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OCCURRENCE OF THE PYRROLIZIDINE ALKALOID MONOCROTALINE IN *CROTALARIA* SEEDS

D.J. PILBEAM, A.J. LYON-JOYCE,

Department of Plant Sciences, University of Leeds, Leeds LS2 9JT, UK

and E.A. BELL

Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AB, UK

ABSTRACT.—The pyrrolizidine alkaloid monocrotaline has been identified in seeds of *Crotalaria aegyptiaca*, *C. cephalotes*, *C. cunninghamii*, *C. nitens*, *C. paulina*, and *C. recta*, but was not detected in extracts prepared from seeds of a further 74 species (detection limit, 0.5%). Monocrotaline has not been found previously in the seeds of these six species, but they are closely related taxonomically to the species already shown to contain the alkaloid in their seeds.

The pyrrolizidine alkaloid monocrotaline is a hepatotoxic carcinogen (1) that can inhibit the growth of transplanted tumors in white mice (2,3). Despite the occurrence of other pyrrolizidine alkaloids in many plant genera, monocrotaline has been reported only in species of the genus *Crotalaria* L. (family Leguminosae) and in *Lindelofia spectabilis* Lehm. (Boraginaceae) (4,5).

Most of the *Crotalaria* species containing monocrotaline are positioned in two closely related groupings within the genus, section *Calycinae* Wight and Arn. and section *Crotalaria* subsection *Crotalaria*. These two taxa contain approximately 20% of the 600 *Crotalaria* species, and so the ability to synthesize and accumulate monocrotaline appears to be highly restricted even within *Crotalaria*. Much of the research on the distribution of pyrrolizidine alkaloids in *Crotalaria* has been carried out in India and Australia, however, and many of the indigenous species of these two countries are positioned in sections *Calycinae* and *Crotalaria* subsection *Crotalaria*. It seemed possible that the apparent restriction of the alkaloid to these two taxa might be the result of limited sampling rather than limited alkaloid distribution.

In many instances where monocrotaline has been identified in *Crotalaria* species, the seeds have been the organ investigated, and the alkaloid may accumulate in seeds to high concentration (see table 1).

During the course of an investigation into how the distribution of free amino acids in *Crotalaria* seeds is related to the taxonomy of the genus (6,7), we noted that seeds of species in sections *Calycinae* and *Crotalaria* subsection *Crotalaria* are not characterized by distinctive patterns of free amino acid accumulation, unlike some other sections and subsections of the genus.

However, if the accumulation of monocrotaline in *Crotalaria* seeds is limited to species positioned in these two taxa, this accumulation would constitute a useful taxonomic marker. Consequently, we screened the seeds of 82 species from all the sections and subsections of the genus, and we now report that monocrotaline was found to accumulate to the threshold concentration of our study (0.5%) in seeds of only eight of these species. The taxonomic significance of this restricted distribution of monocrotaline accumulation is discussed.

RESULTS AND DISCUSSION

Seed extracts were co-chromatographed with authentic monocrotaline on thin layers, on paper, and by gc and were subjected to co-ionophoresis on paper in sodium borate buffer.

C. aegyptiaca Benth., *C. cephalotes* Steud. ex A. Rich., *C. cunninghamii* R. Br., *C. ni-*

tens Kunth, *C. paulina* Schrank, and *C. recta* Steud. ex A. Rich. were found to contain at least 0.5% (the threshold concentration for our study) of monocrotaline in their seeds, and this is the first report of the alkaloid being present in seeds of these six species. In addition, seeds of *C. assamica* Benth. and *C. quinquefolia* L. were shown to contain monocrotaline as at least 0.5% of their weight. The alkaloid has previously been isolated from the aerial parts of *C. aegyptiaca* and *C. recta* (8, 9) and has been reported as occurring in seeds of *C. assamica* and *C. quinquefolia* but no concentration was stated (3, 5). Monocrotaline was the major alkaloid present in seeds of all eight species, but in *C. aegyptiaca*, *C. cephalotes*, *C. cunninghamii*, and *C. paulina*, at least one other alkaloid was detected.

Crotalaria species previously shown to contain monocrotaline in their seeds are listed in table 1. Species in which monocrotaline was not found to occur above the concentration threshold of our study are listed in appendix 1.

African species of the genus (430 in number) have been classified by Polhill (10). As this is the most extensive classification of the genus, it is convenient to consider how the species from Asia, Australia, and America may be related to the African species. The works of Wight and Walker-Arnott (11), Bentham (12,13), and J.G. Baker (14), which cover most of the Asian and Australian species, and Senn (15) and de Munk (16), which cover the smaller number of species found in North America and Malaysia, respectively, are used to establish taxonomic relationships.

Of the African species that accumulate monocrotaline in their seeds, *C. aegyptiaca* is positioned in section *Calycinae*; *C. recta* and *C. retusa* are positioned in section *Crotalaria* subsection *Crotalaria*; and *C. cephalotes* is positioned in section *Dispermae* Wight and Arn. (10). The Asian species *C. spectabilis* Roth (*C. sericea* Retz.) is closely related to *C. retusa* (11,12,14-16) as are the Asian species *C. assamica* (12,14,16) and *C. leschenaultii* (11,12,14).

C. quinquefolia and *C. grahamiana*, both from Asia, were positioned close to each other by many authorities (11,12,14), but it was recognized that they differed from other species examined. However, Polhill regards *C. quinquefolia* as being typical of section *Crotalaria* subsection *Crotalaria* (7,17), and *C. grahamiana* may, therefore, also be closely related to species in the subsection. The Australian species *C. cunninghamii* is also positioned in this subsection (7); Bentham (13) positioned *C. cunninghamii* with two other Australian species, *C. novae-hollandiae* DC. and *C. crassipes* Hooker [now regarded as a synonym of *C. novae-hollandiae* subspecies *novae-hollandiae* (18)], but we did not detect monocrotaline in seeds tentatively identified as either *C. crassipes* or *C. novae-hollandiae*, despite the fact that the alkaloid has been reported in plants of *C. novae-hollandiae* subspecies *lasiophylla* (Benth.) A. Lee (19).

The American species *C. nitens* and *C. paulina* have been included in section *Calycinae* (7), although Bentham positioned *C. nitens* close to *C. retusa* (12).

The Asian species *C. leioloba* Bartl. (*C. ferruginea* Grah.) and *C. stipularia* Desv. may also be close to section *Calycinae*. *C. mysorensis* Roth, which has not been found to accumulate monocrotaline to the threshold concentration of our study, is a member of the section (11,12,14), and *C. leioloba* has been positioned close to *C. mysorensis* by de Munk (16). However, de Munk listed only a few species, and the more extensive works of Bentham and Baker (12,14) did not link the two species together. *C. sagittalis* L. (another species that has been found by us not to accumulate monocrotaline) is regarded as being close to section *Calycinae* (7), and *C. stipularia* was positioned with *C. sagittalis* by Bentham (12).

Of the 82 species examined by us, seed extracts of 62 species gave distinct Dragendorff-positive spots on tlc (appendix 1) and so the sensitivity of our technique is adequate to detect pyrrolizidine alkaloids at the high concentrations encountered in seeds

of many *Crotalaria* species. The accumulation of monocrotaline was limited to species in section *Calycinae* (six species tested, three contained monocrotaline), section *Crotalaria* subsection *Crotalaria* (nine species tested, four contained monocrotaline), and section *Dispermae* (three species tested, one contained monocrotaline), and the alkaloid was not detected in any of the 64 species from the other taxa tested.

This is the first report of the alkaloid being present in a species positioned in section *Dispermae*; the section contains a large number of species and should be further investigated. Other than this one report, the ability to accumulate monocrotaline in the seeds is restricted to species in section *Calycinae* and section *Crotalaria* subsection *Crotalaria*. The detection of high concentrations of monocrotaline in the seeds of any *Crotalaria* species may immediately establish that it is closely allied to species positioned in these two taxa.

The two taxa are similar to each other (10), not least in this ability to accumulate monocrotaline, but not all their component species accumulate the alkaloid. It is likely that the ability to accumulate monocrotaline arose once in the genus, and although the plants concerned have diverged and spread widely since that time, they still retain many characteristics in common. Some of these plants have subsequently lost the ability to synthesize or accumulate the alkaloid (represented in our study by those species in sections *Calycinae* and *Crotalaria* subsection *Crotalaria* in which monocrotaline was not detected). It is possible, however, that these non-accumulators have arisen from different stock plants that never were able to synthesize or accumulate the alkaloid, and their morphological similarities to monocrotaline-containing species may have arisen by convergent evolution.

TABLE 1. *Crotalaria* species previously shown to contain monocrotaline in their seeds

Plant	Monocrotaline Concentration	Reference
C. assamica Benth.	[a]	3
C. grahamiana Wight and Arn.	1.89% of seed weight as alkaloids, monocrotaline is major component	24, 25
C. leschenaultii DC.	1.4 g monocrotaline isolated from 100 g seeds	26
C. leioloba Bartl.	0.52% of seed weight as alkaloids, mainly monocrotaline	27
C. quinquefolia L.	[a]	5
C. retusa L.	5.8% of seed weight	28, 29
C. spectabilis Roth	2.9% of seed weight	30, 31
C. stipularia Desv.	0.70% of seed weight as alkaloids, mainly monocrotaline	27

[a]No values for weight of monocrotaline in seeds was given. We detected the presence of monocrotaline in seeds of *C. assamica* and *C. quinquefolia*, and so the concentration of monocrotaline in seeds of these species is greater than the 0.5% threshold of our study.

C. mysorensis Roth 0.09% of seed weight as alkaloids, mainly monocrotaline (32); *C. sagittalis* L. 0.008% yield of monocrotaline from dried fruit (33). Both values below the threshold concentration of our study.

EXPERIMENTAL

Seed samples used are listed in text (species shown to contain monocrotaline) or in appendix 1 (species in which monocrotaline was not detected). *C. aegyptiaca* was supplied by the Botanic Gardens, Hebrew University of Jerusalem; *C. assamica* by the Genetic Resources section, CSIRO Division of Tropical Crops and Pastures, Samford, Australia; *C. cephalotes* by Jardin Botanique de l'Université de Liège; *C. cunninghamii*, *C. novae-hollandiae*, and *C. sagittalis* by Dr. B.A. Krukoff, New York Botanic Garden (vouchers for first two are held at King's Park and Botanic Garden, Perth); *C. nitens* and *C. paulina* by Dr. F. Bisby, Department of Biology, University of Southampton; *C. quinquefolia* and *C. recta* by Dr. R.M. Polhill, Royal Botanic Gardens, Kew. Vouchers are held at the parent organizations unless stated to the contrary. Suppliers of seeds listed in appendix 1 are listed in previous publications (6,7).

Finely ground seed was shaken with 70% ethanol (100 mg cm^{-3}) for 65 h. The supernatant (30 μl) was subjected to tlc (system A), and those samples giving Dragendorff-positive spots at positions close to monocrotaline were subjected to co-chromatography on thin layers (30 μl) (systems A and B), paper (60 μl), and gc (6 μl), and co-ionophoresis on paper (40 μl) with monocrotaline (kindly supplied by Dr. D.H.G. Crout).

TLC.—Silica gel G, 250 μ. Solvents were (A) chloroform-methanol-0.88 aqueous ammonia (85:14:1) v/v/v (20), the Rf of monocrotaline was 0.46; and (B) chloroform-acetone-ethanol-0.88 aqueous ammonia (5:3:1:1) v/v/v/v (21); Rf of monocrotaline: 0.42.

PC.—Whatman No. 1 paper was used with butan-1-ol-acetic acid-water (80:3:17) v/v/v, ascending method (22); Rf of monocrotaline: 0.39.

GC.—Pye Unicam Pu 4500 was used with 1.5 m silanized glass column, ID 4 mm, 3% E-30 on silanized Poropak Q at 205°, 40 cm^3 N_2 min^{-1}, fid attenuation 5 12 (22). Retention time of monocrotaline was 23 min.

IONOPHORESIS.—Shandon Southern L24 was used, with Whatman 3MM paper, sodium borate pH 9.2 buffer at 25 V cm^{-1} for 130 min. Monocrotaline runs as an anion in this buffer, whereas 19 of 27 pyrrolizidine alkaloids tested have been shown to run as cations (23).

All plates and papers were sprayed with Dragendorff reagent for the detection of alkaloids. Monocrotaline concentrations in excess of 0.5% of seed weight were detected.

APPENDIX 1.

Crotalaria species in which monocrotaline accumulation in the seeds was not detected are listed herein. (Species listed here may contain monocrotaline at concentrations below the threshold of our study.)

Species in which alkaloids other than monocrotaline were detected: *C. aculeata* De Wild., *C. anagyroides* Kunth, *C. anthyllopsis* Welw. ex Baker, *C. atrorubens* Hochst. ex Benth., *C. barnabassii* Dinter ex Bak. f., *C. brevidens* Benth., *C. breviflora* DC., *C. capensis* Jacq., *C. cleomifolia* Welw. ex Baker, *C. cuspidata* Taub., *C. cylindrica* A. Rich., *C. damarensis* Engl., *C. deflersii* Schweinf., *C. densicephala* Welw. ex Baker, *C. dissitiflora* Benth., *C. durandiana* Wilczek, *C. germainii* Wilczek, *C. goetzei* Harms, *C. goreensis* Guill. & Perr., *C. guatemalensis* Benth., *C. impressa* Nees, *C. incana* L., *C. juncea* L., *C. laburnifolia* L., *C. laburnoides* Klotzsch, *C. lachnocarpoides* Engl., *C. lachnophora* A. Rich., *C. lachnosema* Stapf., *C. lanceolata* E. Mey., *C. lasiocarpa* Polhill, *C. longirostrata* Hook. & Arn., *C. longithyrsa* Bak. f., *C. lunulata* Heyne, *C. macaulayae* Bak. f., *C. macrocarpa* E. Mey., *C. mauensis* Bak. f., *C. maypurensis* Kunth, *C. medicaginea* Lam., *C. mildbraedii* Bak. f., *C. natalitia* Meissn., *C. novae-hollandiae* DC., *C. ochroleuca* G. Don, *C. ononoides* Benth., *C. orthoclada* Welw. ex Baker, *C. pallida* Ait., *C. peschiana* Duvign. & Timp., *C. pilosa* Miller, *C. podocarpa* DC., *C. rhodesiae* Bak. f., *C. rosenii* (Pax) Milne-Redh. ex Polhill, *C. semperflorens* Vent., *C. sphaerocarpa* Perr. ex DC., *C. stolzii* (Bak. f.) Milne-Redh. ex Polhill, *C. vatkeana* Engl.

Species in which no alkaloids were detected: *C. amoena* Welw. ex Baker, *C. balbi* Chiov., *C. burtii* Bak. f., *C. comanestiana* Volkens & Schweinf., *C. comosa* Baker, *C. cylindrocarpa* DC., *C. deserticola* Taub. ex Bak. f., *C. emarginata* Boj. ex Benth., *C. glauca* Willd., *C. greenwayi* Bak. f., *C. kapirensis* De Wild., *C. kirkii* Baker, *C. lukwangulensis* Harms, *C. petitiana* (A. Rich.) Walp., *C. phylloloba* Harms, *C. pisicarpa* Welw. ex Baker, *C. quartiniana* A. Rich., *C. sagittalis* L., *C. spinosa* Hochst. ex Benth., *C. zanzibarica* Benth.

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