Phytochemistry, 1977, Vol. 16, pp. 477–479. Pergamon Press. Printed in England.

THE NEUROLATHYROGEN, α-AMINO-β-OXALYLAMINOPROPIONIC ACID IN LEGUME SEEDS

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(*Revised received* 17 *September* 1976)

Key Word Index—α-Amino-β-oxalylaminopropionic acid; lathyrism; *Lathyrus*; *Crotalaria*; *Acacia*.

Abstract—The neurotoxic lathyrogen, α-amino-β-oxalylaminopropionic acid (β-oxalyldiaminopropionic acid, ODAP) accumulates in the seeds of 13 species of *Crotalaria* and 17 species of *Acacia* as well as in seeds of *Lathyrus*. The compound was not detected in seeds representing 250 other legume genera—these are listed.

INTRODUCTION

Classical neurolathyrism in man, which continues to be a problem in the subcontinent of India, is characterized by irreversible paralysis of the legs and in extreme cases death. Similar effects are seen in cattle and horses. The disease is most frequently associated with the ingestion of the seeds of *Lathyrus sativus* and less frequently with those of *L. cicera* and *L. clymenum* [1]. The neurotoxic amino acid α-amino-β-oxalylaminopropionic acid (β-oxalyldiaminopropionic acid, ODAP) has been isolated from seeds of *Lathyrus sativus* [2,3], and identified in the seeds of other species of *Lathyrus* including those of *L. cicera* and *L. clymenum* [4]. The compound is synthesized in *L. sativus* by the transfer of an oxalyl group from oxalyl coenzyme A to L-α,β-diaminopropionic acid [5], and it has been suggested that its toxicity in animals may result from lysosomal damage in the brain [6].

In human populations neurolathyrism usually develops when seed meal of one of the *Lathyrus* species concerned, forms a major part of the diet. The proportion of seed meal in the diet, and the period over which the seed meal remains part of the diet prior to the onset of neurological symptoms is very variable [7]. Such variability is in agreement with the finding of Roy and Narasinga Rao [8], that different samples of *L. sativus* seed collected from areas where lathyrism is endemic showed a variation of ODAP content from 0.1 to 2.5%. As *L. sativus* is a good source of protein, contains high concentrations of free L-homoarginine, which can act as a precursor of the essential amino acid lysine in higher animals [9], and grows well in parts of the world where food is in short supply, the development of toxin-free or toxin-low varieties of this legume may provide a more realistic approach to the elimination of lathyrism than the replacement of *L. sativus* by an exotic species less well adapted to local conditions and possibly less acceptable to the local people. Efforts to improve *L. sativus* in this way are being made [10]. In addition to those species of *Lathyrus* which have been reported to contain ODAP [4], the neurotoxin has also been identified chromatographically in the seeds of two species of *Crotalaria* [11]. As part of a general survey of the distribution of uncommon amino acids and particularly of uncommon amino acids with physiological activity in legume seeds we have analysed seeds from over 250 genera of this family, and now report that ODAP has been found in the seeds of 13 species of *Crotalaria* and 17 species of *Acacia*. The identity of the oxalylamino acid has been confirmed by co-chromatography, co-ionophoresis and by isolating it from representative species of *Crotalaria* and *Acacia*. These findings seem to be of economic importance, as certain of the ODAP-containing species of *Crotalaria* are toxic to domestic animals and birds, and as attention is being paid to the possibility of cultivating *Acacia* species as crops for human and animal nutrition in the semi-arid regions of the world.

RESULTS AND DISCUSSION

Legume genera without detectable concentrations of ODAP in the seeds of their species. The number of species of each genus analysed is shown in parentheses.

Papilionoideae

Abrus (2), *Adenodolichos* (1), *Aeschynomene* (1), *Alhagi* (1), *Alysicarpus* (4), *Amorpha* (1), *Amphicarpa* (1), *Amphimas* (2), *Anagyris* (1), *Andira* (1), *Angylocalyx* (1), *Anthyllis* (2), *Aotus* (1), *Astragalus* (9), *Ateleia* (1), *Atylosia* (2), *Baphia* (1), *Baptisia* (1), *Bolusanthus* (1), *Bossiaea* (2), *Brachysema* (1), *Brongniartia* (1), *Burtonia* (1), *Butea* (1), *Cajus* (2), *Calicotome* (1), *Calopogonium* (2), *Calpurnia* (2), *Camoensia* (1), *Canavalia* (2), *Caragana* (8), *Carmichaelia* (3), *Castanospermum* (1), *Centrolobium* (1), *Centrosema* (1), *Chordospartium* (1), *Chorizema* (2), *Cicer* (1), *Cladastris* (1), *Clianthus* (2), *Clitoria* (4), *Colutea* (3), *Coronilla* (1), *Cyamopsis* (1), *Cytisus* (5), *Dalbergia* (3), *Dalea* (2), *Daviesia* (2), *Desmodium* (8), *Dillwynia* (1), *Dioclea* (1), *Dipteryx* (1), *Dorycnium* (1), *Drepanocarpus* (1), *Eriosema* (3), *Erythrina* (10), *Eutaxia* (2), *Flemingia* (2), *Galactia* (2), *Galega* (1), *Genista* (2), *Gliricidia* (1), *Glycine* (2), *Glycyrrhiza* (1), *Gompholobium* (1), *Gonocytisus* (1), *Goodia* (2), *Halimodendron* (1), *Hardenbergia* (2), *Hedysarum* (4), *Hippocrepis* (1), *Hovea* (1), *Humularia* (1), *Hymenocarpos* (1), *Hypocalyptus* (4), *Indigofera* (8), *Jacksonia* (1), *Kennedia* (7), *Lablab* (1), *Laburnum* (1), *Lens* (1), *Leptoderris* (1), *Lespedeza* (3), *Lessertia* (1), *Lonchocarpus* (5), *Lotononis* (1), *Lotus* (1), *Lupinus* (3), *Maackia* (1), *Machaerium* (1), *Medicago* (4), *Melilotus* (1), *Millettia* (5), *Mucuna* (6), *Mundulea* (1), *Myroxylon* (1), *Notospartium* (1), *Onobrychis* (7), *Ononis* (3), *Ophrestia* (1), *Ormocarpum* (1), *Ormosia* (6), *Ornithopus* (1), *Oxylobium* (2), *Oxytropis* (1), *Pachyrizus* (2), *Petalostemon* (2), *Pericopsis* (1), *Phyllota* (1), *Piptanthus* (3), *Piscidia* (1), *Pisum* (1), *Platylobium* (1), *Podalyria* (2), *Poecilanthe* (2), *Pongamia* (1), *Priestleya* (1), *Pseudarthria* (1), *Psophocarpus* (1), *Psoralea* (1), *Pterocarpus* (6), *Pueraria* (2), *Pulteana* (1), *Retama* (1), *Rhynchosia* (3), *Robinia* (1) *Scorpiurus* (2), *Securigera* (1), *Sesbania* (10), *Sophora* (4), *Spartium* (1), *Sphaerophysa* (1), *Stahlia* (1), *Strongylodon* (1), *Stylosanthes* (2), *Sutherlandia* (1), *Swainsona* (5), *Swartzia* (3), *Templetonia* (1), *Tephrosia* (9), *Teramnus* (1), *Tetragonolobus* (2), *Thermopsis* (2), *Tipuana* (2), *Trifolium* (3), *Trigonella* (5), *Uraria* (1), *Vicia* (4), *Vigna* (12), *Viminaria* (1), *Virgilia* (2), *Voandzeia* (1), *Wiborgia* (1), *Xanthocercis* (1), *Xeroderris* (1), *Zornia* 2(2).

Caesalpinioideae

Acrocarpus (1), *Afzelia* (2), *Baikiaea* (1), *Brachystegia* (1), *Brownea* (3), *Bauhinia* (12), *Burkea* (1), *Bussea* (1), *Caesalpinia* (15), *Campsiandra* (1), *Cassia* (19), *Ceratonia* (1), *Cercis* (1), *Colophospermum* (1), *Colvillea* (1), *Cordyla* (1), *Crudia* (2), *Cryptosepalum* (1), *Cynometra* (1), *Daniella* (1), *Delonix* (1), *Dialium* (2), *Eperua* (1), *Erythrophloeum* (2), *Gleditsia* (4), *Guibourtia* (1), *Gymnocladus* (3), *Haematoxylum* (1), *Humboldtia* (1), *Hylodendron* (1), *Hymenaea* (2), *Hymenostegia* (1), *Intsia* (3), *Isoberlinia* (1), *Julbernardia* (2), *Koompassia* (1), *Leonardoxa* (1), *Lysidice* (1), *Macrolobium* (2), *Maniltoa* (1), *Mora* (2), *Pahudia* (1), *Paramacrolobium* (1), *Parkinsonia* (1), *Peltogyne* (2), *Peltophorum* (5), *Petalostylis* (1), *Phanera* (1), *Piliostigma* (1), *Poeppigia* (1), *Prioria* (1), *Pseudocopaiva* (1), *Saraca* (4), *Schizolobium* (2), *Schotia* (2), *Sindora* (3), *Tachigalia* (1), *Talbotiella* (1), *Tamarindus* (1), *Tylosema* (1), *Wagatea* (1).

Mimosoideae

Adenanthera (1), *Albizia* (12), *Amblygonocarpus* (1), *Amblygonocarpus* (1), *Anadenanthera* (1), *Calliandra* (5), *Cathormion* (1), *Desmanthus* (1), *Dichrostachys* (1), *Elephantorrhiza* (1), *Entada* (5), *Enterolobium* (3), *Inga* (1), *Leucaena* (2), *Mimosa* (9), *Neptunia* (1), *Newtonia* (2), *Parapiptadenia* (2), *Parkia* (9), *Pentaclethra* (1), *Piptadenia* (1), *Pithecellobium* (9), *Prosopis* (1), *Samanea* (1), *Schrankia* (1), *Tetrapleura* (1).

Legume genera with species containing ODAP in their seeds:

Crotalaria. Species with high concentrations (>0.25% of ODAP). *C. barkae* Schweinf., *C. incana* L., *C. mauensis* Bak. f., *C. polysperma* Kotschy, *C. quartiniana* A. Rich. Species with low concentrations (<0.25%). *C. burkeana* Benth., *C. doniana* Baker, *C. glauca* Willd., *C. glaucifolia* Baker, *C. lotoides* Benth., *C. pallida* Ait., *C, phylloba* Harms, *C. simulans* Milne-Redh. Species in which ODAP was not detected. *C. aculeata* De Wild., *C. agatiflora* Schweinf., *C. anagyroides* Kunth, *C. argyraea* Welw. ex Baker, *C. axillaris* Ait., *C. balbi* Chiov., *C. barnabassii* Dinter ex Bak. f., *C. berteroana* DC., *C. brevidens* Benth., *C. burttii* Bak. f., *C. capensis* Jacq., *C. caudata* Welw. ex Baker, *C. cleomifolia* Welw. ex Baker, *C. comanestiana* Volkens & Schweinf., *C. crassipes* Hook., *C. cuspidata* Taub., *C. deflersii* Schweinf., *C. elisabethae* Bak. f., *C. emarginata* Boj. ex Benth., *C. goetzei Harms*, *G. grantiana* Harv., *C. greenwayi* Bak. f., *C. guatemalensis* Benth., *C. juncea* L., *C. kirkii* Baker, *C. laburnoides* Klotzsch., *C. lachnophora* A. Rich., *C. lanceolata* E. Mey., *C. lupulina* Kunth, *C. macaulayae* Bak. f., *C. medicaginea* Lam., *C. peschiana* Duvign. & Timp., *C. petitiana* (A. Rich.) B.D. Jackson, *C. podocarpa* DC., *C. pycnostachya* Benth., *C. quinquefolia* L., *C. recta* Steud. ex A. Rich., *C. retusa* L., *C. rosenii* (Pax) Milne-Redh. ex Polhill, *C. sagittalis* L., *C. scassellatii* Chiov., *C. sessiflora* L., *C. spectabilis* Roth, *C. tetragona* Roxb., *C. umbellata* Wight., *C. vallicola* Bak. f., *C. vatkeana* Engl., *C. verdcourtii* Polhill, *C. verrucosa* L., *C. walkeri* Arn., *C. zanzibarica* Benth.

Acacia species with ODAP. All species contained high concentrations >0.25% of ODAP. *A. albida* Delile, *A. ataxacantha* DC., *A. catechu* (L). Willd., *A. confusa* Merr., *A. coulteri* Benth., *A. erubescens* Welw. ex Oliv., *A. ferruginea* DC., *A. galpinii* Burtt Davy, *A. hamulosa* Benth., *A. mellifera* (Vahl.) Benth., subsp. *detinens* (Burch.) Brenan, *A. modesta* Wall., *A. nigrescens* Oliv., *A. polyacantha* Willd. subsp. *campylacantha* (A. Rich.) Brenan, *A. rovumae* Oliv., *A. senegal* (L) Willd., *A. venosa* Hochst. ex Benth., *A. welwitschii* Oliv. subsp. *delagoensis* (Harms) Ross & Brenan. Species in which ODAP was not detected. *A. arabica* (Lam.) Willd., *A. auriculiformis* A. Cunn. ex Benth., *A. binervata* DC., *A. bivenosa* DC., *A. bonariensis* Gill., *A. brevispica* Harms, *A. caesia* Wight & Arn., *A. caesiella* Maiden & Blakely, *A. calamifolia* Sweet ex Lindl., *A. circinnata* F. Muell., *A. clivicola* Pedley., *A. dealbata* Link, *A. dunnii* (Maiden) Turrill, *A. elata* A. Cunn ex Benth., *A. erioloba* E. Mey (*A. giraffae* auct.), *A. farnesiana* (L.) Willd., *A. gerrardii* Benth., *A. glomerosa* Benth., *A.*, *greggii* Gray, *A. holosericea* A. Cunn. ex G. Don., *A. karroo* Hayne, *A. kraussiana* Meisn. ex Benth., *A. longifolia* (Andr.) Willd. var. *longifolia*, *A. mearnsii* De Wild., *A. meisneri* Lehmann ex Meisn., *A. melanoxylon* R. Br., *A. monticola* J. M. Black., *A. nilotica* (L.) Willd. ex Delile subsp. *kraussiana* (Benth.) Brenan, *A. oxycedrus* Sieber ex DC., *A. parramattensis* Tindale, *A. pentagona* (Schumach. & Thonn.) Hook. f., *A. peuce* F. Muell., *A. plumosa* Lowe, *A. polybotrya* Benth., *A. polyphylla* DC., *A. pruinocarpa* Tindale, *A. pyrifolia* DC., *A. riparia* Kunth, *A. schweinfurthii* Brenan & Exell, *A. roemerana* Scheele., *A. sieberana* DC. var. *woodii* (Burtt Davy) Keay & Brenan, *A. stricta* (Andr.) Willd., *A. stuhlmannii* Taub., *A. suaveolens* (Sm.) Willd., *A. tenuissima* F. Muell., *A. tortilis* (Forssk.) Hayne subsp. *heteracantha* (Burch.) Brenan, *A. tumida* F. Muell., *A. velutina* DC., *A. verniciflua* A. Cunn., *A. victoriae* Benth., *A. wrightii* Benth.

Lathyrus. As previously reported high concentrations of ODAP were found in the seeds of 15 species, low concentrations in 6 species and none in 20 species [4].

The presence of ODAP in seeds of the *Crotalaria* species listed indicates that toxicity to mammals and/or birds caused by ingestion of *Crotalaria* plants may be due to the presence of compounds other than pyrrolizidine alkaloids, which are known to be present in many species. *C. burkeana* [12], *C. incana* [13], *C. pallida* (*C. mucronata* Desv., *C. striata* DC.) [14], *C. polysperma* [12] and *C. quartiniana* [12], all of which contain ODAP in their seeds have been reported to be toxic to domestic animals and/or birds. The variability of ODAP content found in seeds of *L. sativus* [8] may be paralleled in other ODAP-containing species and we have listed certain *Crotalaria* species as containing high and others low concentrations of ODAP in their seeds; we have not however analysed a sufficient number of individual seeds from a single species to determine how much variability exists in any single *Crotalaria* or *Acacia* species. Our findings indicate that *Acacia* species which accumulate ODAP in their seeds are those included by Bentham in his series *Vulgares* together with *A. confusa* and *A. albida* which he classified as *Phyllodineae* and *Gummiferae* respectively. The finding of ODAP in seeds of *A. albida* is of particular interest as this species is reported to have "great promise as a forage plant in areas with a prolonged dry season" and the seeds which "contain up to 27% crude protein are eaten by people in Rhodesia during times of famine" [15].

EXPERIMENTAL

Paper ionophoresis. Finely ground seed (200 mg) was shaken with 70% EtOH (1 ml) for 24 hr. After standing for a further 17 hr the suspension was centrifuged and the supernatant subjected to ionophoresis on Whatman 3MM paper (70 V/cm for 30 min) in buffer solns of pH 1.9 and 3.6 [16].

PC. Supernatant soln (0.5 ml) prepared as above was passed through a column (5 × 1 cm) of Dowex 50 W × 8 (H^+ form). After washing with H_2O the amino acids were displaced from the column with 2 M NH_4OH (20 ml). The ammoniacal soln was evaporated to dryness and the residue redissolved in 70% EtOH (0.5 ml). The mixture of crude amino acids was subjected to descending PC on 3MM paper. Solvents used [17] were (1) *n*-BuOH–HOAc–H_2O (12:3:5) for 48 hr; (2) PhOH–H_2O (4:1, w/v) in presence of the vapour of aq. NH_3 (sp. gr. 0.88) for 48 hr; (3) PhOH–EtOH–H_2O (3:1:1, w/v/v), to which 1 vol. of NH_3 (sp. gr. 0.88) was added before use, for 48 hr; (4) MeOH–H_2O–pyridine (20:5:1) for 48 hr. 2D chromatograms were prepared by the ascending method on Whatman No. 1 paper using solvents (1) and (2), 17 hr in each solvent.

Isolation and identification of ODAP. ODAP in extracts of *Crotalaria incana* was isolated by ion exchange chromatography as previously described [18]. ODAP in extracts of *Acacia modesta* was isolated by eluting the compound from paper after ionophoresis at pH 3.6. The purified isolates from *C. incana* and *A. modesta* were co-chromatographed with authentic ODAP in the solvents described. They were subjected to co-ionophoresis with authentic ODAP at pH 1.9, 3.6 and 6.5. The PMR spectra of the isolated and authentic ODAP were identical. On hydrolysis the isolates gave α,β-diaminopropionic acid and oxalic acid which were identified as described previously [11].

Quantitative values. Approximate concentrations of ODAP in seed extracts were determined by simultaneous ionophoresis of the extracts and standard solns of authentic ODAP.

Acknowledgements—We thank the Science Research Council for financial support and Dr. B. A. Krukoff (New York), Dr. R. M. Polhill (Royal Botanic Gardens, Kew), Dr. M. D. Tindale (Royal Botanic Gardens, Sydney, Australia) and Dr. P. R. Shewry (Rothamsted Experimental Station) for gifts of seed.

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