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# FREE AMINO ACIDS IN THE SEEDS OF *ACACIA* SPECIES

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**Key Word Index**—*Acacia*; Leguminosae; amino acids; chemotaxonomy; α-amino-β-oxalylaminopropionic acid; neurotoxin.

**Abstract**—Free amino acids in the seeds of 80 species of *Acacia* have been determined. In addition to confirming the known difference in seed amino acid 'patterns' shown by species of the series *Gummiferae* on the one hand and species of the series *Phyllodineae* and *Botryocephalae* on the other, we have found that species of the series *Vulgares* Benth. show two additional 'patterns'. One of these is common to some of the Afro-Asian species of the series which have been analysed and the other is common to species from America and Africa. One of the 'marker' amino acids of the Afro-Asian group is the neurotoxic lathyrogen α-amino-β-oxalylaminopropionic acid. The possible taxonomic and phylogenetic significance of these results is discussed, with special reference to five species *A. albida*, *A. confusa*, *A. heterophylla*, *A. coulteri* and *A. kauaiensis*.

## INTRODUCTION

The genus *Acacia* (Leguminosae, subfamily Mimosoideae) is a large genus containing 850–900 species, approximately 700 of which are native to Australia; the remainder occur mainly in tropical and subtropical regions of Africa, Asia and America.

Seneviratne and Fowden [1] investigated the free amino acids in the seeds of 34 species of the genus. Most of these were Australian species in the series *Phyllodineae* and *Botryocephalae* Benth., the rest were members of the series *Gummiferae* Benth. They found that seeds of species of the *Gummiferae* characteristically contain *N*-acetyldjenkolic acid and its sulphoxide, while those of the series *Phyllodineae* and *Botryocephalae* accumulate large amounts of albizziine, *S*-carboxyethyl cysteine and its sulphoxide. Many species of these last two groups also contain *S*-carboxyisopropyl cysteine, and α-amino-β-acetylaminopropionic acid. Seneviratne and Fowden concluded that "these characteristics are so clearly defined and constant . . . that one would feel justified in assigning a species to the subgenus *Gummiferae* if it possessed *N*-acetyldjenkolic acid".

We have now analysed seeds of another 80 species of *Acacia*, these have been taken mainly from the series *Vulgares* Benth. but include a few additional representatives of the series *Phyllodineae*, *Botryocephalae*, *Pulchellae* and *Gummiferae*. We report that two further groups of *Acacia* species are characterized by the associations of free amino acids which they accumulate in their seeds. We have attempted to correlate the four biochemically defined sub-genera with the morphological classifications of Bentham [2] and Vassal [3].

## RESULTS AND DISCUSSION

Table 1 shows the distribution of free amino acids in the seeds of 106 species of *Acacia* including 34 previously analysed by Seneviratne and Fowden (marked with an asterisk). Species of Group 1 contain the amino acid N-acetyldjenkolic acid as the 'marker' amino acid. All of these species were classified by Bentham in the series *Gummiferae*, and are included by Vassal in the subgenus *Acacia*. Species of Group 2 contain the combination of 'marker' amino acids *S*-carboxyethylcysteine, *S*-carboxyisopropylcysteine, albizziine and α-amino-β-acetylaminopropionic acid. All species within this group are Australian, except for *A. heterophylla*, and occur in Bentham's series *Phyllodineae*, *Botryocephalae*, and *Pulchellae*, and in Vassal's subgenus *Heterophyllum*, sections *Heterophyllum*, *Uninervea*, and *Pulchelloidae*. *A. heterophylla* is found in the Mascarene Islands and is the only non-Australian phyllodinous species studied in this project. The amino acids pattern of the seeds is identical with those of the Australian species, and this evidence of chemical as well as morphological similarity raises the question of a possible Australian origin for *A. heterophylla*.

Species of Group 3 are mainly of African and Asian origin, they were classified by Bentham as series *Vulgares*, and are placed by Vassal in the subgenus *Aculeiferum* section *Aculeiferum*. *A. ataxacantha* is classified in section *Monacanthaea* by Vassal. Geographical exceptions in this group are *A. coulteri* from Mexico and *A. confusa* from the Pacific Islands and Taiwan. Bentham classified *A. coulteri* as *Vulgares* but Vassal places it in the subgenus *Acacia*. *Acacia confusa* was placed in the series *Phyllodineae* by Bentham while Vassal regards it as a member of the subgenus *Heterophyllum* sect. *Heterophyllum*, subsect. *Globiferae*. Species of Group 3 contain the same 'marker' amino acids as those of Group 2 together with α-amino-β-oxalylaminopropionic acid (the neurolathyrogen previously identified in 24 species of *Lathyrus* [4] and 13 species of *Crotalaria* [5]) and free αβ-diaminopropionic acid. The seeds of some of these species also contain 4-*N*-α-amino-γ-oxalylaminobutyric acid which also occurs in *Lathyrus*

Table 1. Subdivisions of the genus *Acacia* based on seed amino

| Vassal's classification | Bentham's classification | Species | *S*-carboxyethylcysteine | *S*-carboxyethylcysteine sulphoxide | *S*-carboxyisopropylcysteine | α-amino-β-acetylamino-propionic acid |
|---|---|---|---|---|---|---|
| | | | (a) | (b) | (c) | (d) |
| Subgenus *ACACIA* | Series *GUMMIFERAE* | [*A arabica* (Lam.) Willd.] = *A. nilotica* | | | | |
| | | **A. bidwillii* Benth. | | | | |
| | | **A. farnesiana* (L.) Willd. | | | | |
| | | *A. gerrardii* Benth. | | | | |
| | | [*A giraffae* Willd.] = *A. erioloba* E. Mey. | | | | |
| | | **A. grandicornuta* Gerstner | | | | |
| | | **A. karroo* Hayne | | | | |
| | | *A. nilotica* (L.) [Willd. ex.] Del. subsp. *kraussiana* (Benth.) Brenan | | | | |
| | | **A. robusta* Burch. | | | | |
| | | *A. sieberana* DC. var. *woodii* (Burtt-Davy) Keay & Brenan | | | | |
| | | *A. stuhlmannii* Taub. | | | | |
| | | **A. suberosa* A. Cunn. ex Benth. | | | | |
| | | **A. tortilis* (Forsk.) Hayne ssp. *heterocantha* (Burch.) Brenan | | | | |
| | Series *PHYLLODINEAE* | | (a) | (b) | (c) | (d) |
| Subgenus *HETEROPHYLLUM* | Subseries ii. *Continuae* | *A. peuce* F. Muell. | + + | | + | + + |
| Sections *Heterophyllum* | iii. *Pungentes* | *A. oxycedrus* Sieber ex DC. | + + | | + | + |
| and *Uninervea* | iv. *Calamifolia* | **A. calamifolia* Sweet ex Lindl. | + + | | + | + + |
| | | **A. rigens* A. Cunn. ex G. Don | | | | + + |
| | vi. *Uninerves* | **A. armata* R. Br. | + + + | + + | + + | + + + |
| | | **A. brachybotrya* Benth. | + + + | + + | + | + |
| | | *A. caesiella* Maiden et Blakely | + + | | + | + + |
| | | **A. cultriformis* A. Cunn. ex G. Don | + + + | + + | + + | + + |
| | | **A. gladiiformis* A. Cunn. ex Benth. | + + + | + + | + + | + + |
| | | **A. hakeoides* A. Cunn. ex Benth. | + + + | + + | + | + + |
| | | **A. lineata* A. Cunn. ex G. Don | + + | + + | + | + |
| | | *A. meissneri* Lehm. ex Meisn. | + + | + + | | + |
| | | **A. montana* Benth. | + + + | + + | + + | + |
| | | **A. podalyriifolia* A. Cunn. ex G. Don | + + + | + + | + + | + + + |
| | | *A. prunocarpa* Tindale | + | | | + |
| | | **A. pycnantha* Benth. | + + | + + | + | + + |
| | | *A. pyrifolia* DC. | + + | + | + | + |
| | | **A. retinodes* Schlecht. | + + + | + | + | + + |
| | | **A. salicina* Lindl. | + | + | + | + |
| | | *A. stricta* (Andr.) Willd. | + + | | + | + + |
| | | *A. suaveolens* (Sm.) Willd. | + + + | + | + + | + + |
| | | *A. verniciflua* A. Cunn. | + + + | + | + + | + + |
| | | *A. victoriae* Benth. | + + | | + | + + |
| | | **A. wattsiana* F. Muell. ex Benth. | + + | + + | + | + |
| | vii. *Plurinerves* | *A. binervata* DC. | + + | | + | + |
| | | *A. bivenosa* DC. | + | + | + | + + |
| | | **A. cyclops* A. Cunn. ex G. Don | + | + + | + | + + |
| | | **A. georginae* F. M. Bail. | | | | + |
| | | *A. heterophylla* (Lam.) Willd. | + + + | + | + | |
| | | **A. longifolia* (Andr.) Willd. var. *longifolia* | + + | + | + + | + |
| | | *A. melanoxylon* R. Br. | + + | + | + + | + + |
| | | *A. monticola* J. M. Black | + | + | + | + |
| | | **A. osswaldii* F. Muell. | | + | + | + |
| | | **A. stenophylla* A. Cunn. ex Benth. | | + | | + + |
| | viii. *Juliflorae* | **A. aneura* F. Muell. ex. Benth. | + + + | + | + + | + |
| | | *A. auriculiformis* A. Cunn. | + + | + | | + |
| | | *A. circinnata* F. Muell. | + + + | | + + | + + |
| | | *A. clivicola* Pedley | + + + | | + | + + |
| | | *A. holosericea* A. Cunn. ex G. Don | + + + | + | + | + + |
| | | *A. tenuissima* F. Muell. | + | + | | + |
| | | *A. tumida* F. Muell. | + + | | | + + |
| | *BIPINNATAE* | | | | | |
| | Series ix. *BOTRYOCEPHALAE* | **A. baileyana* F. Muell. | + + + | + + | + + | + |
| | | **A. dealbata* Link | + + + | + + | + + | + + |
| | | **A. decurrens* (J. Wendl.) Willd. | + + | + + | + | + + |
| | | *A. elata* A. Cunn. ex Benth. | + + | | + | + + + |
| | | *A. mearnsii* De Wild. | + | + | | + + |
| | | *A. parramattensis* Tindale | + + + | + | + | + |
| | | *A. polybotrya* Benth. | + + + | | + + | + |
| Subgenus *HETEROPHYLLUM* | Series x. *PULCHELLAE* | *A. browniana* H. Wendle var. browniana | + + + | + | + + | + |
| Section *PULCHELLOIDEA* | | *A. drummondii* Lind. ssp. *elegans* B. R. Maslin | + | + + | + | + |
| | | *A. gilbertii* Meisn. | + + | + | + | + |
| | | *A. lasiocarpa* Benth. var. *sedifolia* (Meisn.) B. R. Maslin | + | + + | + | + |
| | | *A. luteola* B. R. Maslin | + | + + | + | + + |

* Species analysed by Seneviratne and Fowden [1] and Fowden (private communication).

acid patterns compared with subdivisions based on morphology

| Albizzine | Willardine | αβ-diaminopropionic acid | αβ-diaminobutyric acid | α-amino-β-oxalylamino-propionic acid | α-amino-γ-oxalylamino-butyric acid | Unidentified derivative of diaminopropionic acid | Djenkolic acid | Djenkolic acid sulphoxide | *N*-acetyldjenkolic acid | *N*-acetyldjenkolic acid sulphoxide | γ-glutamyldjenkolic acid | Pipecolic acid | 4-OH Pipecolic acid | 5-OH Pipecolic acid | 2,4-*cis*,4,5-*trans*-diOH Pipecolic acid | | |
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| **GROUP 1** | | | | | | | | | | | | | | | | | |
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| **GROUP 2** | | | | | | | | | | | | | | | | | |
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+ = weak; + + = medium; + + + = strong.

Table 1.—

| Vassal's classification | Bentham's classification | Species | *S*-carboxyethylcysteine | *S*-carboxyethylcysteine sulphoxide | *S*-carboxyisopropylcysteine | α-amino-β-acetylaminopropionic acid |
|---|---|---|---|---|---|---|
| | *BIPINNATAE —continued* | | (a) | (b) | (c) | (d) |
| Subgenus *HETEROPHYLLUM* —*continued* | Series *x PULCHELLAE* —*continued* | *A megacephala* B. R Maslin | + | + | | + |
| Section *PULCHELLOIDEA* —*continued* | | *A moirii* E. Pritzel ssp. *moirii* | + | + | + | + |
| | | *A. pentadenia* Lindl | + | + + | + | + |
| | | *A. pulchella* R. Br var *glaberrima* Meisn | + + | + | + | + |
| | | *A. pulchella* R Br. var *pulchella* | + + | + | | |
| | | *A. pulchella* R. Br. var *reflexa* B R. Maslin | + + | + | + | + |
| | | *A. varia* B R. Maslin var *affinis* B. R Maslin | | + + | + | + |
| Subgenus *ACULEIFERUM* | Series *VULGARES* | | (a) | (b) | (c) | (d) |
| Section *Monacanthea* | | *A ataxacantha* DC. | + + + | + | + + + | + + + |
| Section *Aculeiferum* | | *A. catechu* (L.) Willd. | + + + | + + | + | + + + |
| | | *A mellifera* (Vahl) Benth ssp *detinens* (Buch) Brenan | + + + | + | + | + + + |
| | | *A erubescens* [Welw. ex] Oliv | + | | | + + + |
| | | *A. ferruginea* DC. | + + | + | + | + |
| | | *A. galpinii* Burtt-Davy | + + | + | | + + + |
| | | *A. goetzei* Harms ssp goetzei | + + + | + | + | + + |
| | | *A hamulosa* Benth. | + + | | + | + + + |
| | | *A modesta* Wall | + + | + | + | + + |
| | | *A nigrescens* Oliv | + + | + + | + | + + + |
| | | *A polyacantha* Willd ssp. *campylacantha* ([Hochst ex] A Rich) Brenan | + + + | + + | + | + + |
| | | *A rovumae* Oliv | + + | | | |
| | | *A. senegal* (L.) Willd | + + | + | + | + + + |
| | | *A venosa* [Hochst. ex] Benth | + + | + | + | + + + |
| | | *A welwitschii* Oliv. ssp. delagoensis (Harms) Ross & Brenan | + + | | | + + + |
| Subgenus *ACACIA* | | *A coulteri* Gray ex Benth. | + + + | + | + + + | + + |
| Subgenus *HETEROPHYLLUM* | Series *Phyllodineae* vii. *Plurinerves* | *A confusa* Merr | + + | | + + | + + |
| Genus *FAIDHERBIA* | Series *GUMMIFERAE* | *A albida* Delile | | | | + + |
| | | | (a) | (b) | (c) | (d) |
| Subgenus *ACULEIFERUM* Section *Monacanthea* | Series *VULGARES* | *A bonariensis* Gill ex Hook & Arn | | | | |
| | | *A. brevispica* Harms | | | | |
| | | *A. caesia* W & A. | | | | |
| | "pennata" group | *A. kraussiana* Meisn. ex Benth | | | | |
| | | *A schweinfurthii* Brenan & Exell | | | | |
| | | *A pentagona* (Schumach. & Thonn.) Hook. f. | | | | |
| | | *A greggii* Gray | | | | |
| | | *A plumosa* Lowe | | | | |
| | | *A polyphylla* DC. | | | | |
| | | *A. riparia* Kunth | | | | |
| | | *A. roemeriana* Scheele | | | | |
| | | *A. velutina* DC. | | | | + + |
| | | *A. wrightii* Benth. | | | | |
| | | *A. glomerosa* Benth | | | | |

* Species analysed by Seneviratne and Fowden [1] and Fowden (private communication)

species [4]. All the species in this group contained an unidentified derivative of αβ-diaminopropionic acid in their seeds. Many of the species also contained small amounts of 2,4-*cis*-4,5-*trans*-dihydroxypipecolic acid, an imino acid recently isolated from *Derris elliptica* by Marlier *et al.* [6].

The seeds of *A. coulteri* contain all the amino acid markers characteristic of Group 3 and this species appears closely allied chemically to those species in Africa and Asia which were classified as *Vulgares* by Bentham, even though it is a Mexican species. The absence of *N*-acetyldjenkolic acid from *A. coulteri* suggests that it is less closely related to the species in the subgenus *Acacia* (sensu Vassal), than to the species in the subgenus *Aculei ferum*.

The seeds of *A. confusa*, a species which is found in the Pacific Islands and Taiwan, contain all the 'marker' amino acids of Group 3. The identity of its seed amino acid pattern with the patterns found in the seeds of African and Asian species suggests that the island species originated in Asia rather than Australia.

The seeds of *A. albida* contain the 'marker' amino acids of Group 3 with the exception of *S*-carboxyethylcysteine, though this is present in small amounts in the leaves (unpublished observation). The seeds do not contain *N*-acetyldjenkolic acid, the 'marker' amino acid of Group 1 which characterizes species in Bentham's series *Gummiferae*. Bentham included *A. albida* in the *Gummiferae*, while Vassal has placed it in a separate genus *Faidherbia*. We consider that the amino acid pattern in the seeds of *A. albida* is not sufficiently different from other species of Group 3 to warrant its exclusion

*continued*

| Albizziine | Willardine | αβ-diaminopropionic acid | αβ-diaminobutyric acid | α-amino-β-oxalyamino-propionic acid | α-amino-γ-oxalyamino-butyric acid | Unidentified derivative of diaminopropionic acid | Djenkolic acid | Djenkolic acid sulphoxide | *N*-acetyldjenkolic acid | *N*-acetyldjenkolic acid sulphoxide | γ-glutamyldjenkolic acid | Pipecolic acid | 4-OH Pipecolic acid | 5-OH Pipecolic acid | 2,4-*cis*,4,5-*trans*-diOH Pipecolic acid | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | GROUP 2—*continued* | | | | | | | | | | |
| (e) | (f) | (g) | (h) | (i) | (j) | (k) | (l) | (m) | (n) | (o) | (p) | (q) | (r) | (s) | (t) | (u) | (v) |
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| | | | | | | | GROUP 3 | | | | | | | | | | |
| (e) | (f) | (g) | (h) | (i) | (j) | (k) | (l) | (m) | (n) | (o) | (p) | (q) | (r) | (s) | (t) | (u) | (v) |
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| +++ | | + | | ++ | | | + | | | | | | + | + | | | |
| | | | | | | | GROUP 4 | | | | | | | | | Unknown | |
| | | | | | | | | | | | | | | | | 1 | 2 |
| (e) | (f) | (g) | (h) | (i) | (j) | (k) | (l) | (m) | (n) | (o) | (p) | (q) | (r) | (s) | (t) | (u) | (v) |
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| | | | | | | | + | | | | + | | | | | + | + |
| | | | | | | | | | | | | | | | | | + |

+ = weak; ++ = medium; +++ = strong.

from this group (which contains species classified by Bentham as *Vulgares*, and by Vassal as subgenus *Aculeiferum*, section *Aculeiferum*).

The species listed in Group 4 occur in Africa and in south and central America, they are classified by Bentham as *Vulgares* and by Vassal as subgenus *Aculeiferum*, sect. *Monacanthaea*. Free amino acids do not accumulate in high concentrations in the seeds of Group 4 species but two unidentified amino acids occur in several species of the Group. One of these (unknown 1, Table 1) is common to *Acacia* species of the 'pennata' group. The seeds of *A. velutina* are exceptional in containing significant concentrations of α-amino-β-acetylaminopropionic acid, while those of *A. riparia* Kunth. contain γ-glutamyldjenkolic acid and both the 'unknowns'. Trace amounts of djenkolic acid were present in a few species in the group.

To summarize, the data presented in Table 1 show that the species placed by Bentham in the *Gummiferae* and by Vassal in his subgenus *Acacia* are biochemically as well as morphologically distinct from all other species of the genus. The data also indicate that the morphologically distinct groups from Australia (treated by Bentham as *Phyllodineae*, *Botryocephalae* and *Pulchellae*, and by Vassal as sections *Heterophyllum*, *Uninervea* and *Pulchelloidea* of the subgenus *Heterophyllum*) are nevertheless related to each other and distinguished from non-Australian species by their common amino acid biochemistry. The species *A. heterophylla* from the Mascarene Islands has an identical amino acid pattern to the Australian species. The distinctive 'patterns' given by species of Groups 3 and 4 support the view that those species which were placed together by Bentham in his series *Vulgares* should more properly be separated into

two groups of the type which Vassal has proposed (subgenus *Aculeiferum*, section *Aculeiferum* and subgenus *Aculeiferum*, sect. *Monacanthea*).

The presence of oxalylamino acids in and the absence of acetyldjenkolic acid from the seeds of *A. coulteri* supports Bentham's opinion that this species is related to other species in his series *Vulgares*. The 'pattern' is in fact identical with the pattern given by species which Vassal has placed in section *Aculeiferum*, sub genus *Aculeiferum* (which Bentham included in his series *Vulgares*). In respect of the amino acids in its seeds it does not resemble species of Vassal's subgenus *Acacia* [*Gummiferae* (Benth.)]. The same oxalylamino acid pattern in the seeds of *A. confusa* suggests that this Pacific Island species originated from Asia rather than from Australia, no Australian species containing oxalylamino acids having been found.

## EXPERIMENTAL

*Paper ionophoresis.* 200 mg finely ground seed was shaken with 70% EtOH (1 ml) for 24 hr. After standing for a further 17 hr the suspension was centrifuged and subjected to ionophoresis on Whatman 3MM paper (70 V/cm for 30 min) in buffer solns of pH 1.9 and 3.6 [7].

*2D-Paper chromatography.* Supernatant soln (0.5 ml) prepared as above was passed through a column (5 × 1 cm) of cation exchange resin (Dowex 50W × 8) in the $H^+$ form. After washing with $H_2O$ amino acids were displaced from the column with 2N $NH_4OH$ (20 ml) The ammoniacal soln was evaporated to dryness and residue redissolved in 70% EtOH (0.5 ml). The soln of mixed amino acids (0.01 ml) was chromatographed on Whatman No. 1 paper using the ascending method. Solvents used were *n*-BuOH–HOAc–$H_2O$ (12:3:5) followed by PhOH–$H_2O$ (4:1, w/v) in the presence of $NH_3$ [8].

*Development of papers.* All papers were developed with ninhydrin (0.2% w/v in 95% aq. $Me_2CO$). Ehrlich's reagent [8] was used as a second reagent to confirm the presence of α,β-diaminopropionic acid and α,γ-diaminobutyric acid.

*Identification of amino acids.* Amino acids were identified from their $R_f$ values and ionic mobilities and by co-chromatography with authentic compounds. Oxalylamino acids were isolated by elution from ionophoresis papers, hydrolysed and products identified as previously described [9]. αβ-Diaminopropionic acid and α,γ-diaminobutryic acid gave characteristic colour changes (purple to green and brown-purple to blue-green) when papers were developed successively with ninhydrin and Ehrlich's reagent.

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*Note added in proof* Recently analysed seeds of the following Australian species.

*A. alata*, R. Br., *A. drewiana* W. V. Fitzg. ssp *drewiana*., *A. empelioclada* B. R. Maslin., *A. insolita* E. Pritzel., *A. lateriticola* B. R. Maslin., *A. leioderma* B. R. Maslin and *A. myrtifolia* Wild, gave the characteristic Australian pattern of Group 2.

The seeds of the Hawaiian species *A. kauaiensis* Hbd. contained α-amino-β-oxalylaminopropionic acid characteristic of Group 3 suggesting that this species like the other Pacific island species *A confusa* is of Asiatic rather than Australian origin.

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