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'UNCOMMON' AMINO ACIDS IN THE SEEDS OF 64 SPECIES OF CAESALPINIEAE

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Abstract—Characteristic associations of free amino acids occur in the seeds of various groups of species within the Caesalpinieae. *Guilandina* species are distinctive in accumulating 4-ethylideneglutamic acid in their seeds, *Gymnocladus* and *Gleditzia* species in accumulating isomers of 3-hydroxy-4-methylglutamic acid, *Bussea* species in accumulating azetidine-2-carboxylic acid, *Peltophorum* species in accumulating a previously undescribed imino acid tentatively identified as a derivative of 4-hydroxypipecolic acid.

INTRODUCTION

Many species of the Leguminosae contain 'uncommon' amino acids in their seeds and the accumulation of one or more of these compounds may be characteristic of a particular species or group of species, usually a genus or sub-genus [1]. In 1972 Nulu and Bell [2] reported the presence of γ-methylglutamic acid, γ-methyleneglutamic acid and γ-ethylideneglutamic acid in seeds of *Guilandina crista* and the following year Watson and Fowden [3] isolated two previously unknown aromatic amino acids 3-hydroxymethylphenylalanine and 4-hydroxy-3-hydroxymethylphenylalanine from the seeds of *Caesalpinia tinctoria*. These two amino acids were only detected in *C. tinctoria* but Watson and Fowden found five substituted glutamic acids (including those previously found in *Guilandina crista*) and six imino acids in the seeds of various species of this and 11 related genera which are placed together according to Hutchinson's classification [4] in group 5 of the subfamily Caesalpinioideae. The presence of azetidine-2-carboxylic acid as the "principal component of the soluble nitrogen fraction" in the seed of the one species of *Bussea* analysed (*B. massaiensis*) was one of the most interesting of Watson and Fowden's findings. The same imino acid was detected as a minor component in seeds of *Parkinsonia aculeata* and in higher concentrations in the seedlings of this and other species.

The acquisition of other species of the same group including seven additional species of *Guilandina* led us to make a wider survey. Hutchinson [4] regards species of *Guilandina* as members of *Caesalpinia* and not as members of a separate genus, whereas Gillis and Proctor [5] prefer to treat *Guilandina* as a subgenus of *Caesalpinia* on morphological grounds.

RESULTS AND DISCUSSION

The results of the survey are set out in Table 1. It will be seen that with one exception seeds of all *Guilandina* species are characterized by the presence of high concentrations of γ-ethylideneglutamic acid, some species also contained γ-methylglutamic and γ-methyleneglutamic acids. The only species of the subgenus *Guilandina* whose seeds contained none of the γ-substituted glutamic acids was *C. solomonensis*. This species along with *C. spinosa* and *C. nuga* contained high concentrations of an unidentified neutral amino acid which gave a grey-purple colour with ninhydrin; *C. spinosa* also contained large amounts of baikiain, which was also found by Watson and Fowden [3] in *C. tinctoria*. Seeds of several species of the genus *Caesalpinia* contained pipecolic acid, frequently as the only non-protein amino acid detected. However, in seeds of *C. palmeri*, γ-methylglutamic acid was found although this species does not belong to the subgenus *Guilandina*.

The six species of *Mezoneuron* and three species of *Pterolobium* analysed were lacking in distinctive accumulations of amino acids though pipecolic acid occurred in some.

Some species of the genera *Hoffmannseggia*, *Cordeauxia*, *Haematoxylon* and *Schizolobium* accumulated γ-methyleneglutamic acid in their seeds, while seeds of *Wagatea spicata* contained γ-ethylglutamic acid. The seeds of 3 species of *Bussea* contained high concentrations of azetidine-2-carboxylic acid, found in *Bussea massaiensis* by Watson and Fowden [3]. The seeds of no other legume species (from 300 genera analysed to date) have been found to accumulate this imino acid and the presence of high concentrations of this imino acid may be a unique characteristic of *Bussea* seeds.

The three species of *Gymnocladus* all contained high concentrations of (2*S*,3*S*,4*R*)-3-hydroxy-4-methylglutamic acid and lower concentrations of (2*S*,3*R*,4*S*)-3-hydroxy-4-methylglutamic acid, first reported in *Gymnocladus dioicus* by Dardenne *et al.* [6–8]. Seeds of *Gymnocladus burmanicus* contained in addition an unidentified neutral amino acid which gave a similar ninhydrin colour reaction to the hydroxymethylglutamic acids. All *Gymnocladus* species contained pipecolic acid, and two of them *G. dioicus* and *G. chinensis* contained both the *trans* and *cis* isomers of 5-hydroxypipecolic acid

Table 1. The non-protein amino acids in the seeds of Caesalpinieae species

	γ-Ethylideneglutamic acid (a)	γ-Methylglutamic acid (b)	γ-Methyleneglutamic acid (c)	γ-Ethylglutamic acid (d)	Pipecolic acid (e)	4-Hydroxypipecolic acid (f)	5-Hydroxypipecolic acid, *trans* isomer (g)	5-Hydroxypipecolic acid, *cis* isomer (h)	Baikain (i)	Azetidine-2-carboxylic acid (j)	3-Hydroxy-4-methylglutamic acid G-1 (k)	3-Hydroxy-4-methylglutamic acid G-2 (l)	Unknown glutamic acid derivative (m)	3-Hydroxyproline (n)	Unknown neutral amino acid (o)	Derivative of 4-hydroxy-pipecolic acid (p)
CAESALPINIA																
C. (Guilandina) grisebachiana O Kuntze	+++	++	++													
C (Guilandina) bonduc (L) Roxb. (*Guilandina crista* auct)	++	+++	+		+											
C (Guilandina) divergens Urban	+++	++			++											
C (Guilandina) glaucophylla Urban	+++	+	+		+											
C (Guilandina) major (Medic) Dandy and Exell	++	+++														
C (Guilandina) ovalifolia Urban	++				++											
C (Guilandina) ciliata Urban	+++				+											
C (Guilandina) melanosperma Urban	+++				++											
C (Guilandina) minax Hance	++				++		++									
C (Guilandina) portoricensis (Britton & Wilson) Alain	+++															
C (Guilandina) volkensii Harms	+++				+++		++									
C (Guilandina) solomonensis Hattink															++	
C palmeri S Watson			+++		++											
C spinosa (Molina) O. Kuntze					++	++			+++						+++	
C crista L (*C nuga* (L) Ait)					++										+++	
C gilliesii (Hook) Dietr					+		+									
C decapetala (Roth) Alston (*C sepiaria* Roxb)					++											
C sinensis (Hemsl) Vidal																
C. sappan L																
C coriaria (Jacq.) Willd																
C digyna Rottl.					+											
C paraguariensis (Parodi) Burkart					+											
C pulcherrima (L.) Swartz																
C trothae Harms																
C welwitschiana (Oliv) Brenan																
MEZONEURON																
M kauaiense (Mann) Hillebrand					+											
M andamanicum Prain					+											
M sumatranum (Roxb) Wight & Arn																
M benthamianum Baill																
M angotense Oliv																
PTEROLOBIUM																
P stellatum (Forssk) Brenan					+											
P microphyllum Miq																
P. micranthum Gagnep																
HOFFMANNSEGGIA																
H jamesii Torrey & Gray																
H burchellii (DC) Benth.			++		+											
CORDEAUXIA																
C. edulis Hemsl			++													
HAEMATOXYLUM																
Haematoxylum sp.		++	++													
SCHIZOLOBIUM																
S parahybum (Vell.) Blake (*S excelsum* Vog.)			+													
GYMNOCLADUS																
G dioicus (L) Koch					+		++	+			+++	+				
G chinensis Baill					+		+	+			+++	++				
G burmanicus C E Parkinson					+		+				++	+	+++			
GLEDITSIA																
G amorphoides (Griseb) Taub					++		++	++			+					
G triacanthos L forma *inermis* (L) Zab					++		++	+				+				
G macracantha Desf					++		++	++				+				
G. ferox Desf					++		++	+				+				
DELONIX																
D regia (Hook) Raf.														++		
D. elata (L) Gamble																
PARKINSONIA																
P. aculeata L																
P africana Sond.					+	++										
COLVILLEA																
C racemosa Hook														+		
BUSSEA																
B massaiensis (Taub.) Harms					+	++	++			+++				++		
B occidentalis Hutch		+								+++				++		
B goswelleri Bak f					+	+	++			+++				++		
CERCIDIUM																
C floridum Gray					+	++										
C microphyllum (Torrey) I. Johnston																
WAGATEA																
W spicata (Dalz) Wight				++												
PACHYELASMA																
P tessmannii Harms					+	+										
STACHYOTHYRSUS																
S staudtii Harms																
PELTOPHORUM																
P africanum Sond						+										+++
P. dubium (Spreng.) Taub						+										+++
P ferrugineum Benth						+										+++
P inerme (Roxb.) Naves						+										+++
P pterocarpum (DC) Heyne						+										+++

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

recently reported by Despontin *et al.* [9]. Both isomers of 5-hydroxypipecolic acid were also found in seeds of 4 species of the genus *Gleditsia* and trace amounts of the 3-hydroxy-4-methylglutamic acids previously found in *Gymnocladus* species were also present.

An unusual and possibly unique chemical character was found in the seeds of *Peltophorum*. All species were found to accumulate a strongly acidic ninhydrin-reacting compound which has been found in no other plant species. The isolation and characterisation of this compound will be described later but is tentatively identified as a phosphate ester of 4-hydroxypipecolic acid.

EXPERIMENTAL

Paper ionophoresis. Finely ground seed (200 mg) was shaken with 1 ml 70% EtOH for 24 hr. The suspension was allowed to stand for 1 hr and 0.01 ml of the supernatant was subjected to ionophoresis on Whatman 3 mm paper (70 V/cm for 30 min) in buffer solutions of pH 1.9 and 3.6 [10].

2D paper chromatography. 0.5 ml of supernatant solution 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 water the amino acids were displaced from the column with 20 ml 2N NH_3. The ammoniacal solution was evaporated to dryness and the residue redissolved in 0.5 ml 70% EtOH. 0.01 ml of this solution 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. All papers were developed with ninhydrin (0.2% w/v in 95% aqueous acetone). Amino acids were identified from their R_f values and ionic mobilities and by co-chromatography with authentic compounds.

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REFERENCES

1. Bell, E. A. (1966) in *Comparative Phytochemistry* (Swain, T. ed.) pp. 195–209. Academic Press, London.
2. Nulu, J. R. and Bell, E. A. (1972) *Phytochemistry* **11**, 2573.
3. Watson, R. and Fowden, L. (1973) *Phytochemistry* **12**, 617.
4. Hutchinson, J. (1964) *The Genera of Flowering Plants.* Clarendon Press, Oxford.
5. Gillis, W. T. and Proctor, G. R. (1974) *J. Arnold Arbor.* **55**, 425.
6. Dardenne, G. A., Casimir, J., Bell, E. A. and Nulu, J. R. (1972) *Phytochemistry* **11**, 787.
7. Dardenne, G. A., Bell, E. A., Nulu, J. R. and Cone, C. (1972) *Phytochemistry* **11**, 791.
8. Evrard, G., Durant, F. and Dardenne, G. (1974) *Crist. Struct. Commun.* **3**, 65.
9. Despontin, J., Marlier, M. and Dardenne, G. (1977) *Phytochemistry* **16**, 387.
10. Bell, E. A. and Tirimanna, A. S. L. (1965) *Biochem. J.* **97**, 104.