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FREE AMINO ACIDS IN *CROTALARIA* SEEDS

DAVID J. PILBEAM* and E. ARTHUR BELL

Department of Plant Sciences, University of London, King's College, 68 Half Moon Lane, London, SE24 9JF, U.K.

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Key Word Index—*Crotalaria*; Leguminosae; chemotaxonomy; free amino acids; pyrrolizidine alkaloids; seeds.

Abstract—The free amino acids of seeds of 163 species of *Crotalaria* have been identified. Their pattern of distribution is compared with recent classifications of the genus and the possible ecological significance of their presence is discussed. Attention is drawn to the occurrence of toxic amino acids.

INTRODUCTION

The genus *Crotalaria* L. (Leguminosae) comprises *ca* 500 species. These occur in tropical and subtropical areas throughout the world, but the majority of species are found in Africa. The genus has recently been studied extensively by Milne-Redhead and then Polhill, resulting in the characterisation of 432 African species, and the arrangement of these into 11 natural sections [1–3]. This classification replaces the earlier work of Bentham, J. G. Baker, E. G. Baker and Wilczek [4–7], and has been modified by Bisby and Polhill to give 8 natural sections [8].

Although a few *Crotalaria* species are part of the human diet in some parts of the world, many species are known to be toxic to man and livestock. *C. aridicola* Domin. causes oesophageal disease of horses [9], while *C. barkae* Schweinf. (*C. zimmermannii* Bak. f.) is a cattle poison [10]. *C. berteroana* DC. (*C. fulva* Roxb.) may be a component of bush-teas which cause veno-occlusive disease of humans in Jamaica [11]. *C. burkeana* Benth. causes crotalariosis in cattle; the seeds are toxic to sheep [10, 12]. *C. dura* Wood & Evans causes jaagsiekte in horses; sheep are also affected [10, 12]. The plants of *C. juncea* L. are toxic to horses and the seeds have been shown to be toxic to sheep [10]. *C. pallida* Ait. is toxic to goats, and probably cattle and sheep also; the seeds are toxic to fowl [10, 12, 13]. Ingestion of *C. retusa* L. plants caused Kimberley horse disease, and the seeds are highly toxic to chickens and pigs [14, 15]. *C. rhodesiae* Bak. f. is toxic to sheep, and probably cattle also [10]. Plants and pods of *C. sagittalis* L. caused Missouri bottom disease of horses [13], while *C. spectabilis* Roth caused severe losses of cattle, horses, fowl and swine in U.S.A. [13]. In addition to these species, many others have been suspected of being toxic.

Toxicity has been shown to be due to the presence of pyrrolizidine alkaloids in *Crotalaria* plants and seeds in many cases, and the alkaloids of *ca* 45 *Crotalaria* species have been analysed. In more than 30 of these species the alkaloids of the seeds have been identified. *Crotalaria* seeds, in common with seeds of many other legumes, contain free amino acids in high concentration, yet despite the importance of amino acids as possible toxins, potential nutrients or pyrrolizidine alkaloid precursors, little work has been carried out on their distribution in the genus.

Bell noted the presence of the neurotoxin α-amino-β-oxalylaminopropionic acid in seeds of *C. incana* L. and *C. pallida* Ait. [16], and the presence of this amino acid in seeds of an additional 11 species was later reported [17]. α-Amino-β-oxalylaminopropionic acid is common to those *Lathyrus* species which cause classical neurolathyrism [18, 19], and the 13 *Crotalaria* species which contain the compound were recognised as potential causes of this disease. The isolation of δ-hydroxynorleucine from seeds of *C. juncea* L. [20], and its identification in seeds of *C. tetragona* Roxb. [21] have been followed by its identification in seeds of a further 9 *Crotalaria* species [22]. The amino acid isowillardiine has been isolated from seeds of *C. ochroleuca* G. Don [23].

We have investigated the free amino acid content of seeds of 163 species of *Crotalaria*, and we find that a classification of the genus based on the distribution of these free amino acids mirrors the classification of the genus proposed by Polhill and Bisby [2, 3, 8]. Predictions about the occurrence of toxic amino acids in species not examined in this study are made on the basis of their position in the scheme drawn up by these 2 workers. In addition to the amino acids already identified in the genus α-amino-β-acetylaminopropionic acid, α-amino-γ-oxalylaminobutyric acid, α-oxalylamino-γ-aminobutyric acid, α-oxalylamino-β-aminopropionic acid, α,γ-diaminobutyric acid, γ-glutamyltyrosine and pipecolic acid were detected. The level of alkaloids in seeds of 85 species was also recorded.

RESULTS AND DISCUSSION

Overall amino acid patterns in the genus

The free amino acids present in *Crotalaria* seeds are shown in Tables 1 and 2. Seed extracts were first subjected to high voltage paper ionophoresis at pH 1.9 and 3.6, and where samples were large enough 2D paper chromatography was carried out. Table 1 lists those

* Present address: Department of Plant Sciences, Baines Wing, University of Leeds, Leeds, LS2 9JT, U.K.

Table 1. Free amino acids and alkaloids in *Crotalaria* seeds

	ODAP	ODAB	A3	ADAP	DAB	Glu tyr	Isowill	Nle(5OH)	Pip	Asp	Glu	Ser	Gly	Asn	Ala	Val	Leu/Ileu	Am but	Arg	Thr	Tyr	Pro	Alkaloid		Size (g)	Country of origin
Section *Grandiflorae* (Bak. f.) Polhill																										
P 22 *C. rosenii* (Pax) Milne-Redh. ex Polhill						2				2	2	T?	T?	1	1			T?	1				H	N4, 1	0.0426	Ethiopia
K 62 *C. agatiflora* Schweinf.						1				1	2	T	T	T	2	T	T	T	T				M	N4,T?	0.0253	Zaire
P 88 *C. agatiflora* Schweinf.						2	2			2	2	T	T	2	2	T?	T	1	2				H			
K 290 *C. agatiflora* Schweinf.						2				1	2	1	2	T	1	T	1	T	1				H	N4,T	0.0246	Kenya
P 89 *C. agatiflora* subsp. *engleri* (Harms ex Bak. f.) Polhill	T			1		2				2	3	T	T	2	2	T?		T	1				H	N4,T, A12,1, A13,T, A14,1		
P 90 *C. agatiflora* subsp. *erlangeri* Bak. f.						2				2	3	1	T	1	2	T?	T	T	T?				H	N4,T		
P 91 *C. agatiflora* subsp. *imperialis* (Taub.) Polhill						2				2	3	1	T	1	2	T?	T	T?	T?				H	N4,T, A11,T		
D 324 *C. agatiflora* subsp. *imperialis*					1					2	3	1	2	1	2	2	3	2	2	2			H	N4,1, N1,1	0.0132	
K 292 *C. grandibracteata* Taub.	T			3+		2				1	2	1	1	1	2	T	T		T?		T		H		0.0453	Tanzania
P 174 *C. lebrunii* Bak. f.	T			3+		1	2			2	2	1	T	2	3	1	1	1	2				H	N4,T		
P 13 *C. laburnifolia* L.	1			2		2		T?		2	2	T	T	2	1		T	T?	2				H	B1,1	0.0264	Kenya
P 14 *C. laburnifolia* L.	1			2		2		1		2	2	T	T	2	1		T	T?	2		1		H	B1,1	0.0251	Kenya
P 20 *C. laburnifolia* L.	T			1		2	1			2	2	T		2	2	T?	T	T	1				H	B1,2	0.0214	Ethiopia
P 167 *C. laburnifolia* L.				2		1		2	T	2	2	1	1	2	2	T	T	T	2		T		*			
K 279 *C. laburnifolia* L.						1	2			1	2	T	T	T	1			T?	T?				H	B1,T	0.0203	Kenya
K 280 *C. laburnifolia* L.	1			3		2				1	2	T	T	2	1	1	1	1	2		T?		M	BI,T?, N4,T?	0.0215	Kenya
D 330 *C. laburnifolia* L.	1			3				3		2	2	2		2	3+	2	2	2	3		2		M			
P 119 *C. capensis* Jacq.		T?		3+		1	3			3	3	2	1	2	3	T	T	2	3	T?		2	H			
C 294 *C. capensis* Jacq.				3		T	1			T	2	T	T	T	2	T?	T?			T?			L	N4,T?	0.0183	S. Africa
P 206 *C. pallidicaulis* Harms				T		1	2			2	2	T	T	1	2	T		T	1				H	N4,T, A14,1		
P 191 *C. monteiroi* Taub. ex Bak. f.						1	3			2	2	T	T?	1	1	T		T?	1				+			
P 56 *C. barnabassii* Dinter ex Bak. f.	T?			T?	T	T				2	1	1	T?	1	1			1	1				M		0.0186	Tanzania
P 107 *C. barnabassii* Dinter ex Bak. f.	T			2	T	1				2	3	1	T	1	2	T?		1	2				M	A10,1		
Section *Chrysocalycinae* (Benth.) Bak. f. subsection *Incanae* (Benth.) Bisby & Polhill																										
D 329 *C. goodiiformis* Vatke	1	1		2				1	1	2	2	2		2	3		3	1	2	1			H	B4,1	0.0063	
P 58 *C. simulans* Milne-Redh.	1	2		2						1	2		2	2	2		2	1	1				*	N4,2		Tanzania
P 237 *C. simulans* Milne-Redh.	2	2		3						1	2		2	3	2	2		T	1				M		0.0149	
P 54 *C. doniana* Baker	1	T								2	1		1	2	1			T	2				*	N4,1		Nigeria
P 21 *C. quartiniana* A. Rich.	1	1	1	2						1	1	T		1	T	T?			T?				L	N13,1	0.0029	Ethiopia
K 281 *C. mauënsis* Bak. f.	2	3	3	3+						1	2	T	T	2	1	T	T	1	2				L		0.0181	E. Africa
P 2 *C. polysperma* Kotschy	1	2		3	2					1	1	1		1	1				T	T			*			Tanzania
P 48 *C. burkeana* Benth.	2	2	1		1			2		1	2	1	T	1	3	2	T	2	3				*	N4,2		S. Africa
P 36 *C. barkae* Schweinf.	1	1						1		1	1	1		1	2		T		2				*	A7,2		Zambia
P 38 *C. phylloloba* Harms					1			2		2	2	T	1	1	2	1	1	2	3	T	T		*	A5,2		Tanzania
P 52 *C. phylloloba* Harms					1			2		2	2	T?	T	'2	2	2		2	3	1			0	A5,T?	0.0052	Tanzania
P 11 *C. incana* L.	1	2	1	3						1	1	1	T	1	1	T?		T?	1				L		0.0047	Kenya
P 12 *C. incana* L.	2	3		3						1	2	1	1	2	1			T?	2				H		0.0049	Kenya
P 17 *C. incana* L.	1	3		3						1	2	T	T	2	1			T?	1				H		0.0041	Ethiopia
P 19 *C. incana* L.	T	2		1						1	1	T	T?	1	T			T?	T				L		0.0054	Ethiopia
P 28 *C. incana* L.	1	3	T	2	T?					1	2	1	T	1	1	T		T?	T	T			M	A5, T	0.0065	Kenya
K 278 *C. incana* L.	1	2		3+						T	2	T	T	1	1	T?	T	T?	T?				L		0.0064	Kenya
K 311 *C. incana* L.	2	3		3						T	1	T	T	1	1	T?		T?	T?	T?			L		0.0082	Honduras
D 320 *C. incana* L.	1	3		1	1					1	2	1	T	T	1	1	1	1	2	T?			0	N4,T?	0.0044	
P 39 *C. lotoïdes* Benth.	2	2	2							2	2		1	1	2	T	T	T?	3				*	N4,2	0.0033	S. Africa
Section *Chrysocalycinae* (Benth.) Bak. f. subsection *Stipulosae* (Bak. f.) Bisby & Polhill																										
P 196 *C. natalitia* Meissn.						2				1	2	1	T	1	2			1	1				H	Al *or*, 3	0.0070	
P 227 *C. rhodesiae* Bak. f.						1				2	2	T	T	1	2			T	T				H	Al *or*, 3	0.0065	
P 130 *C. cylindrocarpa* DC.						1				1	2	T	T	1	2			T					L		0.0059	
P 152 *C. goreënsis* Guill. & Perr.						2		1		2	2	T	T	T	2			1	2				M		0.0119	
K 308 *C. goreënsis* Guill. & Perr.		T			1			1	2	3	2	T	T	3+	3+	1		2	3	T		2	M	A9,T?	0.0029	
P 41 *C. podocarpa* DC.						2				2	2	T	T	1	1			T?	T?				M		0.0323	Tanzania
P 217 *C. podocarpa* DC.						1		1		2	2	T	T	1	2	T		1	2				M	B4,1	0.0191	
P 132 *C. damarensis* Engl.									2	3	1			2	2		T	T	1				H	B4,2, A1 *or*, 3	0.0136	
M 33 *C. lachnophora* A. Rich.						2	T	T?	1	2	2	T	T	2	2	T		T	T	T			H	Al *or*, 2, N4,T	0.0174	Zambia
C 299 *C. lachnophora* A. Rich.						2				2	2	T	T	2	2		T?	T?	2	T			M	A9,T?	0.0189	Angola
P 154 *C. grandistipulata* Harms						1				1	2	1	T	1	2			T	T				H	Al *or*, 3	0.0282	
Section *Chrysocalycinae* (Benth.) Bak. f. subsection *Glaucae* (Benth.) Bisby & Polhill																										
P 171 *C. lachnosema* Stapf				T?	2	T				1	2		T	T	1								M		0.0089	
K 310 *C. lachnocarpoides* Engl.	1	2	T?	2						T	T			T	1								L		0.0063	Malawi
P 134 *C. densicephala* Welw. ex Baker				T	2	T				2	2	T	T	2	1								L			
P 30 *C. goetzei* Harms							T			1	1		T?	1	2								H	N4,1	0.0147	Zambia
P 151 *C. goetzei* Harms					2	T?		1		2	2	T	T	T	2								H	B4,2		
P 148 *C. gazensis* Bak. f.				T	2					2	2		T	1	1								M			
P 43 *C. glaucifolia* Baker						2		2		2	2	1	1	2	3					1			*	N4,2, B1,2		Zambia
P 47 *C. glauca* Willd.								T?		2	2	1		1	2		1		2	1		T	*	N4,2, B1,1		Tanzania

Table 1.—*continued*

	ODAP	ODAB	A3	ADAP	DAB	Glu tyr	Isowill	Nle(5OH)	Pip	Asp	Glu	Ser	Gly	Asn	Ala	Val	Leu/Ileu	Am but	Arg	Thr	Tyr	Pro	Alkaloid		Size (g)	Country of origin
C 298 *C. glauca* Willd.					T?					1	1	T	T	1	2			T?	1				0		0.0021	Angola
P 204 *C. orthoclada* Welw. ex Baker					T					2	2		T	1	1			T	1				H		0.0058	
C 301 *C. orthoclada* Welw. ex Baker										1	2	T	T	1	1		T	T	T				M		0.0058	Angola
P 59 *C. caudata* Welw. ex Baker										T?	T	1		T				T	1				L		0.0027	Zambia
P 213 *C. pisicarpa* Welw. ex Baker						1				2	2		T	2	1			T	2				M	B4,1	0.0106	
Section *Chrysocalycinae* (Benth.) Bak. f. subsection *Chrysocalycinae*																										
P 181 *C. macrocalyx* Benth.					T?	1				1	1	T	T	1	2		T	T	1				M		0.0050	
Section *Hedriocarpae* Wight & Arn. subsection *Hedriocarpae*																										
P 144 *C. fischeri* Taub.				2		T	2			2	2	T	T	2	2	T	T	1	2				H	N4,1		
P 35 *C. verdcourtii* Polhill			T?	T?		2				2	2		1	2	2	1	1	T	2	1		T	*	N4,1, B1,1, A7,2, A9,2		Kenya
P 49 *C. deflersii* Schweinf.						T				1	1	T	T	1	1	T	T	T?	1	T			M		0.0110	Kenya
P 18 *C. comanestiana* Volkens & Schweinf.						1				1	2	1	T	T	3			T?	T				L		0.0131	Ethiopia
P 248 *C. trifoliata* Bak. f.						T				2	2	1	1	2	3	2	2	2	2	1			+	N4,1, B1,T, A5,T		
P 23 *C. burttii* Bak. f.						2				2	2	T	T	1	2			1	1				L		0.0013	Tanzania
P 16 *C. pycnostachya* Benth.						2				2	2	T	T	2	2			T?	2				0		0.0028	Ethiopia
D 331 *C. pycnostachya* Benth.						T				1	2	1		T	2	T	1	1	2	T			0		0.0018	
Section *Hedriocarpae* Wight & Arn. subsection *Macrostachyae* (Benth.) Bisby & Polhill																										
P 15 *C. pallida* Ait.	T?					2	3			2	2	T	T	1	2	T?		T?	1				M	N4,T?	0.0084	Kenya
P 27 *C. pallida* Ait.						1	3			T	2	T	T	1	T			T?	T				L	N4,T?	0.0086	Nigeria
P 205 *C. pallida* Ait.						1	3			1	1		T		1				T?				H		0.0094	
K 282 *C. pallida* Ait.						2	3			1	2		T?	T?	1		T?	T?	T		T?		L	A9,T?	0.0109	Zambia
K 314 *C. pallida* Ait.						1	2			T	1	T	T	T?	1	T?	T?	T?	1		T?		*	A9,T?		
P 230 *C. rogersii* Bak. f.				T?		T	3			2	2		1	2	3	2	2	2	2	1			*	N4,2		
P 195 *C. naragutensis* Hutch.		T?				T	2			2	2	T	T	2	2	T	T	1	3	2			*	A9,T?, B4,1		
P 101 *C. argyraea* Welw. ex Baker						T?				2	2	T?	T	2	2	T		T?	3				*			
K 309 *C. argyraea* Welw. ex Baker						2				2	3	T	T?		1		T?	T					H	A9,T?, A1 *or*, 3	0.0203	Namibia
P 200 *C. ochroleuca* G. Don					T	1	2			2	2	T	T	1	1	T		T	2				+	A9,T		
C 293 *C. ochroleuca* G. Don						1	2			T?	2	T?	T?	T?	T		T		1				L		0.0060	Tanzania
C 300 *C. ochroleuca* G. Don						2	3			T	1	T	T	T	1	T?	T?	T?	T	T			L	A9,T?	0.0066	Angola
P 7 *C. brevidens* Benth.	T?					2	2			1	2	1	T	2	1	T?			2				H	B1,T	0.0034	Kenya
K 274 *C. brevidens* var. *intermedia* (Kotschy) Polhill						1	1			1	1		T	T	T				2				L		0.0045	Kenya
D 323 *C. dewildemaniana* Wilczek						T	1			2	2	1	T	T	3	1	1	1	2	T			0	N4,T?	0.0035	
K 76 *C. zanzibarica* Benth.						1	1			1	1	1	1		2	T	T	T	1	T			L	N4, T	0.0031	
K 77 *C. zanzibarica* Benth.						T	T			1	1	1	1		2	T	T	T	1				L	N4,T	0.0031	
P 5 *C. kirkii* Baker						2	2			1	2	T	T	1	T			T?	2	T			L		0.0032	Tanzania
K 70 *C. lanceolata* E. Mey.						1	2			2	2		1	1	2	T	T	T	1	T			L		0.0020	
K 64 *C. emarginata* Boj. ex Benth.				T?		T?	2			1	1	T	T		2		T	T?	T	T			L	N4,T?	0.0031	
P 6 *C. balbi* Chiov.						1	1			1	1	T	T	1	1		T		1				*	N4,1, N12,1	0.0018	Kenya
P 10 *C. vallicola* Bak. f.	T?				T	2	2			2	2	T	1	2	2	T?	T	T	3				L	B1,T	0.0042	Kenya
K 277 *C. cylindrica* A. Rich.						2	2			2	2	T	T	1	1	T?	T?	T?	3				L		0.0024	Kenya
P 32 *C. cleomifolia* Welw. ex Baker	T					T	3			T	1	T?	T?	T	T			T	1				M		0.0052	Kenya
P 8 *C. vatkeana* Engl.						1	2			1	1	T	T	1	T				1				*	N4,1		Kenya
P 26 *C. vatkeana* Engl.						2	3			1	1	T	T	1	1	T	T	T?	2	T			M	N4,T	0.0032	Kenya
K 284 *C. vatkeana* Engl.						1	2			2	2	T	T?	1	1	T	T	T?	3				H	N4,T?	0.0022	Kenya
P 3 *C. petitiana* (A. Rich.) Walp.	T?					2	2			2	2	T	T	2	2			T?	2				H		0.0038	Tanzania
P 9 *C. petitiana* (A. Rich.) Walp.						2	2			2	2	T	T	2	2			T?	2				M		0.0041	Kenya
P 211 *C. petitiana* (A. Rich.) Walp.						1	2			1	1		T	1	1	1		1	2				M			
C 297 *C. comosa* Baker				T		1	3			2	2		T	2	3	1	2	1	2				L	N4,T, B4,1	0.0034	Angola
C 296 *C. anthyllopsis* Welw. ex Baker	T					1			2	2	2	T?	T	2	2				1				L	BI,3, A6,T, A9,T?	0.0041	Angola
K 291 *C. distantiflora* Bak. f.	T			3+		2				1	2	1	1	T	2		T	T?	T				0	N4,T, A9,T?	0.0022	Tanzania
Section *Calycinae* Wight & Arn.																										
P 203 *C. orixensis* Rottl. ex Willd.										1	1	1	1	1	1		T?	T	1				*	Y1,2		
P 25 *C. juncea* L.							T?	3+		1	1	T?	T?	T	T	T		T?	2	2			0		0.0437	India
Q 34 *C. juncea* L.								3+		T	1	T	T?		1	T	T	T?	1	T?			0		0.0395	Pakistan
K 69 *C. juncea* L.							T?	3+		1	1	T	T		2		T	T?	1	T?			0		0.0398	
K 312 *C. juncea* L.								3+		1	2	T	T		1	T	T?	T	2	T?			L		0.0564	Honduras
Section *Crotalaria* subsection *Crotalaria*																										
P 187 *C. mildbraedii* Bak. f.						1				1	2		T	T	2	T?			T				M		0.0113	
P 179 *C. lukwangulensis* Harms						1				1	2		T	1	2	T	T	T?	T?				M	N4,1	0.0198	
P 60 *C. axillaris* Ait.						2		1		T				1	T			1	T				H		0.0379	Tanzania
P 104 *C. axillaris* Ait.						2				1	2		T	T	1		T	T	T				+			
P 50 *C. scassellatii* Chiov.						2				1	1	T	T		T				T?				M	N4,T?, A6,T, A8,T	0.0188	Kenya

Table 1.—*continued*

	ODAP	ODAB	A3	ADAP	DAB	Glu tyr	Isowill	Nle(5OH)	Pip	Asp	Glu	Ser	Gly	Asn	Ala	Val	Leu/Ileu	Am but	Arg	Thr	Tyr	Pro	Alkaloid		Size (g)	Country of origin
P 232 *C. scassellatii* Chiov.						2				2	2	T?	T		1	T	T	T	1				+			
P 29 *C. recta* Steud. ex A. Rich.								T		1	1	T	T	1	1			1	1				H		0.0204	Kenya
P 225 *C. recta* Steud. ex A. Rich.						T?				T	2		T	1	T	T		T	1				H		0.0164	
K 304 *C. recta* Steud. ex A. Rich.								T?		T	1	T		2	2	T	T?	T	2				L		0.0169	Angola
P 226 *C. retusa* L.						T?		1		1	2	T	T	1	1	T			T?				H		0.0249	
C 302 *C. retusa* L.										T	1	T		T	1			T?	1				L		0.0175	Angola
K 316 *C. retusa* L.										T	1	T			1	T?	T?	T					H		0.0237	India
P 44 *C. verrucosa* L.								2		2	2		1	2	3	2	2	T	3	2	1	2	L	A15,T	0.0146	
P 254 *C. verrucosa* L.								2		3	1		2	2	3	2		2	3				H			
Section *Crotalaria* subsection *Longirostres* (Benth.) Polhill																										
D 321 *C. deserticola* Taub. ex Bak. f.						T				2	2		1	1	2	2	2	1	2				L	N4,T	0.0020	
P 4 *C. greenwayi* Bak. f.						2				2	2	T		1	T				T				L		0.0014	Tanzania
P 40 *C. laburnoïdes* Klotzsch						2				1	1	T	T	1	1			T?	1				M		0.0043	Kenya
P 234 *C. senegalensis* (Pers.) Bacle ex DC.						2				2	2		T	T	1			T	2				+			
P 215 *C. platysepala* Harv.								2		2	2		1	1	3	2	2	2	3+				+	N4,2		
S 82 *C. peschiana* Duvign. & Timp.				T		2		2		2	2	2	T	2	3+	1	2	2	2	T?			H	N4,T, N1,2, B4,1	0.0030	
P 53 *C. macaulayae* Bak. f.					2	T				3	T		T	2	2		T	1	2				L	B1,T	0.0026	Zambia
K 65 *C. grantiana* Harv.										2	2	T?	1	1	2			T	3	T			L		0.0030	
P 161 *C. kapirensis* De Wild.						2				2	2	1	1	1	2	T	1	1	2	T			*			
C 295 *C. kapirensis* De Wild.					T	1				T	T			T	1	T?	T	T?	T?	T			0	N4,T?		
S 81 *C. aculeata* De Wild.					T	2			T	1	2	T	T	2	2	2	1	T	2				H	N4,T, B4,1	0.0045	
P 87 *C. aculeata* De Wild.						2				2	2		T	1	1	T			1				*			
C 303 *C. spinosa* Hochst. ex Benth.						2				1	2	1	T	1	2	T	1	T	1				0	N4,T?, B4,1	0.0045	Angola
D 322 *C. spinosa* Hochst. ex Benth.										1	2	T	T		T	T?	T	T	T				0		0.0019	
Section *Dispermae* Wight & Arn.																										
P 220 *C. prolongata* Baker						2			1	2	2	2		2	2			1	1	2			+	B4,1		
P 42 *C. cuspidata* Taub.					T	1				2	2		1	1	2		T	T	2	T			0	B1,T	0.0019	Zambia
P 55 *C. elisabethae* Bak. f.					T	T				2	2	T	T	1	2			1	2				L	B1,2	0.0011	Zambia

Key. ODAP: α-amino-β-oxalylaminopropionic acid (with α-oxalyl isomer); ODAB: α-amino-γ-oxalylaminobutyric acid (with α-oxalyl isomer); ADAP: α-amino-β-acetylaminopropionic acid; DAB: α,γ-diaminobutyric acid; Glu tyr: γ-glutamyltyrosine; Isowill: isowillardiine; Nle(5OH): δ-hydroxynorleucine; Pip: pipecolic acid; Am but: γ-aminobutyric acid; other amino acids: standard symbols. 3 2 1 T—Amino acid detected; estimation of concentration. T?—Concentration of amino acid so low that chromatograms and ionophoresis papers do not show clear spots. Absence of symbols—Amino acid not detected. H M L 0 —Concentration of alkaloids as estimated after development of papers with Dragendorff reagent (high, medium, low, none). +—Alkaloid detected on ninhydrin-developed papers, but no development with Dragendorff reagent was carried out. *—No data. C, D, K, P, Q, S—Seed supplier (Crout, Dossaji, Krukoff, Polhill, Qureshi, Shewry). A, B, N 1–15—Unidentified amino acids. A6–15—Possibly γ-glutamyl amino acids. Y1—Compound occupies a position often associated with lactones on ionophoresis at pH 3.6, pale yellow after ninhydrin-development. A1 *or*—Alkaloid which gives bright orange colour after ninhydrin-development. Size—Seeds of species in sections *Dispermae* and *Geniculatae* were noted to be small; subsequently average seed weights were determined, but in many cases the average is based on very few seeds.

Table 2. Free amino acids and alkaloids in *Crotalaria* seeds (limited data)

	ODAP	ODAB	DAB	Glu tyr	Pip	Asp	Glu	Gly	Asn	Ala	Am but	Arg	Alkaloids	Size (g)
Section *Chrysocalycinae* (Benth.) Bak. f. subsection *Incanae* (Benth.) Bisby & Polhill														
P 137 *C. doniana*	2	2				1	1	1	T?	2	T	T	M	0.0083
P 224 *C. quartiniana*	2	2				1	1	T	1	2	1	1	H	0.0030
P 115 *C. burkeana*	2	2	2			2	2	2	T	3	2	3	L	0.0105
P 212 *C. phylloloba*			2			3	2	1	T	2	T	T	M	0.0088
Section *Chrysocalycinae* (Benth.) Bak. f. subsection *Stipulosae* (Bak. f.) Bisby & Polhill														
P 244 *C. stolzii* (Bak. f.) Milne-Redh. ex Polhill				T		2	T	1	2	2	1	3	H A1 *or*, 3	0.0018
Section *Chrysocalycinae* (Benth.) Bak. f. subsection *Glaucae* (Benth.) Bisby & Polhill														
P 129 *C. cordata* Welw. ex Baker			2			2	2	2	2	3+	2	3+	H	
P 169 *C. lachnocarpoïdes*			2	1		2	2		1	2		1	M	0.0110
P 94 *C. amoena* Welw. ex Baker			2			T	1	T	T	1	1	1	L	

Table 2.—*continued*

	ODAP	ODAB	DAB	Glu tyr	Pip	Asp	Glu	Gly	Asn	Ala	Am but	Arg	Alkaloids	Size (g)
P 176 *C. glaucifolia*				T?		2	2	T?	1	1	T	1	L	0.0109
P 150 *C. glaucoïdes* Bak. f.						3	2	*	*	*	3	3+	+	0.0011
P 96 *C. anisophylla* Welw. ex Baker						2	1	T	T	1	T	2	L	
P 149 *C. glauca*			T			2	1	1	1	2	T	3	M	
P 255 *C. vialis* Milne-Redh.						2	2	T	2	2	1	3	L	0.0033
P 121 *C. caudata*			1			1	2	T	1	T	T	2	L	
P 214 *C. pisicarpa*				T?		2	1	1	T	1	1	2	L	0.0028
Section *Chrysocalycinae* (Benth.) Bak. f. subsection *Tetralobocalyx* (Harms) Bisby & Polhill														
P 198 *C. nigricans* Baker				T?		T	1	1	T	1		2	L	0.0024
Section *Chrysocalycinae* (Benth.) Bak. f. subsection *Chrysocalycinae*														
P 127 *C. confusa* Hepper				1		2	2	1	1	2	1	2	*	
P 192 *C. mortonii* Hepper				1		2	2	1	2	2	1	3	*	
P 209 *C. perrottetii* DC.					T?	3	2	2	1	2	1	2	*	
P 201 *C. ononoïdes* Benth.				2		2	2	1	2	2	2	3	*	
Section *Hedriocarpae* Wight & Arn. subsection *Hedriocarpae*														
P 159 *C. inopinata* (Harms) Polhill						2	2	T	2	2	1	2	*	
P 133 *C. deflersii*				T		2	2	1	2	2	2	3	+	
P 116 *C. burttii*				T		2	2	*	*	*	T	2	*	
P 228 *C. rhynchocarpa* Polhill				2		2	2	*	2	2	T	1	+	
P 231 *C. saltiana* Andr.						2		T	2	2	T	2	*	
P 210 *C. persica* (Burm. f.) Merrill				2		1	2	*	*	1	*	1	+	
P 222 *C. pycnostachya*			1	1		2	2	T	T	2		1	*	0.0015
P 223 *C. pycnostachya*			T	2		2	1	T	T	1		T	*	0.0017
Section *Hedriocarpae* Wight & Arn. subsection *Macrostachyae* (Benth.) Bisby & Polhill														
P 160 *C. brevidens*				1		1	2	1	T	2	T	2	M	0.0044
P 257 *C. zanzibarica*				T	T	1	1	T	1	1	1	1	*	
P 172 *C. lanceolata*				2		1	1	T	T?	1	T	1	*	
P 140 *C. emarginata*				2		1	2		T	1	T	1	*	
P 184 *C. mesopontica* Taub.				1		2	2	T	1	1	T	1	*	
P 106 *C. balbi*				1		1	2	T	T	1		2	*	
P 158 *C. impressa* Nees						3	2	1	1	2	1	3	*	
P 110 *C. bernieri* Baill.				1		1	1	1	1	2	T	2	*	
P 250 *C. vallicola*				1		1	1	T	2	2	1	2	M	0.0032
P 123 *C. chrysochlora* Bak. f. ex Harms					2	2	3	2	T	3	1	3	*	
P 124 *C. cleomifolia*				T		T	2	T?	T	1	T	1	M	0.0049
P 216 *C. plowdenii* Baker	T?					3	2	2	2	3	2	3	+	
P 125 *C. comosa*				T		2	2	T	1	1	T	2	*	
P 126 *C. comosa*				T		1	1	1	1	2	T	2	*	
P 253 *C. vasculosa* Wall. ex Benth.	1	T?			T	2	2	T	2	2	2	2	*	
P 86 *C. abbreviata* Bak. f.						2	2	1	2	3	2	3	+	
P 98 *C. anthyllopsis*		T?		T?	2	2	2	T	T	2	1	2	+	
P 131 *C. cylindrostachys* Welw. ex Baker				T?		2	2	T	1	2	T	2	+	
P 249 *C. ukambensis* Vatke				1		2	2	T	1	1	T	2	+	
P 188 *C. massaiënsis* Taub.						2	2	1	1	1	1	2	*	0.0005
P 177 *C. lotiformis* Milne-Redh.				1		1	2	T?	1	1	T	2	*	
P 233 *C. schinzii* Bak. f.				1		2	2	1	2	2	T	3	*	
P 136 *C. distantiflora*						2	2	T	1	2	1	2	*	
P 243 *C. steudneri* Schweinf.	T					2	2	T	2	2	T	2	*	
P 111 *C. boehmii* Taub.	2					2	2	T	T	1	1	1	*	
P 239 *C. spartea* Baker	2					2	3	1	1	1	T	3	*	
Section *Geniculatae* Polhill														
P 240 *C. spartioïdes* DC.	1			1		2	2	T	*	1	1	2	*	
P 155 *C. heidmannii* Shinz						2	2	T	T	1	T	2	*	
P 241 *C. sphaerocarpa* Perr. ex DC.				2		2	3	T	1	1	T	3	*	0.0044
P 186 *C. microphylla* Vahl				1	2	3	2	1	2	2	1	3	*	
P 113 *C. boranica* Harms ex Bak. f.				2		2	2	1	2	2	2	3	*	
P 252 *C. vanmeelii* Wilczek	2					2	1	T	T	1	1	2	*	
P 247 *C. teretifolia* Milne-Redh.	2					2	2	T	1	1	1	2	*	
P 189 *C. minutissima* Bak. f.						3	2	1	2	2	1	3	*	
Section *Calycinae* Wight & Arn.														
P 99 *C. arenaria* Benth.						1	2		2		T?	T	+	
P 118 *C. calycina* Schrank						2	1	2	2	2	1	3	+	
P 157 *C. juncea*						2	2		1	2	1	2	L	

Table 2.—*continued*

	ODAP	ODAB	DAB	Glu tyr	Pip	Asp	Glu	Gly	Asn	Ala	Am but	Arg	Alkaloids	Size (g)
Section *Crotalaria* subsection *Crotalaria*														
P 245 *C. tabularis* Bak. f.			1	2		1	2	T	1	2	T	1	H	0.0147
P 142 *C. fascicularis* Polhill			T	T		1	2	T	1	T	T	1	H	0.0072
P 173 *C. emarginella* Vatke				T		1	2	T?	T?	T	T?	T	+	0.0025
Section *Crotalaria* subsection *Longirostres* (Benth.) Polhill														
P 135 *C. deserticola*				2		2	2	T	T	2		1	+	
P 190 *C. miranda* Milne-Redh.			1	2		2	2	1	2	3	T	2	*	
P 168 *C. laburnoïdes*				2		1	2	T	2	2		T	+	
P 117 *C. subcaespitosa* Polhill				2		3	2	1	2	3	T	2	L	0.0016
P 146 *C. friesii* Verdoorn			1	2	1	2	2	1	2	3	2	2	+	
P 180 *C. macaulayae*	T	T				3	2	1	2	3+	2	3+	+	
P 103 *C. aurea* Dinter ex Bak. f.					2	3	2		2	3	2	3+	* Y1, 1	
P 202 *C. oöcarpa* Baker				2		2	2	1	1	2	1	2	+	
P 256 *C. virgulata* Klotzsch						2	2	1	1	2	2	2	*	
P 242 *C. spinosa*			1	2		2	2	T	2	3	T	2	*	
P 246 *C. teixeirae* Torre				T		3	2	T	2	2	2	3	+	
Section *Dispermae* Wight & Arn.														
P 235 *C. cuspidata*			2	2		2	2	T	1	2	T	2	H	0.0030
P 193 *C. morumbensis* Bak. f.				2		2	2	T	1	1	T	1	+	
P 194 *C. morumbensis* Bak. f.	1			1		2	2	T	2	1	1	2	+	
P 97 *C. annua* Milne-Redh.						2	2	2	2	2	1	3	*	
P 272 *C. seemeniana* Harms				1		T	1	T	T	1	T	2	*	
P 141 *C. exelliana* Wilczek				1		2	2		2	1	1	2	*	
P 147 *C. gamwelliae* Bak. f.				2		2	2		1	1		1	*	
P 145 *C. florida* Welw. ex Baker				T		1	2	T	1	2		1	*	
P 182 *C. malangensis* Bak. f.						2	1	T	T	1	1	2	*	
P 166 *C. kutchiensis* Bak. f.						1	T	T	1	2	1	2	+	
P 153 *C. graminicola* Taub. ex Bak. f.						1	2	T	1	1	1	3	*	
P 175 *C. lepidissima* Bak. f.						2	1	T	1	1	1	2	*	
P 100 *C. argenteo-tomentosa* Wilczek					1	1	2	2	2	2	T	2	*	
P 105 *C. axillifloroïdes* Bak. f. ex Wilczek						2	2	T	2	2	1	3	+	
P 251 *C. vandenbrandii* Wilczek					2	2	2	1	1	2	1	2	+	
P 163 *C. kipandensis* Bak. f.						2	T		T	T	1	3	*	
P 164 *C. kipandensis* Bak. f.				1		2	2	T	1	1	1	3	*	
P 122 *C. cephalotes* Steud. ex A. Rich.						2	2	T	2	1	T	2	*	
P 138 *C. elisabethae*	T			1		1	1	T	1	1	1	3	*	
P 139 *C. elisabethae*	T			1		1	2	T	1	1	1	2	*	
P 114 *C. bredoi* Wilczek				T?		2	2		2	2	T?	2	+	
P 108 *C. basipeta* Wilczek					2	2	2	1	2	3	1	3	*	
P 208 *C. passerinoïdes* Taub.		2			2	3	2	2	2	2	2	3	*	
P 165 *C. kuiririensis* Bak. f.				1	1	2	2	1	1	2	1	2	+	
P 120 *C. carsonii* Bak. f.				T?		2	2	1	2	2	T	2	*	
P 143 *C. filicaulis* Welw. ex Baker			2	T?		1	2	T	1	1	1	2	*	
P 156 *C. hyssopifolia* Klotzsch				T		T	1		*	T	T	2	*	
P 93 *C. alexandri* Bak. f.						2	2	1	2	2	1	3	*	
P 109 *C. bequaertii* Bak. f.						2	1	1	2	2	1	2	*	

For Key to abbreviations, see Table 1.

species on which all the procedures were carried out and Table 2 lists the species on which only ionophoresis was performed. Consequently the only amino acids listed in Table 2 are those which can be clearly separated by high voltage paper ionophoresis at pH 1.9 and pH 3.6. Any samples which were not exhausted during these studies were subjected to further ionophoresis at pH 3.6, and the papers were sprayed with Dragendorff reagent in order to detect alkaloids.

Species in the section *Grandiflorae* contain high concentrations of free amino acids and alkaloids. All of the species contain γ-glutamyltyrosine and many species contain acetyldiaminopropionic acid in high concentration. Oxalyldiaminopropionic acid is also present in many of the species. *C. laburnifolia*, which can be split into 5 subspecies, shows considerable variation between accessions, whereas *C. agatiflora*, which can also be divided into 5 subspecies, shows less variation. *C. agatiflora* subsp. *imperialis* sample 324 and subsp. *engleri* differ significantly from the other *C. agatiflora* accessions as subsp. *engleri* contains acetyldiaminopropionic acid and oxalyldiaminopropionic acid, which are common to those *Grandiflorae* species positioned immediately after *C. agatiflora* in Polhill's classification, whilst sample 324 contains diaminobutyric acid. The *C. laburnifolia* accessions differ in the presence or absence of oxalyldiaminopropionic acid, acetyldiaminopropionic acid, γ-glutamyltyrosine and isowillardiine.

There is a considerable amount of variation in section *Chrysocalycinae*. In subsection *Incanae* all the species examined, with the exception of the 3 accessions of *C. phylloloba*, contain oxalyldiaminobutyric acid and oxalyldiaminopropionic acid, and acetyldiaminopropionic acid also occurs in most species. Those 3 accessions contain none of these amino acids, but at least 2 were shown to contain the unidentified amino acid A5 which was not detected in any other *Crotalaria* species except *C. incana* (sample 28), where it is present in such low concentration that its identification is tentative. The unidentified amino acid A3 is common to species in subsection *Incanae*, and as it runs close to oxalyldiaminobutyric acid and oxalyldiaminopropionic acid on 2D paper chromatograms it may possibly be another oxalylamino acid. It gives a characteristic brown colour with ninhydrin.

C. incana shows little variability, although in S. America, material referred to as *C. incana* exhibits a great deal of polymorphism [2]. However, with the exception of sample 311, which came from Honduras, all the accessions examined here were of African origin. *C. quartiniana* is reminiscent of species in subsection *Chrysocalycinae* and also species in section *Calycinae* [2], but its amino acid pattern is typical of subsection *Incanae*. The overall pattern of the subsection is very constant, and the shrubby species *C. goodiïformis*, *C. simulans* and *C. mauënsis* exhibit few differences to the herbaceous species.

γ-Glutamyltyrosine and isowillardiine were not detected in species in subsection *Incanae*. However, γ-glutamyltyrosine is present in all of the species in subsection *Stipulosae* except *C. damarensis* and one sample of *C. goreënsis*. According to Polhill, *C. damarensis* is very closely related to *C. podocarpa* [2], and the presence of the alkaloid Al *or*, which is only found in species in subsection *Stipulosae*, gives confirmation that its taxonomic position is correct.

The occurrence of this alkaloid in only a few species in subsection *Stipulosae* leads us to suggest that some further subdivision of section *Chrysocalycinae* could be envisaged. Polhill acknowledged the existence of 2 sub-groups of section *Chrysocalycinae* in South America [2], and examination of more South American species ought to result in changes being made to the existing classification. However, the overall amino acid pattern of subsection *Stipulosae* is constant between species and so division of this taxon on the basis of alkaloid distribution may only be applicable at a level lower than the rank of subsection.

As well as containing γ-glutamyltyrosine, species in subsection *Stipulosae* differ from subsection *Incanae* in having lower levels of free amino acids and no acetyl- or oxalyl- amino acids in most cases. The level of alkaloids is also higher in subsection *Stipulosae*.

Species in subsection *Glaucae* are similar to species in subsection *Stipulosae* except that more of them contain acetyldiaminopropionic acid and diaminobutyric acid and fewer contain γ-glutamyltyrosine. The first-mentioned species in the subsection are close to subsection *Stipulosae*, yet *C. goetzei*, *C. gazensis* and allies are very similar to the first-mentioned woody species of subsection *Incanae* [2]. *C. gazensis* is noticeable for containing diaminobutyric acid and a trace of acetyldiaminopropionic acid. These are common to subsection *Incanae*, but diaminobutyric acid occurs in subsection *Incanae* as its oxalyl derivatives, and oxalylamino acids were not detected in *C. gazensis*. Also, other species in subsection *Glaucae* which are not close to subsection *Incanae* contain these 2 amino acids. Free diaminobutyric acid is particularly common to the first species in subsection *Glaucae*, yet it is not common in subsection *Stipulosae*. One sample of *C. lachnocarpoïdes*, which is a species positioned close to subsection *Stipulosae*, has an amino acid pattern which is typical of subsection *Incanae*. This accession (No. 310) may have been incorrectly identified.

Information on subsections *Tetralobocalyx* and *Chrysocalycinae* is limited. *C. nigricans*, the only species in subsection *Tetralobocalyx*, contains γ-glutamyltyrosine, and has a low level of free amino acids and alkaloids. Of the 5 species in subsection *Chrysocalycinae* examined, 4 contain γ-glutamyltyrosine and none were shown to contain oxalyldiaminobutyric acid or oxalyldiaminopropionic acid.

There is very little variation in amino acid patterns between species in section *Hedriocarpae*. Members of subsection *Hedriocarpae* mostly contain γ-glutamyltyrosine and no isowillardiine, whereas members of subsection *Macrostachyae* mostly contain both of these compounds. This difference reinforces the decision not to merge the 2 subsections into a single entity [8], but the overall similarity in amino acid distribution between the 2 subsections is an indication that they are correctly positioned in the same section.

A few species in subsection *Macrostachyae* contain oxalyldiaminopropionic acid, but, with the exception of the closely related species *C. boehmii* and *C. spartea*, this is always in low concentration. The position of *C. boehmii* is ambiguous as in many features it resembles species in section *Geniculatae*, but its affinity to section *Hedriocarpae* subsection *Macrostachyae* is not in doubt [8].

Acetyldiaminopropionic acid, diaminobutyric acid and oxalyldiaminobutyric acid are mostly lacking from section *Hedriocarpae*, although *C. distantiflora* and *C. fischeri* contain acetyldiaminopropionic acid in high and medium concentration respectively. *C. distantiflora*, an unspecialised species in subsection *Macrostachyae*, shows slight similarities to *C. stolzii*, a member of section *Chrysocalycinae* subsection *Stipulosae* which itself shows similarities to species in section *Grandiflorae* [8]. The presence of acetyldiaminopropionic acid, oxalyldiaminopropionic acid and γ-glutamyltyrosine in *C. distantiflora* is of interest as this is the only species in which these amino acids were detected together outside section

Grandiflorae. *C. fischeri* is also unusual in being the only species in subsection *Hedriocarpae* which contains isowillardiine, and the abandonment of section *Hedriocarpae* subsection *Priotropis* (Wight & Arn.) Polhill, in which it was once positioned [2, 8], has led to a greater diversity of characters occurring in subsection *Hedriocarpae*.

C. verdcourtii and *C. deflersii* are outlying members of section *Hedriocarpae* [8], but although *C. verdcourtii* contains traces of acetyldiaminopropionic acid and the unknown amino acid A3, their amino acid distribution otherwise conforms to the subsection *Hedriocarpae* pattern.

C. pallida and *C. brevidens* are both species of which different varieties exist [2], but the accessions tested here show very little variability in amino acid distribution. *C. pallida* sample 314 was submitted to us as *C. striata* DC., and the decision of Polhill to include this species, with others, in the concept of *C. pallida* Ait. is not challenged by our data.

C. argyraea sample 309 was almost certainly misidentified as it has an amino acid pattern typical of section *Chrysocalycinae* subsection *Stipulosae*. It contains the alkaloid Al *or* which is only found in section *Chrysocalycinae* subsection *Stipulosae* and it differs from sample 101, a *C. argyraea* accession which has a pattern typical of section *Hedriocarpae* subsection *Macrostachyae*.

Little information is available for section *Geniculatae*. Some species, in particular the closely related *C. vanmeelii* and *C. teretifolia*, contain oxalyldiaminopropionic acid. *C. spartioides* contains both oxalyldiaminopropionic acid and γ-glutamyltyrosine, an occurrence which, with the exception of a few species in section *Hedriocarpae* subsection *Macrostachyae* and some in section *Dispermae*, is associated with section *Grandiflorae*. The differences in amino acid patterns between species in the section reflect the view that it is not a very natural group [2].

Little information is available for section *Calycinae*. All the species analysed lack γ-glutamyltyrosine, diaminobutyric acid or diaminopropionic acid derivatives and free diaminobutyric acid. The amino acid pattern of *C. juncea* is different from that of any other *Crotalaria* species examined as the concentration of δ-hydroxynorleucine is very high (up to 2% of the seed weight [21]), and this may reflect the fact that *C. juncea* has been the most extensively cultivated *Crotalaria* species. This cultivation may also account for the fact that although alkaloids have been isolated from *C. juncea* seeds [24]), their concentrations are too low for them to have been detected in this study.

C. juncea is intermediate between section *Calycinae* and section *Crotalaria* subsection *Crotalaria* [2], but no obvious relationship is apparent in the amino acid data. Species in section *Crotalaria* subsection *Crotalaria* mostly contain γ-glutamyltyrosine, which is lacking from *C. juncea* and other species in section *Calycinae*, and their amino acid levels are generally low. Acetyldiaminopropionic acid, oxalyldiaminobutyric acid and oxalyldiaminopropionic acid are entirely lacking from subsection *Crotalaria*, and only *C. tabularis* and *C. fascicularis* contain free diaminobutyric acid. The level of alkaloids in the subsection is predominantly high.

The pattern of amino acid distribution in subsection *Longirostres* is very similar to that of subsection *Crotalaria*, but the concentration of the amino acids is higher in subsection *Longirostres*. Most species in subsection *Longirostres* contain γ-glutamyltyrosine, a few contain diaminobutyric acid, but acetyldiaminopropionic acid, oxalyldiaminobutyric acid and oxalyldiaminopropionic acid are almost entirely lacking. *C. macaulayae* sample 180, was the only sample shown to contain oxalylamino acids, but this species exhibited some variation, as *C. macaulayae* sample 53 did not contain oxalylamino acids whereas it did contain diaminobutyric acid and γ-glutamyltyrosine, both of which were lacking from sample 180. *C. macaulayae* had its closest link with *C. juncea* in Bisby & Polhill's study, but this was because *C. macaulayae* is one of the least advanced members of subsection *Longirostres* [8]. No trace of oxalylamino acids, diaminobutyric acid or γ-glutamyltyrosine has been found in *C. juncea*, and *C. macaulayae* does not contain δ-hydroxynorleucine.

Section *Crotalaria* subsection *Longirostres* is similar to section *Dispermae* [2], but unfortunately there is a lack of information for the latter section. There is a great deal of variation between species in the section, γ-glutamyltyrosine occurring in a noticeable fraction of the species and diaminobutyric acid, oxalyldiaminobutyric acid and oxalyldiaminopropionic acid occurring in a few species. The diversity of amino acid patterns matches the diversity of morphological forms which occur within the section, and the only constant feature is the high level of arginine which occurs in many species.

The amino acid and alkaloid patterns which are distinctive for certain taxa are displayed in Table 3. If seeds of unidentified *Crotalaria* species are subjected to chemical tests of a similar nature to those described here, their taxonomic rank could be elucidated purely on the basis of amino acids present, concentration of alkaloids and weight of seeds if they belong to the following taxa: section *Grandiflorae* (most species), section *Chyroscalycinae* subsection *Incanae*, section *Hedriocarpae* subsection *Macrostachyae* and section *Crotalaria* subsection *Crotalaria* (most species).

Table 3. *Crotalaria* taxa with characteristic amino acid and alkaloid patterns

Section and subsection	ODAP	ODAB	ADAP	Glu tyr	Isowill	Alk. concn	Seed size
Grandiflorae	T	–	+	+	+/–	High	Large
Chrysocalycinae: Incanae	+	+	+	–	–		
Chrysocalycinae: Stipulosae	–	–	–	+	–		
Hedriocarpae: Hedriocarpae	–	–	–	+	–		
Hedriocarpae: Macrostachyae	T/–	–	–	+	+		Small
Crotalaria: Crotalaria	–	–	–	+	–	High	Large
Crotalaria: Longirostres	–	–	–	+	–		

Biosynthetic aspects

Oxalyldiaminobutyric acid and oxalyldiaminopropionic acid are probably formed in developing *Crotalaria* seeds by the addition of an oxalyl group to α,γ-diaminobutyric acid and α,β-diaminopropionic acid. An oxalyl-coenzyme A synthetase has been isolated from *Lathyrus sativus* seeds, and incubation of this enzyme with the appropriate substrates resulted in the formation of oxalyl derivatives of glycine, alanine, serine, homoserine and lysine, as well as α,γ-diaminobutyric acid and α,β-diaminopropionic acid [25]. In *Lathyrus sativus* seeds, the oxalyl group is added specifically to the β-amino group of α,β-diaminopropionic acid [26], and no evidence of the enzymic formation of α-oxalylamino-β-aminopropionic acid has been found. As α-oxalylamino-γ-aminobutyric acid and α-oxalylamino-β-aminopropionic acid are usually present in those *Lathyrus* seeds which also contain the corresponding ω-oxalyl derivatives, it has been suggested that these α-oxalyl derivatives are formed by an isomeric rearrangement which is chemical rather than enzymic [27].

The oxalyldiaminobutyric acid and the oxalyldiaminopropionic acid of *Crotalaria* seeds were both resolved into pairs of ninhydrin-reacting compounds upon high voltage paper ionophoresis at pH 3.6. The most acidic compound of each pair (that moving the greatest distance towards the anode) was found to correspond to the relevant ω-oxalyl derivatives, and the least acidic compounds corresponded to the α-oxalyl derivatives. The ω-oxalyl derivatives occur in much higher concentration than the α-oxalyl derivatives in all *Crotalaria* species which contain oxalyl derivatives, and this situation also occurs in *Lathyrus latifolius* seeds [27].

Crotalaria species which contain α,γ-diaminobutyric acid in the free form without its oxalyl derivatives being present may have lost the ability to add oxalyl groups to amino acids during the evolution of the genus. Only 3 accessions (10, *C. vallicola*, 56 and 107, *C. barnabassii*) were found to contain free diaminobutyric acid and the oxalyl derivatives of diaminopropionic acid without oxalyl derivatives of diamonobutyric acid being present, and in all 3 instances the concentrations of diaminobutyric acid and oxalyldiaminopropionic acid are so low that the oxalyl derivatives of diaminobutyric acid may be present, but at a concentration too low for detection.

α,β-Diaminopropionic acid is not found free in *Crotalaria* seeds (except perhaps at concentrations too low for detection in this study) and neither is it found free in *Lathyrus* seeds. Any available diaminopropionic acid in developing seeds of *Crotalaria* and *Lathyrus* species is obviously utilised quickly, by conversion to the oxalyl derivatives or to other compounds. In *Crotalaria* one such alternative compound is α-amino-β-acetylaminopropionic acid, an amino acid which has previously been isolated from seeds of species of the legume genera *Acacia* [28] and *Schrankia* [29]. Addition of [^{14}C]-diaminopropionate to *Acacia podalyriaefolia* seedling extracts established that an extensive conversion of α,β-diaminopropionic acid into its β-acetyl derivative occurs in this species [30], and the high concentrations of α-amino-β-acetylaminopropionic acid which occur in many *Crotalaria* seeds may account for the absence of diaminopropionic acid.

α-Amino-γ-acetylaminobutyric acid was not detected in any *Crotalaria* seeds, yet α,γ-diaminobutyric acid has been shown to be acetylated in *Lathyrus latifolius* seedlings [31]. The acetylating system in *Crotalaria* is not necessarily specific for α,β-diaminopropionic acid, however, as there are very few *Crotalaria* species in which free α,γ-diaminobutyric and α-amino-β-acetylaminopropionic acid occur together, and the absence of α-amino-γ-acetylaminobutyric acid from the genus may be due to an absence of available α,γ-diaminobutyric acid in those species which contain an acetylating system. Many species (e.g. those in section *Chrysocalycinae* subsection *Incanae*) contain α-amino-β-acetylaminopropionic acid and oxalyl derivatives of α,γ-diaminobutyric acid, but here preferential addition of oxalyl groups to the α,γ-diaminobutyric acid may be removing it from the acetylation system. Also, α-amino-γ-acetylaminobutyric acid is difficult to detect with the 2D paper chromatography system used in this study as it runs close to δ-hydroxynorleucine. The unidentified amino acid B4 runs close to the position taken up by δ-

Table 4. Distribution of enzyme systems in the genus *Crotalaria*

Section and subsection	Oxalylation	Acetylation	Synthesis of DAP	Synthesis of DAB	Synthesis of Glu tyr	Synthesis of Isowill
Grandiflorae	+	+	most spp.	–	all spp.	few spp.
Chrysocalycinae: Incanae	all spp.	all spp.	all spp.	all spp.	–	–
Chrysocalycinae: Stipulosae	?	?	?	–	all spp.	–
Chrysocalycinae: Glaucae	–	+	few spp.	many spp.	few spp.	–
Chrysocalycinae: Chrysocalycinae	?	*	?	–	most spp.	*
Hedriocarpae: Hedriocarpae	?	?	?	–	most spp.	–
Hedriocarpae: Macrostachyae	+	+	+	–	most spp.	most spp.
Geniculatae	+	*	+	–	half the spp.	*
Calycinae	?	*	?	–	–	*
Crotalaria: Crotalaria	?	?	?	2 spp.	most spp.	–
Crotalaria: Longirostres	–	?	?	few spp.	most spp.	–
Dispermae	few spp.	*	+	few spp.	half the spp.	*

+, Present; –, absent or not functioning; ?, unknown (as DAP does not occur free in *Crotalaria* seeds, the absence of ADAP and ODAP does not permit conjecture as to whether the DAP synthesising system, the acetylating system or the oxalylating system are absent); *, lack of data.

It is conjectured that if the oxalylating system is present, available DAB is oxalylated. Species not typical of groups have been ignored.

acetylornithine in this system, and so further investigation may possibly reveal the occurrence of a range of acetyl amino acids in *Crotalaria* seeds. One dimensional paper chromatograms of *C. mauënsis* (sample 281) extracts run in the solvent systems given bore no ninhydrin-reacting compound near the position taken up by α-amino-γ-acetylaminobutyric acid, and so in this species at least the α,γ-diaminobutyric acid present in the seeds occurs as its oxalyl derivatives.

The amino acid isowillardiine (β-uracil-3-yl-aminopropionic acid) is only present (with very few exceptions) in *Crotalaria* seeds which also contain γ-glutamyltyrosine. In pea seedlings isowillardiine arises from addition of a side chain, probably derived from serine or *O*-acetylserine, to a preformed pyrimidine ring derived from the orotate pathway [32]. The synthesis of γ-glutamyltyrosine has been catalysed by a γ-glutamyltransferase isolated from *Phaseolus vulgaris* fruits [33]. This enzyme facilitates the transfer of the γ-glutamyl group of glutathione to many amino acids, a reaction which results in the formation of a γ-glutamyl 'dipeptide' and cysteinylglycine. Glycine and serine are interconverted in microorganisms, plants and animals in a reaction catalysed by serine hydroxymethyltransferase (EC 2.1.2.1) [34], and so the side chain of isowillardiine may be derived from the cysteinylglycine formed during the biosynthesis of γ-glutamyltyrosine.

The synthesis of α,β-diaminopropionic acid in plants may involve *O*-acetylserine or cysteine and ammonia as precursors, but this has still to be proved. The differential occurrence of acetyldiaminopropionic acid, oxalyldiaminopropionic acid and isowillardiine in *Crotalaria* seeds may, therefore, be the result of minor changes occurring to one biosynthetic pathway of which serine and *O*-acetylserine are components. However, in *Acacia podalyriaefolia* seedlings, serine does not act as a precursor of α-amino-β-acetylaminopropionic acid or willardiine (β-uracil-1-yl-α-aminopropionic acid), 2 normal constituents of the seeds and seedlings [30], and so alternative pathways to diaminopropionic acid and also isowillardiine may occur in *Crotalaria*.

Information concerning the probable distribution of enzyme systems which catalyse the biosynthesis of some free amino acids in *Crotalaria* species is summarised in Table 4.

Chemotaxonomic aspects

The genus may be split into 2 groups on the basis of flower complexity [2, 8], and the occurrence of acetyl and oxalyl amino acids is largely confined to the group whose species bear flowers with an untwisted keel, or, where the keel is twisted, the standard appendages run on to the claw. With the exception of 3 species in section *Dispermae*, 1 species in section *Crotalaria* subsection *Longirostres* and 3 species in section *Geniculatae*, oxalyl amino acids are not found in those species which bear more complex flowers (species in sections *Calycinae*, *Crotalaria*, *Dispermae* and *Geniculatae*, and acetyldiaminopropionic acid was only detected in one species in this group.

Section *Chrysocalycinae* subsection *Incanae* shows great similarity in morphological characters to many other sections and subsections, and for this reason has been regarded as being centrally positioned in the classification of the genus [2]. Section *Grandiflorae* and section *Crotalaria* subsection *Crotalaria* are nearest in form to section *Chrysocalycinae* [2, 8], and the amino acid pattern of section *Grandiflorae* is very similar to that of section *Chrysocalycinae* subsection *Incanae*. The main differences in amino acid distribution between these 2 taxa are: (i) γ-glutamyltyrosine occurs in most species in section *Grandiflorae* and is entirely absent from section *Chrysocalycinae* subsection *Incanae*; (ii) the ability to synthesise diaminobutyric acid may be almost entirely missing from section *Grandiflorae*, as very few species in this section contain the free amino acid or its oxalyl derivatives, whereas the oxalyl derivatives of diaminobutyric acid are found in section *Chrysocalycinae* subsection *Incanae*. The presence of γ-glutamyltyrosine, which is common throughout the genus, in section *Grandiflorae* is an indication that the section may be intermediate between section *Chrysocalycinae* subsection *Incanae* and other taxa, but the absence of diaminobutyric acid shows that it is probably not intermediate between section *Chrysocalycinae* subsection *Incanae* and sections *Crotalaria* and *Dispermae*, despite its reported close similarity in morphological features (other than flowers) to section *Crotalaria* subsection *Crotalaria* [2]. Although free diaminobutyric acid occurs in subsections *Longirostres* and *Crotalaria* of section *Crotalaria*, its oxalyl derivatives do not, and so the oxalylating system is presumably missing from the section. This absence of the oxalylating system underlines the differences between section *Grandiflorae* and section *Crotalaria* subsection *Crotalaria*, and furthermore it is an indication that the reported similarity between section *Chrysocalycinae* subsection *Incanae* and section *Crotalaria* subsection *Crotalaria* [2] does not extend to the amino acid pattern.

The similarity between sections *Grandiflorae* and *Hedriocarpae* [8] is not restricted to morphological characters, but is apparent in the amino acid data also. The ability to synthesise diaminopropionic acid and acetylate and oxalylate it is present in both sections, whereas the ability to synthesise diaminobutyric acid is almost entirely lacking from both. Most species in both sections contain γ-glutamyltyrosine, and isowillardiine, which is highly characteristic of section *Hedriocarpae* subsection *Machrostachyae*, is only found in these 2 sections.

There is a lack of information concerning the free amino acids of sections *Calycinae*, *Dispermae* and *Geniculatae*. Section *Calycinae* shows few similarities to species in other taxa due to the absence of γ-glutamyltyrosine, free diaminobutyric acid and diaminobutyric acid and diaminopropionic acid derivatives, but section *Geniculatae* shows similarities to sections *Grandiflorae* and *Hedriocarpae* as γ-glutamyltyrosine and oxalyldiaminopropionic acid are both present whereas diaminobutyric acid and its oxalyl derivatives are not. However, section *Geniculatae* exhibits considerable variation between species, and only 1 species in the section was found to contain both γ-glutamyltyrosine and oxalyldiaminopropionic acid. There is also a great deal of variation between species in section *Dispermae*, but the reported similarity between the section and section *Crotalaria* subsection *Longirostres* is partly borne out by our data. However, the presence of an oxalylating ability in some species in section *Dispermae* is an indication that it may have closer links with other taxa than previously reported.

The amino acid pattern of section *Chrysocalycinae* subsection *Incanae* varies considerably from those of section *Chrysocalycinae* subsections *Stipulosae*, *Glaucae*, *Tetralobocalyx* and *Chrysocalycinae*. The chromosome number of many species in subsection *Incanae* is 2n = 14, whereas the usual number in the genus is 2n = 16 [35], and on the basis of these 2 characters the subsection merits elevation to the rank of section. Also, the removal of subsection *Glaucae* from section *Chrysocalycinae* may be warranted by the common occurrence of free diaminobutyric acid in this subsection and its absence from subsections *Stipulosae* (except 1 sample) and *Tetralobocalyx*. The occurrence of sub-groups within section *Chrysocalycinae* has already been noted, and further studies on species from S. America and Asia may lead to a reclassification of the entire section *Chrysocalycinae*.

The presence or absence of δ-hydroxynorleucine has not been useful as a taxonomic marker for any groups of species. Its separation from other neutral amino acids on the 2D paper chromatography and high voltage paper ionophoresis systems used in this study is not very good, and so its presence is difficult to detect.

The relationships between sections and subsections based on the presence or absence of other amino acids are shown in Fig. 1.

Ecological aspects

Species which contain high concentrations of non-protein amino acids in the seeds may have been selected due to the toxicity of many of these compounds. α-Amino-β-oxalylaminopropionic acid is toxic to chicks [36], young rats, guinea pigs, dogs [37], monkeys [38] and yeasts [39], α-amino-γ-oxalylaminobutyric acid is toxic to chicks [37], α,γ-diaminobutyric acid is toxic to rats [40] and larvae of the southern cowpea weevil, *Callosobruchus maculatus* [41], δ-hydroxynorleucine is toxic to the fungi *Alternaria alternata* and *Phoma lingcam* [42] and isowillardiine is toxic to *Callosobruchus maculatus* larvae [41].

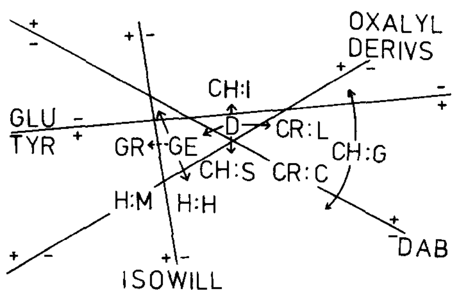

Fig. 1. Relationships between sections and subsections based on the distribution of oxalyl amino acids, α,γ-diaminobutyric acid and its derivatives, γ-glutamyl tyrosine and isowillardiine. GR, section *Grandiflorae*; CH:I, CH:S, CH:G, section *Chrysocalycinae* subsections *Incanae*, *Stipulosae* and *Glaucae*; H:H, H:M, section *Hedriocarpae* subsections *Hedriocarpae* and *Macrostachyae*; GE, section *Geniculatae*; CR:C, CR:L, section *Crotalaria* subsections *Crotalaria* and *Longirostres*; D, section *Dispermae*. Species in section *Dispermae*, and also, to a lesser extent, species in section *Chrysocalycinae* subsection *Glaucae* and section *Geniculatae*, exhibit considerable variation from the patterns common to these groups. The position of section *Geniculatae* cannot be fixed due to the lack of data concerning the distribution of isowillardiine in this section.

Many of the pyrrolizidine alkaloids which have been isolated from *Crotalaria* plants and seeds are known to be toxic to a wide range of organisms, and seeds containing these alkaloids or the amino acids listed above will be subjected to a low level of predation from organisms to which the compounds are toxic. The embryos, cotyledons and testae of seeds of *C. juncea* and *C. spectabilis* Roth, species which contain high concentrations of free amino acids and alkaloids respectively, were separated by dissection, and the seed components were extracted individually with ethanol. The extracts were analysed on the 2D paper chromatography and high voltage paper ionophoresis systems given, and the free amino acids of both species and the alkaloids of *C. spectabilis* were shown to be present in both embryos and cotyledons but almost entirely absent from the testae. (The alkaloids of *C. juncea* were at a concentration too low for detection in this study.) It is probable that the free amino acids and alkaloids of other species are also present in the embryos and cotyledons, but not the testae, of the seeds, and if some of the amino acids and alkaloids are toxic, they will afford protection to the species against insects which utilise seeds as a food source for their larvae by laying eggs in them and pathogenic microorganisms which penetrate seeds when the testa is damaged.

Although amino acids are precursors of pyrrolizidine alkaloids there is no noticeable correlation between alkaloid and free amino acid levels in *Crotalaria* seeds, except in species in section *Crotalaria* subsection *Crotalaria* and section *Chrysocalycinae* subsection *Incanae*. In the first subsection, free amino acid levels are low and alkaloid levels are high and in the second subsection, free amino acid levels are high and alkaloid levels are generally low. However, in section *Grandiflorae* both free amino acid and alkaloid levels are high, but the levels of these compounds may be a function of the effects that they both have on potential predators, and of the size of the seeds. Seeds of species in section *Crotalaria* subsection *Crotalaria* and section *Grandiflorae* are some of the largest seeds in the genus, and so represent such abundant supplies of nutrients to predators that the potential predation pressure is very high. The seeds of species in section *Crotalaria* subsection *Crotalaria* are possibly protected by high levels of alkaloids, which may be toxic, and seeds of species in section *Grandiflorae* are protected by high levels of free amino acids and alkaloids. However, although the levels of free amino acids in section *Grandiflorae* are high, the levels of free amino acids which are toxic are low, and this may also be true of the alkaloids present.

Seeds of species in section *Chrysocalycinae* subsection *Incanae*, which are generally of average size for the genus, contain low levels of alkaloids but high levels of toxic, free amino acids. Seeds of species in section *Hedriocarpae* subsection *Macrostachyae*, which are generally small, contain low levels of free amino acids and alkaloids, and of the amino acids present only isowillardiine and the oxalyl derivatives of diaminopropionic acid (which occur at low concentration in a few species) are known to be toxic.

The role of some amino acids as nitrogen stores may also have caused their accummulation in *Crotalaria* seeds to be selected for. This function may be fulfilled by diamino-acids and can possibly be ascribed to γ-glutamyltyrosine. Arginine, which occurs in high concentration in seeds of many species in section *Dispermae*, constitutes a supply of readily utilisable nitrogen, and as seeds of these species are very small the presence of a much more concentrated supply of nitrogen than would be stored in proteins may be essential in order for enough nitrogen to be available for seedling growth.

Crotalaria species in which amino acids toxic to mammals and birds are present in the seeds are listed in Table 5. Seeds and seed-bearing plants of these species are potentially toxic to livestock and poultry, and where domesticated animals are exposed to them feeding tests ought to be carried out. The presence of one or more of these amino acids in the seeds (and therefore in the seed-bearing plants) could cause the reported toxicity of *C. barkae* plants and *C. pallida* plants and seeds. In addition to the species listed in Table 5, the seeds and seed-bearing plants of species which contain high levels of pyrrolizidine alkaloids may also be toxic.

Table 5. *Crotalaria* species in which the toxic amino acids α-amino-β-oxalylaminopropionic acid, α-amino-γ-oxalylamino-butyric acid and α,γ-diaminobutyric acid are present in the seeds

Species	ODAP	ODAB	DAB
C. aculeata			(T)
C. agatiflora	(T)		(1)
C. amoena			2
C. anthyllopsis	T		
C. barnabassii	T		T
C. boehmii	2		
C. caudata			(1)
C. cleomifolia	(T)		
C. cordata			2
C. cuspidata			T-2
C. densicephala			2
C. distantiflora	(T)		
C. elisabethae	T		(T)
C. fascicularis			T
C. filicaulis			2
C. friesii			1
C. gazensis			2
C. glauca	T*		(T)
C. glaucifolia	T*		
C. goetzei			(2)
C. goreënsis		(T)	(1)
C. grandibracteata	T		
C. kapirensis			(T)
C. laburnifolia	T-1		
C. lachnocarpoïdes	(1)	(2)	(2)
C. lachnosema			2
C. lebrunii	T		
C. macaulayae	(T)	(T)	(2)
C. miranda			1
C. morumbensis	(1)		
C. orthoclada			(T)
C. pallida	T*		
C. passerinoïdes		2	
C. pycnostachya			(T-1)
C. spartea	2		
C. spartioïdes	1		
C. spinosa			(1)
C. steudneri	T		
C. tabularis			1
C. teretifolia	2		
C. vallicola			(T)
C. vanmeelii	2		
C. vasculosa	1		
All species in section *Chrysocalycinae*	1-2	2-3	(1-2)
subsection *Incanae*, except *C. phylloloba*	T*		1-2

( )—Amino acid not detected in some accessions of the relevant species. *—Amino acid detected previously [17], but concentration below the threshold of this study.

EXPERIMENTAL

Paper ionophoresis. Finely ground seed was shaken with 70% EtOH (100 mg/ml) for 65 hr. Supernatant (30 μl) was subjected to ionophoresis on Whatman 3MM paper (70 V/cm for 30 min) in buffer solns of pH 1.9 and 3.6 [43].

2D-Paper chromatography. Supernatant (120 μl) 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 [44].

Development of papers. Ionophoresis papers and chromatograms were developed with ninhydrin (0.2%, w/v, in 95% aq. Me_2CO) or Dragendorff reagent [45].

Identification of α-amino-β-acetylaminopropionic acid. Seed extracts of *C. mauënsis* (Sample 281) were co-chromatographed with a sample of authentic α-amino-β-acetylaminopropionic acid (supplied by Dr. C. S. Evans) using the 2D system above and also 1D systems, descending method, of Whatman No. 1 paper and the solvent systems PhOH–EtOH–H_2O + aq. NH_3 immediately before use (3:1:1:1, w/v/v/v) and MeOH–H_2O–Py (20:5:1) [44]. The amino acid was isolated by preparative paper ionophoresis at pH 1.9 and was hydrolysed with 6 N HCl at 110° for 3 hr. The hydrolysate was co-chromatographed with α,β-diaminopropionic acid on the 2D and 1D chromatography systems described, and was subjected to co-ionophoresis at pH 1.9 and 3.6. The hydrolysate gave the characteristic green colouration of α,β-diaminopropionic acid when papers were developed with Ehrlich reagent [44] after ninhydrin, and gave an orange colouration with $FeCl_3$ soln, a positive test for acetate ions [46]. The isolated amino acid was also co-chromatographed with α-amino-β-acetylaminopropionic acid on the 2D and 1D systems given.

Identification of γ-glutamyltyrosine. C. agatiflora (sample 290) seeds (59 g) were ground in a hammer mill and extracted overnight with 70% EtOH (300 ml). The supernatant was passed through Dowex 50-X8, 50–100 U.S. Mesh, [H^+] form, (2.6 × 10 cm), and the column effluent was used to extract the seed material. The procedure was repeated until the seed residue was exhausted (4×). Amino acids were displaced with 1 N Py (400 ml) and the eluate was evapd to dryness at room temp. Preparative ionophoresis was carried out at pH 1.9 on the amino acid mixture dissolved in H_2O (10 ml), and the isolated amino acid was co-chromatographed on the 2D system given and subjected to co-ionophoresis at pH 1.9 and 3.6 with authentic γ-glutamyltyrosine kindly provided by Dr. M. F. Wilson. Hydrolysis in 6 N HCl at 110° overnight yielded glutamate and tyrosine in 1:1 ratio (as shown using an LKB Model 4101 automatic amino acid analyser, resins and buffers as previously described [47]). A dansyl-derivative of the amino acid [48, 49] hydrolysed to dansyl-glutamate and tyrosine.

Identification of other amino and imino acids. Amino and imino acids were identified from their R_f values and ionic mobilities, and by co-chromatography with authentic standards. (α-Amino-γ-oxalylaminobutyric acid, α-oxalylamino-γ-amino-butyric acid, α-amino-β-oxalylaminopropionic acid and α-oxalylamino-β-aminopropionic acid from *Lathyrus latifolius* [27], δ-hydroxynorleucine from *Crotalaria juncea* [21], iso-willardiine from *C. ochroleuca* [23]. (supplied by Dr. D. H. G.

Crout). α,γ-Diaminobutyric acid gave a characteristic blue-green colour on development of papers with Ehrlich reagent after ninhydrin; pipecolic acid gave characteristic fluorescence with UV light after ninhydrin development.

Seed identification. Suppliers of seeds are listed in Tables 1 and 2. Vouchers for samples supplied by Dr. Polhill are held at the Royal Botanic Gardens, Kew.

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