Phytochemistry, Vol. 28, No. 3, pp. 817–820, 1989.
Printed in Great Britain.

0031-9422/89 $3.00+0.00

CYANOGENESIS IN *ACACIA* SUBGENUS *ACULEIFERUM*

E. E. Conn, D. S. Seigler,* B. R. Maslin† and J. Dunn

Department of Biochemistry and Biophysics, University of California, Davis, CA 95616, U.S.A.; *Department of Plant Biology, University of Illinois at Champaign-Urbana, Urbana, IL 61801, U.S.A.; †Western Australian Herbarium, Baron-Hay Court, South Perth, Western Australia 6151

(*Received* 9 *August* 1988)

Key Word Index—*Acacia* subgenus *Aculeiferum*; Leguminosae; cyanogenic glycosides; prunasin; sambunigrin; proacacipetalin; chemotaxonomy.

Abstract—Cyanogenic glycosides are for the first time identified from members of *Acacia* subgenus *Aculeiferum*. The aromatic glycosides prunasin and sambunigrin occur in *A. caffra* and *A. hereroensis*. These African species are placed in section *Aculeiferum* subsection *Polyacanthae*. These data support other evidence derived from pollen and free amino acids in seeds that subgenus *Aculeiferum* is more closely related to the predominantly Australian subgenus *Phyllodineae* than to the pan-tropical subgenus *Acacia*.

INTRODUCTION

About 60 species of *Acacia* have been reported as cyanogenic, 15 species from subgenus *Acacia* [1, 2] and 45 species from subgenus *Phyllodineae* [3]. Although cyanogenesis has been reported in a few species of subgenus *Aculeiferum*, until now no cyanogenic compounds have been isolated or characterized from members of this group [1]. Biochemically the genus *Acacia* is of special interest because it is one of only three or four genera known to synthesize cyanogenic compounds from more than one precursor amino acid; at least nine cyanogenic compounds have been reported from cyanogenic members of the genus. As reviewed elsewheres [1, 2, 4] cyanogenic species from subgenus *Acacia* contain cyanogens derived from the branch chain amino acids valine, leucine and isoleucine, while cyanogenic members of subgenus *Phyllodineae* contain cyanogens biosynthesized from phenylalanine. This paper reports the isolation and characterization of cyanogens from two cyanogenic species of subgenus *Aculeiferum*. In order to place our results in the proper taxonomic context, we discuss here the classification and distribution of subgenus *Aculeiferum*.

Five major classification schemes for *Acacia* subgenus *Aculeiferum* are outlined in Table 1. The subgenus was established in 1972 by Vassal [5] and it incorporated Bentham's [6, 7] series *Vulgares* and *Filicinae*. As detailed below Vassal recognized three sections and a number of subordinate taxa within subgenus *Aculeiferum*. In subsequent classifications of the subgenus by Pedley [8, 9], only two sections and no subordinate taxa were recognized; these two classifications provide too few infrageneric categories to be useful in the present discussions. Furthermore, Pedley's 1986 [9] classification uses a system of nomenclature which is too recent to have been fully evaluated. We have therefore decided to adopt Vassal's [5] scheme even though this classification is not universally accepted. Ross [10], for example, discussed a number of inadequacies in relation to the African species. A problem we encountered was that Vassal included only 16 of the approximately 150 species of *Aculeiferum* in his classification. Furthermore, seedling characters are needed to positively assign species to infrageneric groups. Therefore, we have discussed below our placement of cyanogenic species which were not included in Vassal's original classification. Notwithstanding these inadequacies Vassal's classification is a phylogenetic scheme and does provide, through its hierarchy of infrageneric categories, a useful framework in which to discuss our results. The relationship between Vassal's and Bentham's classifications of subgenus *Aculeiferum*, (syn. series *Vulgares*) is discussed in Vassal [5, 11] and Ross [10].

Vassal [5] divided the pan-tropical subgenus *Aculeiferum* into section *Aculeiferum* (distributed in Africa and Asia), section *Monacanthea* (distributed in Africa, Asia and the Americas) and later [12] section *Filicinae* (endemic to the Americas). A single sub-section (*Polyacanthae*) was recognized within section *Aculeiferum*, while section *Monacanthea* was divided, on seedling characters, into sub-section *Cryptocotylae* (endemic to the Americas) and sub-section *Phanerocotylae*. The latter sub-section was then divided into an Old and New World group, namely series *Gerontogeae* and series *Americanae* respectively. Subordinate taxa were not recognized within section *Filicinae*. An outline of Vassal's classification is given in Table 1. The world-wide distribution of subgenus *Aculeiferum* is shown in Ross [13, Fig. 2].

Our placement of the 10 species of subgenus *Aculeiferum* reported or known to be cyanogenic is shown in Table 2. Of the ten, only three, *A. berlandieri*, *A. caffra* and *A. greggii*, are included in Vassal's [5] classification, viz. *A. berlandieri* (section *Monacanthea* sub-section *Phanerocotylae* series *Americanae*), *A. caffra* (section *Aculeiferum* sub-section *Polyacanthae*) and *A. greggii* (section *Monacanthea* sub-section *Cryptocotylae*). Vassal [5] originally placed *A. coulteri* in subgenus *Acacia* but on account of its seed amino acids [14] he subsequently transferred the species to subgenus *Aculeiferum* where he noted that it "has no clear relationships with the *Americanae Spiciflorae* Benth. neither with the three sections already

Table 1. Classification schemes for subgenus *Aculeiferum*

Bentham [7]	Bentham [6]	Vassal [5]	Pedley [8]	Pedley [9]
Genus *Acacia*	Genus *Acacia*	Genus *Acacia*	Genus *Acacia*	Genus *Senegalia*
Ser. *Vulgares*	Ser. *Vulgares*	Subgenus *Aculeiferum*	Subgenus *Aculeiferum*	Sect. *Senegalia*
S. ser. *Diacanthae*	S. ser. *Gerontogeae Spiciflorae*	Sect. *Monacanthea*	Sect. *Spiciflorae*	Sect. *Filicinae*
S. ser. *Ataxacanthae*	A. *Triacanthae*	S. sect. *Cryptocotylae*	Sect. *Filicinae*	
S. ser. *Nudiflorae*	B. *Diacanthae*	S. sect. *Phanerocotylae*		
S. ser. *Concinnae*	C. *Ataxacanthae*	Ser. *Americanae*		
S. ser. *Pennatae*	S. ser. *Americanae Spiciflorae*	Ser. *Gerontogeae*		
Ser. *Filicinae*	S. ser. *Americanae Capitulatae*	Sect. *Aculeiferum*		
	S. ser. *Gerontogeae Capitatae*	S. sect. *Polyacanthae*		
	Ser. *Filicinae*	Sect. *Filicinae**		

Abbreviations: Sect. = Section; S. sect. = Subsection; Ser. = Series; S.ser. = Subseries.
*Formalized in Guinet and Vassal [12].

recognized." We have therefore included *A. coulteri* in Table 2 within an informal monotypic group although the similarity of this species to *A. acatlensis* suggests that it should be placed in the same sub-section. Using data derived from Robbertse and Schijff [15] and Ross [10] it is possible, with reasonable confidence, to place the three African species, *A. chariessa*, *A. hereroensis* and *A. welwitschii*, within section *Aculeiferum* sub-section *Polyacanthae*. Because seedling characters are unknown for the American species, *A. acatlensis* and *A. roemeriana*, our infra-sectional placement for these is less certain. They have been referred to sub-section *Phanerocotylae* series *Americanae* and sub-section *Cryptocotylae* because of the number of pinnae given in Bentham [6]. Based on the description in Macbride [16] *A. klugii* appears to belong to subgenus *Aculeiferum* and would be placed in sub-section *Phanerocotylae* series *Americanae*.

RESULTS AND DISCUSSION

The occcuuurrence of cyanogenesis in subgenus Aculeiferum

Of the approximate 150 known species of *Acacia* subgenus *Aculeiferum*, only 10 species are known or thought to be cyanogenic, (Table 2). Our testing of herbarium specimens has shown that at least some specimens of *A. caffra*, *A. chariessa*, *A. hereroensis*, *A. welwitschii* and *A. klugii* release hydrogen cyanide and of these we have characterized the cyanogenic compounds in *A. caffra* and *A. hereroensis* (see below). Except for *A. klugii* these species belong to section *Aculeiferum* and are distributed in southern Africa (Angola southwards, see [10]). The poorly known species *A. klugii* is an exception in that it is placed in section *Monacanthea* and grows in South America (Peru). The remaining five species in Table 2 occur in the New World. Of these, *A. greggii*, *A. berlandieri* and *A. roemeriana* (all section *Monacanthea*) are widely distributed in southwestern U.S. and northern Mexico. *Acacia acatlensis* (section *Monacanthea*) and *A. coulteri* (section unknown) occur primarily in southern Mexico, but *A. coulteri* is more widely distributed and is found in other parts of Mexico as well. Although these two species are readily distinguished in the field, identification of herbarium materials is often difficult.

The results of cyanide tests by the Feigl–Anger method on the last two species proved to be perplexing. In the field, fresh materials of *A. acatlensis* gave a moderately strong positive test for hydrogen cyanide whereas those of *A. coulteri* did not. In the laboratory, fresh air-dried leaf material of these two species occasionally gave weakly positive tests. Clearly, both the taxonomic relationships of these two species and their close relatives and cyanogenesis in this group of plants needs to be more thoroughly examined.

Chemical identification

Acacia caffra contained a mixture of sambunigrin and prunasin in the ratio of 19:1. Similarly, *A. hereroensis* contained these compounds in a ratio of 89:11. Both species lack an endogenous β-glucosidase capable of hydrolysing these glucosides.

The co-occurrence of sambunigrin and prunasin in both species was expected in view of results obtained in an earlier study of the cyanogenic glycosides in the subgenus *Phyllodineae* [3]. As in that study, there is no evidence that racemization during isolation of the cyanogens in *A. caffra* and *A. hereroensis* was responsible for the mixture observed.

Taxonomic interpretations

Currently the most generally accepted classification of *Acacia* is the one summarized by Vassal [17] in which the genus is viewed as comprising three subgenera, namely, *Acacia*, *Aculeiferum* and *Phyllodineae* (syn. *Heterophyllum*). In recent years there have been suggestions that these subgenera might best be regarded as distinct genera [18–20] and recently the generic groups were formally recognized [9], i.e. *Acacia*, *Senegalia* and *Racosperma* respectively. A summary of the relationships between the main classification schemes for *Acacia* is presented in Maslin [21].

Cyanogenic glycosides within *Acacia* are derived from both aromatic and aliphatic amino acids. Species of subgenus *Aculeiferum* and subgenus *Phyllodineae* utilize the aromatic amino acid phenylalanine to produce sambunigrin and/or prunasin. This biochemical pathway occurs in a number of different plant families [3]. Because of its wide taxonomic distribution, this pathway is considered to be primitive; however, there is evidence at least within subgenus *Phyllodineae* that the ability to accumulate sambunigrin and prunasin is an advanced character

Table 2. Species of *Acacia* subgenus *Aculeiferum* reported/known to be cyanogenic

Infrageneric group		Species in which cyanogenesis has been recorded		
Name	Distribution	Name	Cyanogen present	Notes
Sect. *Aculeiferum*				
S. sect. *Polyacanthae*	Africa/Asia	*A. caffra*	Sambunigrin: prunasin (19:1)	The one herb. UCB specimen tested was moderately HCN positive, viz. UCB 14095424; the six herb. JRAU specimens tested were moderately to strongly HCN positive, viz. P. J. Robbertse 904, J. Pretorius 1042, B-E. van Wyk 2043, 2044, 2045, and B-E. and M. van Wyk 67; see present text for discussion of cyanogenesis in this species.
		A. chariessa	Unknown	The one herb. UCB specimen tested was weakly HCN positive, viz. UCB M010533.
		A. hereroensis	Sambunigrin: prunasin (89:11)	The one herb. UCB specimen tested was moderately HCN positive, viz. UCB M279547; 7 of the 13 herb. PRE and JRAU specimens tested were weakly to strongly cyanogenic, viz. D. C. Biggs 220 (PRE, weak), J. W. Snyman 75 (PRE weak), J. W. Morris 1121 (PRE, weak), M. Muller and H. Kolberg 2108 (PRE, weak), M. Mason 2626 (PRE, strong), M. C. Rutherford A33 (PRE, moderate), R. Seydel 4082 (PRE, moderate); see present text for discussion of cyanogenesis in this species.
		A. welwitschii	Unknown	The one herb. UCB specimen tested was strongly HCN positive, viz. UCB M085858.
Sect. *Monacanthea*				
S. sect. *Cryptocotylae*	Americas	*A. greggii*	Unknown	Doubtfully cyanogenic [1]
		A. roemeriana	Unknown	One plant field-tested HCN positive by D.S.S. [27] but numerous subsequent testings of other individuals in the field and herbarium material have not reconfirmed this result.
S. sect. *Phanerocotylae*				
Ser. *Americanae*	Americas	*A. acatlensis*	Unknown	Reported cyanogenic in [28]; two of the 7 herb. UCB specimens tested were HCN positive, viz. UCB 933107 and M285706; see present text for discussion of this species.
		A. berlandieri	Unknown	One plant field-tested HCN positive by D.S.S. [27] but numerous subsequent testings of other individuals in the field and herbarium material have not reconfirmed this result.
		A. klugii	Unknown	The one herb. UCB specimen tested was weakly HCN positive, viz. UCB 710059.
Ser. *Gerontogeae*	Africa/Asia	None known		
Sect. *Filicinae*	Americas	None known		
Sect. Unknown	Americas	*A. coulteri*	Unknown	All 8 herb. UCB specimens tested were HCN negative.

[3]. Members of subgenus *Acacia*, on the other hand, utilize the aliphatic amino acids leucine, isoleucine and valine to produce a series of cyanogenic compounds, i.e. linamarin, lotaustralin, proacacipetalin, epiproacacipetalin, heterodendrin, proaciberin and 3-hydroxyheterodendrin [for review see 1]. Only very rarely does heterodendrin occur in subgenus *Phyllodineae* [3]. In subgenus *Acacia* the most common cyanogenic glycoside is proacacipetalin; this compound does not appear to occur elsewhere in the plant kingdom.

A phenetic interpretation of these data suggest that subgenus *Aculeiferum* and subgenus *Phyllodineae* are more closely related to each other than either is to subgenus *Acacia*. This particular clustering of subgenera within *Acacia* is supported by other independent lines of evidence. Guinet [22] showed that the pollen of subgenus *Acacia* (colporate apertures and columellar exine) differs markedly from that of subgenus *Aculeiferum* and *Phyllo-*

dineae (porate apertures and a granular exine). Evans *et al.* [14] and Bell and Evans [23] showed that the free amino acids in seeds of subgenus *Acacia* were very different from those found in subgenus *Aculeiferum* and subgenus *Phyllodineae* and that the biochemical distinctions between the latter two subgenera were minor, perhaps involving only one or a few genes. Guinet and Vassal [12] analysed data from pollen, seed/seedlings, chromosomes and gross morphology and again showed that subgenus *Aculeiferum* and subgenus *Phyllodineae* were related and that subgenus *Acacia* was distinct. A diagrammatic representation of these subgeneric affinities is given in Fig. 2 of Pedley [9].

Thus, a number of lines of evidence, including cyanogenic glycosides, support the division of *Acacia* into two groups, namely, subgenus *Acacia* vs subgenus *Aculeiferum Phyllodineae*. In 1981, Pedley [19] had suggested this two-way division of *Acacia*, but five years later [9] he effected a three-way split of the genus by recognizing *Acacia* (= subgenus *Acacia*), *Senegalia* (= subgenus *Aculeiferum*) and *Racosperma* (= subgenus *Phyllodineae*). Using evidence derived from cyanogenic glycosides as well as from other sources (noted above), it would seem that of the two alternatives, the former one where two genera are recognized would be the more acceptable. However, we are not advocating a split of *Acacia* at the present time because we feel that any taxonomic re-organization of the genus should only be undertaken in the context of a broader study of genera considered closely related to *Acacia*. According to Guinet [24] these would include genera of the tribe Ingeae (*Calliandra sens. str.* excluded) and those of the *Piptadenia* group in the tribe Mimoseae. As far as cyanogenic glycosides are concerned, Seigler *et al.* [4] noted that none have been characterized for any Mimosoid legumes other than *Acacia*. Future work should aim at identifying the cyanogens within genera of Mimosoideae (especially the tribe Ingeae) which will permit us to determine whether the synthesis and accumulation of sambunigrin and prunasin is a shared derived feature. If so, this will establish a close evolutionary relationship between subgenus *Aculeiferum* and subgenus *Phyllodineae*.

EXPERIMENTAL

Herbarium survey. Specimens examined in this survey are located at the herbarium of the University of California at Berkeley (herb. UCB). A list of the species examined, together with the institution number of the specimen is available on request to EEC. When multiple specimens of the same species exist, material from more than one specimen was taken for testing for cyanogenesis.

Plant material. Leaf material of *A. caffra* and *A. hereroensis* collected in South Africa was air-dried before shipment by air to the United States for extraction and purification of the cyanogens. Vouchers of these two species are the following: *A. caffra*: Ben-Erik van Wyk 2044 (JRAU); *A. hereroensis*: H. Kolberg s.n., 5 Aug. 1986 (PRE).

Procedures. Herbarium material was tested for the presence of cyanogenic glycosides as follows: small samples (10–25 mg) of foliage were removed from herbarium specimens and ground in a small glass vial. A few drops of an enzyme mixture consisting of almond emulsin (0.05%) (Sigma Chemical No. G-8625) and flax seed linamarase [25] (0.1 units per ml) in 0.1 M Pi buffer, pH 5.5 were then added to the homogenate and Feigl–Anger test papers [26] were employed to detect any HCN released.

The cyanogenic glycosides in the bulk leaf material were extracted and purified as described previously [2]. The cyanogens were identified as sambunigrin and prunasin by comparison of their ^{1}H NMR spectra with authentic compounds. The relative amounts of the two epimers were determined from integration of the absorption peaks for the cyanohydrin hydrogen (1H, *s*) at δ6.07 (sambunigrin) and at δ5.93 (prunasin) in Me_2CO-d_6.

Acknowledgements—Dr Jim Ross (herb. MEL) is thanked for checking the identity of African herb. UCB specimens which gave a positive test for cyanogenesis. We are particularly indebted to Dr Ben-Erik van Wyk for collecting bulk material of *A. caffra* and H. Kolberg for collecting bulk material of *A. hereroensis*. Dr Steve Hopper is thanked for constructive criticism on parts of the manuscript. Supported by the U.S.–Australia Cooperative Science Program, Division of International Programs, National Science Foundation and NSF Grants PCM-81-04497 (E. E. Conn) and BSR-82-15274 (D. S. Seigler).

REFERENCES

1. Seigler, D. S. and Conn, E. E. (1982) *Bull. Int. Group Study Mimosoideae* **10**, 32.
2. Maslin, B. R., Conn, E. E. and Dunn, J. E. (1985) *Phytochemistry* **24**, 961.
3. Maslin, B. R., Dunn, J. E. and Conn, E. E. (1988) *Phytochemistry* **27**, 421.
4. Seigler, D. S., Maslin, B. R. and Conn, E. E. (1989). in *Advances in Legume Biology* (Stirton, C. H. and Zarucchi, J. L., eds) Monographs in Systematic Botany, Missouri Botanical Garden, St. Louis, MO (in press).
5. Vassal, J. (1972) *Bull. Soc. Hist. Nat. Toulouse* **108**, 125.
6. Bentham, G. (1875) *Trans. Linn. Soc., London* **30**, 335.
7. Bentham, G. (1842) *London J. Botany* **1**, 318.
8. Pedley, L. (1978) *Austrobaileya* **1**, 75.
9. Pedley, L. (1986) *Bot. J. Linn. Soc.* **92**, 219.
10. Ross, J. H. (1979) *Mem. Bot. Survey S. Africa* **44**, 1.
11. Vassal, J. (1986) *Candollea* **41**, 113.
12. Guinet, Ph. and Vassal, J. (1978) *Kew Bull.* **32**, 509.
13. Ross, J. H. (1981) *Bothalia* **13**, 389.
14. Evans, C. S., Qureshi, M. Y. and Bell, E. A. (1977) *Phytochemistry* **16**, 565.
15. Robbertse, P. J. and Schijff, H. P. van der (1971) *Mitt. Bot. Staatssamml. Munchen* **10**, 170.
16. Macbride, J. F. (1943) *Field Mus. Nat. Hist., Bot. Ser.* **13** (3), 78.
17. Vassal, J. (1981) in *Advances in Legume Systematics*, Part 1 (Polhill, R. M. and Raven, P. H., eds), p. 169. Royal Botanic Gardens, Kew, England.
18. Guinet, Ph. (1969) *Inst. Fr. Pondichery, Trav. Sec. Sci. Tech.* **9**, 1.
19. Pedley, L. (1981) *Bull. Int. Group Study Mimosoideae* **9**, 42.
20. Pedley, L. (1983) *Bull. Int. Group Study Mimosoideae* **11**, 29.
21. Maslin, B. R. (1987) *Bull. Int. Group Study Mimosoideae* **15**, 108.
22. Guinet, Ph. (1981) in *Advances in Legume Systematics*, Part 2 (Polhill, R. M. and Raven, P. H., eds), p. 835. Royal Botanic Gardens, Kew, England.
23. Bell, E. A. and Evans, C. S. (1978) *Nature* **273**, 295.
24. Guinet, Ph. (in prep.) The genus *Acacia* (Leguminosae: Mimosoideae): its affinities as borne out by its pollen characters [to be given at IBC, Berlin].
25. Coop, I. E. (1940) *N. Z. J. Sci. Tech.* **22B**, 71.
26. Tantisewie, B., Ruijgrok, H. W. L. and Hegnauer, R. (1969) *Pharm. Weekblad.* **104**, 1341.
27. Seigler, D. S. (1976) *Proc. Okla. Acad. Sci.* **56**, 95.
28. Seigler, D. S., Dunn, J. E., Conn, E. E. and Holstein, G. L. (1978) *Phytochemistry* **17**, 445.