Maslin 2734 (PERTH)

Table 1. (*Continued*)

Subgenus	Section (Pedley 1978)	Cyanogenic taxon
		A. aff. *blakei* Pedley and Maslin
		A. aff. *citrinoviridis* Tind. and Maslin
		A. aff. *kempeana* F. Muell.
		A. aff. *macdonnelliensis* Maconochie

Taxa listed here released HCN from their foliage in the presence of added β-glucosidase. Glucoside composition was respective subgenera and sections. Species numbers include both described and undescribed taxa and refer only to prunasin. Abbreviations for herbaria (column 6): AD, State Herbarium of South Australia, Adelaide; BRI, Queensland California Herbarium, Davis.

*This species showed a very weak Feigl–Anger reaction and should be re-examined.

†Determined by GLC.

‡The identity of these plants is uncertain but they appear to be hybrids involving *A. schinoides* (and possibly

§Low levels of apparently aliphatic material also detected in this sample.

‖There are two variants of *A. signata*. B. R. Maslin 5158 represents the typical variant; E. E. Conn 18–82 represents

¶This is a repeat analysis of B. R. Maslin 5357 which originally showed S:P = 50:50.

to identify the cyanogenic glycosides present, and to investigate the extent to which the biochemical results can be correlated with taxonomy.

RESULTS AND DISCUSSION

The occurrence of cyanogenesis in Australian Acacia *species*

Forty-five species including 5 taxa of uncertain rank, (for the purpose of this paper these taxa are called species), representing about 5% of the Australian *Acacia* flora, have been shown to release HCN from their foliage in the presence of added β-glucosidase (Table 1). This total results from our testing of 3427 herbarium specimens and 1023 living plants. For many species relatively few specimens were tested, and when considered that many taxa seem to be polymorphic for cyanogenesis (see below), it is probable that we have missed some cyanogenic species in this survey. Since many of the 45 species lack an endogenous β-glucosidase capable of releasing HCN from the cyanogenic precursor (see below), it is stressed that a β-glucosidase was added in all tests (see Experimental).

Approximately 96% of the 833 Australian species of *Acacia* were tested during the course of this survey. Details of the herbarium specimens and living plants examined are given in ref. [21, Maslin B. R., Conn E. E. and Hall unpublished results]. Forty-three of the 45 cyanogenic species occurred in subgenus *Phyllodineae* and two in subgenus *Acacia*. *Acacia albizioides*, the sole Australian representative of subgenus *Aculeiferum*, was not tested. Within subgenus *Phyllodineae*, 37 cyanogenic species occurred in section *Juliflorae* (i.e. 17% of the 219 Australian species contained within this section), five in section *Botrycephalae* (i.e. 14% of a 36 species total) and one in section *Pulchellae* (i.e. 4% of a 27 species total). No cyanogenic species were detected in sections *Phyllodineae* (352 species), *Plurinerves* (178 species), *Lycopodiifoliae* (11 species), and *Alatae* (10 species).

Clearly section *Juliflorae* contains the majority of cyanogenic Australian species of *Acacia*. Species belonging to this section are usually characterized by cylindrical flower-heads and multi-nerved phyllodes. In the absence of a meaningful infra-sectional classification of the *Juliflorae*, most cyanogenic spcies are here referred to two informal groups. Group 1 contains *A. gracillima*, *A. lysiphloia* and *A. trachycarpa*. Using Bentham's [22] classification of the *Juliflorae* these species would probably be best included in subseries *Rigidulae*. *Acacia chisholmii* and *A. monticola* are closely related to the above taxa, but they are apparently acyanogenic [21]. The members of Group 1 are characterized by phyllodes with usually two–three, widely spaced, obscure longitudinal nerves and by bark which is red and which exfoliates in narrow shavings that curl retroresely from each end. Colloquially this characteristic bark type is known as 'Minni Ritchi'. Group 1 species are distributed in the northern Arid Zone and adjacent subtropical areas of Australia [23, 24]. It is suggested that species of this Group are taxonomically less advanced than those of the following group. Group 2 includes the remaining 34 *Juliflorae* species listed in Table 1, except *A. gonocarpa*. Group 2 species would be included in subseries *Stenophyllae* and *Falcatae* [22]. There are many acyanogenic species within these subseries. Species of Group 2 are characterized by phyllodes which possess numerous closely approximating longitudinal nerves and , with the exception of *A. rhodophloia*, their bark is not 'Minni Ritchi'. This phyllode nervation type is common in the arid/semi-arid parts of the continent and it is considered to represent a derived condition. Group 2 species are most commonly found in the north-west Arid Zone (i.e. the Pilbara region of Western Australia), the northern wheat-belt region of semi-arid Western Australia and near the McPherson-Macleay Overlap region [25] on the Queensland-New South Wales border [21, 24]. *Acacia gonocarpa* cannot be easily accommodated within either Group 1 or 2 although it is probably most closely related to the former even though it does not have 'Minni Ritchi' bark. It is distributed in northern Australia [24]. The centres of species-richness for cyanogenic *Juliflorae* species coincide with many of the centres of richness for the section as a whole [13].

Major cyanogen present	S:P ratio (NMR)	Voucher specimen
Sambunigrin	98:2	L. Pedley 4952 (BRI)
Unknown		B. R. Maslin 4567 (Perth)
Unknown		D. Hewett 39 (Perth)
Unknown		J. Curnow 624 (BRI)

determined by NMR unless otherwise stated. Species are listed alphabetically under their Australia (Maslin and Hnatiuk, unpublished). Abbreviations (column 5): S, sambunigrin; P, Herbarium, Brisbane; Perth, Western Australian Herbarium, Perth; UCD, University of

A. deanei subsp. *paucijuga* – see text for further details).

the widespread, common, wheatbelt variant which resembles *A. yorkrakinensis*.

It is noted that we have not detected any cyanogenic *Juliflorae* species referable to Bentham's [22] subseries *Dimidiatae*. This group is considered to contain some of the most primitive members of section *Juliflorae*. Also, cyanogenesis appears not to occur in section *Plurinerves*. This section is closely related to the *Juliflorae* [5, 6, 26–28] and in his recent classification Pedley [11] combines the two groups.

Within section *Pulchellae*, cyanogenesis occurs in only a single species, *A. pulchella*. Taxonomically the *Pulchellae* is a diverse section [29]. Its relationship to other sections within subgenus *Phyllodineae* is not clear [11]; however, some suggestions of affinities to the phyllodinous sections *Juliflorae* and *Plurinerves* have been made [6, 30, 31]. *Acacia pulchella* is regarded as one of the most advanced members of the *Pulchellae* (Maslin, B. R., unpublished data). It is an extremely variable species with five varieties currently recognized, all of which contain cyanogenic members. The species is widespread in the more temperate areas of south-west Western Australia [21, 24].

Five species of section *Botrycephalae* are listed in Table 1 as cyanogenic. However, *A. deanei* subsp. *deanei* showed a very weak Feigl–Anger reaction and this taxon should be re-examined. The taxon listed as *A.* (?) *schinoides* may well represent a hybrid involving *A. schinoides* and possibly *A. deanei* subsp. *paucijuga*. The glucoside determination for this taxon was based on material collected from two plants cultivated at the Canberra Botanic Gardens (vouchered by CBG 8317443 and CBG 8500770). In ref. [21] CBG 8317443 was referred to *A. schinoides* and CBG 8500770 to *A. deanei* subsp. *paucijuga*. Trindale and Roux [27] considered the *Botrycephalae* to be a primitive group. However, Pedley [11] regarded it as derived, having evolved from within the 'racemosae group' of section *Phyllodineae*. The *Botrycephalae* is endemic in the more temperate parts of eastern Australia [13].

Two Australian members of subgenus *Acacia* are cyanogenic, namely, *A. pachyphloia* and *A. sutherlandii*. Aspects of cyanogenesis in *A. pachyphloia* are discussed in ref. [16]. Plants of *A. farnesiana* in Queensland have been shown to be cyanogenic [21], but as this species is probably an early introduction into Australia [12], it is not included in the present study. Cyanogenesis in *A. farnesiana* is discussed in ref. [32]. Although subgenus *Acacia* is poorly represented in Australia (6 species), it is very common in Asia, Africa and the New World. References to cyanogenesis in extra-Australian members of subgenus *Acacia* are given in ref. [9, 33].

Characterization of cyanogenic glycosides in Australian species of Acacia

Of the 45 species determined as cyanogenic using the Feigl–Anger test, glucosides have been characterized for 32 species by NMR and one by GLC (Table 1). *Acacia pachyphloia* and *A. sutherlandii* (subgenus *Acacia*) possessed proacacipetalin, a glycoside presumably derived from the aliphatic amino acid, leucine [16]. Within species from subgenus *Phyllodineae*, however, we identified both prunasin and sambunigrin which are glucosides presumed to be derived from the aromatic amino acid phenylalanine. The proportions of these two glucosides in species from subgenus *Phyllodineae* ranged from pure prunasin through varying mixtures of prunasin and sambunigrin to pure sambunigrin. Nevertheless, two general trends seem to be evident. In species from section *Botrycephalae*, the major cyanogen present was prunasin (either 100% as in the case of *A. parramattensis* and *A. polybotrya* or 96% in *A. deanei* subsp. *paucijuga*). Samples of *A. ? schinoides* showed both 96% and 100% prunasin. With few exceptions species from sections *Juliflorae* and *Pulchellae* showed a mixture of sambunigrin and prunasin (often around the proportion 4:1) with the former glycoside predominating. The exceptions to this trend were *A. pulchella* var. *glaberrima* (Wannamal variant) in section *Pulchellae*, the only taxon encountered with 100% sambunigrin, *A. caroleae*, *A. olgana*, *A. sibina* and *A. trachycarpa* (all in section *Juliflorae*) with 50% or less sambunigrin and *A. gracillima* (section *Juliflorae*) which had values for sambunigrin both above and below 50%.

The frequent co-occurrence of sambunigrin and prunasin in single species, often in a ratio approximating 4:1, was a surprising finding in this study. While co-occurrence of these epimers in a single species has been

described previously [34], it is not a common observation. Moreover, many early reports of a mixture of sambunigrin and prunasin, known as prulaurasin, in *Prunus laurocerasus* [35] are undoubtedly the result of racemization during isolation of the prunasin which this plant contains. A detailed study of the recemization has shown the process to be facilitated by mild alkaline conditions encountered with reagents such as barium- or calcium hydroxide employed in the earlier studies [36].

That the mixtures of sambunigrin and prunasin observed in this study actually occur in the plant and are not the result of racemization during isolation is indicated by the following: (i) The isolation procedure employed for all species was identical and yet 100% prunasin was obtained from *A.? schinoides* while 100% sambunigrin was isolated from *A. pulchella* var. *glaberrima* (Wannamal variant). (ii) If racemization were occurring, one would expect the equilibrium mixture of 57% sambunigrin and 43% prunasin to be obtained in at least some of the species [36]; no species exhibited that equilibrium ratio within experimental error. (iii) In the case of *A. atkinsiana* the standard extraction procedure was varied by (a) omitting the treatment with Pb^{2+}; (b) extracting the plant material with cold methanol instead of hot ethanol; (c) extracting with cold methanol and omitting the treatment with Pb^{2+}. These variations produced the following rates: (a) 79:21; (b) 81:19; (c) 81:19. The standard procedure gave 4:1. (iv) (*R*)-Amygdalin (500 mg) was added to a homogenate prepared by grinding 120 g of *A. melanoxylon*, a non-cyanogenic species, in boiling 95% EtOH. When the cyanogen was then isolated using the standard procedure, the final product contained only (*R*) amygdalin.

We have no information as to the process by which different species of *Acacia* could accumulate different ratios of sambunigrin to prunasin. Whether this represents a difference in the capacity of the plant to synthesize the two isomers, or whether equal amounts are formed initially and there are different rates of degration in the intact plant is not known.

Chemotaxonomic interpretations

Some authors have suggested that possibly all plants can be shown to release trace amounts of HCN [37, 38]. If this is true then the mere presence of cyanogenesis has little taxonomic value and can not be used as evidence of relationships between taxa. As pointed out by Hegnauer [39], the systematic and ecological value of cyanogenesis is related to the plant's ability both to synthesize and accumulate cyanogenic constituents. Further taxonomic information can be obtained from information on the different types of the cyanogenic glycosides and their biosynthetic pathways.

This study has shown that within Australian species of *Acacia* there exist cyanogenic glycosides derived from two fundamentally different biochemical pathways. One compound, proacacipetalin, is produced from the apliphatic amino acid L-leucine and is found in species of subgenus *Acacia*. The occurrence of the aliphatic glucosides in *A. pulchella* var *reflexa* and possibly also *A. exilis* (both subgenus *Phyllodineae*) was unexpected and requires further study. The other compounds, prunasin and sambunigrin, are produced from the aromatic amino acid L-phenylalanine and are found in subgenus *Phyllodineae*. Proacacipetalin is unique to subgenus *Acacia* and is not known from elsewhere within the plant kingdom [40]. Its taxonomic significance has already been discussed [16]. Prunasin and sambunigrin on the other hand occur in a number of plant families, e.g. Asteraceae, Caprifoliaceae, Fabaceae, Myoporaceae, Myrtaceae, Oleaceae, Olinaceae, Rosaceae, Rutaceae, Saxifragaceae and Scrophulariaceae [41].

Within subgenus *Phyllodineae*, cyanogenesis is not an attribute which characterizes entire taxonomic groups. Instead, this character occurs at relatively low frequencies in only sections *Botrycephalae*, *Juliflorae* and *Pulchellae*. It is absent from sections *Alatae* (sister-group to the *Pulchellae*), *Phyllodineae* (sister-group to the *Botrycephalae*), *Plurinerves* (sister-group to the *Juliflorae*) and *Lycopodiifoliae*. This systematic distribution of cyanogenesis within subgenus *Phyllodineae* suggests that these compounds were either present in the ancestors of the subgenus and there was differential loss or repression of the biosynthetic pathways or the accumulation mechanism, or that they evolved several times (or the ability to accumulate the compounds evolved several times) independently in some species and sections.

The widespread distribution of prunasin and sambunigrin within the plant kingdom (see above) implies that the biochemical pathways leading to the production of these compounds are primitive. Likewise, the distribution of these aromatic glycosides within subgenus *Phyllodineae* also suggests primitiveness because they occur in sections which are considered not particularly closely related. If these pathways are primitive, it leaves open the question as to why cyanogenic species within subgenus *Phyllodineae* occur principally in groups that have many advanced characters and which predominate in areas of recent evolutionary divergence. In addressing this question it needs to be emphasized that biosynthesis and accumulation of secondary products are separate processes [42]. Also, it needs stressing that these cyanogenic glycosides are biosynthesised from protein amino acids, so unless there is selective pressure for their retention, they are likely to be quickly lost. It therefore seems most probable that within sections *Botrycephalae*, *Juliflorae* and *Pulchellae* there has been selection for the ability to *accumulate* cyanogenic glycosides and that this is a recently acquired, polyphyletic character. It is considered unlikely that the ability to accumulate cyanogenic constituents was a character once more widespread in subgenus *Phyllodineae* and which has been subsequently lost. Were this the case it might be expected that we would have encountered cyanogenic species more randomly distributed among the sections. Because the sections containing cyanogenic taxa also contain many acyanogenic species, it is suggested that there has been a selective, secondary repression of the ability to accumulate cyanogenic glycosides.

Alternatively the uneven distribution of cyanogenesis within subgenus *Phyllodineae* may bring into question the existing taxonomy of this group. It could be argued that those sections in which cyanogenesis occurs are more closely related to one another than those in which it is absent. Such an argument implies that the biosynthesis of the aromatic glycosides prunasin and sambunigrin is a monophyletic, derived character. However, a classification in which sections *Botrycephalae*, *Juliflorae* and *Pulchellae* formed a monophyletic group distinct from the other sections of the subgenus would be counter to current taxonomic evidence based on other criteria [11].

Enzymatic aspects of cyanogenesis

Cyanogenic plants usually contain two enzymes which are responsible for the release of HCN when the plant tissue is disrupted. These are a substrate-specific β-glucosidase which, brought in contact with its substrate, produces glucose and an α-hydroxynitrile that, in turn, dissociates rapidly in the presence of hydroxynitrile lyase to form HCN (1). In this property, most of the cyanogen-containing species of Australian *Acacia* are atypical, as first noted in ref. [43]. Of the 33 species of living plants which were examined for the presence of these enzymes, only five (viz. *A. atkinsiana*, *A. blakei*, *A. olgana*, *A. stowardii* and *A. sutherlandii*) rapidly produced HCN in the absence of added enzymes (in ref. [21] *A. deanei* subsp. *paucijuga* and *A. lasiocalyx* were erroneoulsy included in this group). The other species either failed to release HCN or did so only very slowly unless almond emulsin was added. After 24 hr a few of the species (e.g. *A. caroleae*, *A. gracillima*, *A.* ? *schinoides*) in this latter group yielded weakly-positive reactions with Feigl–Anger paper suggesting that they might possess enzyme in trace amounts or possess a non-specific β-glucosidase which, with time, can hydrolyse the cyanogen present. They clearly lack, however, a more substrate-specific β-glucosidase capable of rapidly hydrolysing the cyanogens.

Very little is known concerning variations and/or polymorphism within these enzyme systems. The only population which we examined from this viewpoint was the one from which B. R. Maslin 5503 (*A. pulchella* var. *glaberrima*—Wannamal variant) was collected. Of the seven plants tested for enzyme, six reacted positive (one within 1 hr, five within 6 hr) and one reacted negative. These results strongly suggest that this variety is polymorphic for enzyme. Data on a wider range of species is clearly required.

Quantitative aspects and the potential for toxicity

The cyanogenic species of *Acacia* described in this study varied widely in their content of cyanogenic material with several species containing less than 1.0 μmol/g (Table 1). The highest value observed was in *A. caroleae* (= *A. doratoxylon* var. *angustifolia*) which contained 90 μmol/g. This concentration and that in several other species (*A. signata* 'yorkrakinensis' variant 71 μmol/g; *A. cheelii*, 62 μmol/g; *A. exilis*, 57 μmol/g; *A. atkinsiana*, 54 μmol/g; *A. binervia*, 52 μmol/g; *A. sutherlandii*, 51 μmol/g) resemble the concentration in *A. sieberiana* var. *woodii* (Natal camelthorn) reported to have caused mortalities in sheep in South Africa [44]. These concentrations are also similar to that in *Sorghum bicolor*, a plant well known for its toxicity to livestock [1]. As discussed in ref. [45], there is a considerable range of variation in the amount of HCN produced, not only between species but also from within the same species. It is interesting to note that Everist [2] attributed cyanide poisoning of livestock under field conditions only to *A. binervia*, one or more species in the *A. cunninghamii* complex and *A. sparsiflora*. (In the past there has been considerable confusion concerning the name *A. cunninghamii*. This name has been applied in an extremely broad sense to *A. concurrens*, *A. crassa*, *A. cretata*, *A. leiocalyx*, *A. longispicata* and *A. tropica* [7, 47]. As noted by Everist [2], it is now not possible to determine with certainty the correct identity of plants described as toxic under the name *A. cunninghamii*. The specimen labelled *A. cunninghamii* at herb. UCB and found to be cyanogenic was subsequently redetermined by L. Pedley (personal communication) as *A. blakei* [21].) Feeding tests demonstrating the toxicity of *A. binervia* have been described [46]. Since *A. binervia*, *A. cheelii* and *A. signata* ('yorkrakinensis' variant) lack an active β-glucosidase while *A. blakei* (in the *A. cunninghamii* complex) *A. atkinsiana* and *A. sutherlandii* contain such an enzyme, it appears that additional factors are involved in determining which cyanogenic species of *Acacia* may be toxic to livestock. Aspects of toxicity within Australian species of *Acacia* are discussed in ref. [45].

EXPERIMENTAL

The procedures used in the sampling, testing and vouchering of herbarium specimens and living plants for cyanogenesis are described in ref. [21]. The procedure employed in extraction, purification and identification of the cyanogenic compound from foliage (leaves and phyllodes) is detailed in ref. [16]. Quantitative analysis were performed on small samples (0.1–0.5 g) of plant material as follows. The material was frozen in liquid N_2 and pulverized. After quantitative transfer to an Erlenmeyer flask (25 or 50 ml) fitted with a centre well, Pi buffer (2–5 ml of 0.1 M, pH 6.8) and a mixture of β-glucosidases (flax seed linamarase [48] and almond emulsin, Sigma G-8625) were added in an amount sufficient to hydrolyse the cyanogenic material present in the sample. NaOH (0.5 ml, 1.0 M) was placed in the centre well, the flask sealed and sample placed on a shaker-incubator (30°, 18–24 hr). At that time the NaOH in the centre well was removed and analysed for cyanide by the colorimetric method of ref. [49].

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