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The chemotaxonomic significance of prunasin in *Buchenroedera* (Fabaceae — Crotalarieae)

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Lotononis and *Buchenroedera* are the only genera of the tribe Crotalarieae so far known to be cyanogenic. A recent survey (van Wyk 1989) has shown that at least 57 species of the former and four of the latter react positively to the Feigl–Anger test. Together with obvious similarities to *Lotononis* in morphology, chromosome cytology and alkaloids this raises doubts about the generic status of *Buchenroedera*. The chemical basis for cyanogenesis in *Buchenroedera* is here reported for the first time. A glucoside from the leaves of *B. multiflora* Eckl. & Zeyh. was isolated and identified as prunasin, a compound previously detected in *L. crumanina* Burch. ex Benth. This result shows that cyanogenesis is homologous in *Buchenroedera* and *Lotononis* and provides further evidence that the two genera should be combined.

Lotononis en *Buchenroedera* is sovêr bekend die enigste genera van die tribus Crotalarieae wat sianogenies is. 'n Onlangse opname (Van Wyk 1989) het getoon dat ten minste 57 spesies van eersgenoemde genus en vier spesies van laasgenoemde positief reageer op die Feigl–Anger-toets. Saam met ooglopende ooreenkomste met *Lotononis* in morfologie, chromosoomsitologie en alkaloïede ontstaan twyfel oor die generiese status van *Buchenroedera*. Die chemiese basis van sianogenese in *Buchenroedera* word hier vir die eerste keer gerapporteer. 'n Glukosied is uit die blare van *B. multiflora* Eckl. & Zeyh. geïsoleer en geïdentifiseer as prunasien, 'n verbinding wat voorheen in *L. crumanina* Burch. ex Benth. waargeneem is. Hierdie resultaat toon dat sianogenese in *Buchenroedera* en *Lotononis* homoloog is en bied verdere aanduiding dat die twee genera gekombineer behoort te word.

Keywords: *Buchenroedera*, cyanogenesis, generic relationships, *Lotononis*, prunasin

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Introduction

In a preliminary survey of cyanogenesis in several genera and species of the tribe Crotalarieae (van Wyk 1989), only two genera were found to react positively to the spot test of Feigl & Anger (1966) as modified by Tantisewie *et al.* (1969), i.e. four species of *Buchenroedera* Eckl. & Zeyh. and 57 species of *Lotononis* (DC.) Eckl. & Zeyh. This similarity was interpreted as supporting evidence for the idea that *Buchenroedera* is indistinct from *Lotononis* at the generic level. Morphological evidence (Polhill 1976), chromosome numbers (van Wyk & Schutte 1988) and alkaloids (van Wyk & Verdoorn 1988, 1989) have shown that *Buchenroedera* is almost identical to the section *Krebsia* (Eckl. & Zeyh.) Benth. of *Lotononis* except for the shape of the fruit. Using chromatographic methods, Fikenscher & Hegnauer (1981) reported that prunasin was the cyanogenic glucoside of *Lotononis crumanina* Burch. ex Benth. This is the only evidence for the source of HCN in *Lotononis* — none of the other four species previously reported to be cyanogenic (Watt & Breyer-Brandwijk 1962) have been studied by analytical methods.

In this paper we report on an investigation into the chemical basis for cyanogenesis in *Buchenroedera*. In the Fabaceae, prunasin is less common than linamarin and lotaustralin and we argue that the presence of prunasin would leave little doubt that cyanogenesis is homologous in *Buchenroedera* and *Lotononis*.

Materials and Methods

Buchenroedera multiflora Eckl. & Zeyh. was chosen for this study because of its rapid positive reaction to the Feigl–Anger test. [Voucher specimen: Zuurberg National Park, E. Cape, *B. & M. van Wyk 1523* (JRAU, PRE)].

An enzyme preparation consisting of 1 mg β-glucosidase (prepared from sweet almonds; Boehringer-Mannheim) in 1 cm^3 phosphate buffer (0.5 mol dm^{-3}, pH 6.8) was used to test for the presence of the cyanogen throughout the following procedures. Release of HCN was detected with paper strips prepared by the method of Feigl & Anger (1966) as modified by Tantisewie *et al.* 1969.

Fresh leaves and twigs (100 g) were boiled in 95% ethanol for several hours. After filtration, the crude ethanolic extract was evaporated to dryness, dissolved in H_2O and extracted 4× with an equal volume of chloroform. The H_2O was removed from the aqueous phase under reduced pressure to yield 1.27 g of a brown syrup. A small quantity of this material reacted positively to the Feigl–Anger test after hydrolysis with β-glucosidase, indicating the presence of cyanogen in the extract. Most of the material was used to isolate the glycosides and sugars by column chromatography (see below). For isolation by paper chromatography, we followed a similar procedure to that described by Spencer & Seigler (1980). A small quantity of the brown syrup was chromatographed on paper (Whatman 3MM)

in Me_2CO–H_2O (5:1). Strips 1 cm wide (Spencer & Seigler 1980) were tested for the release of HCN. The cyanogenic fractions (R_f *ca.* 0.9) were combined, eluted with H_2O, and chromatographed a second time in MeCOEt–Me_2CO–H_2O (15:5:3). The cyanogenic fractions (R_f *ca.* 0.8) were again combined and eluted with H_2O. The eluent was divided into two equal portions and hydrolysed with β-glucosidase (releasing HCN) or HCl (no release of HCN). After hydrolysis, the two fractions were extracted 2× with equal volumes of diethyl ether. The aqueous phases both reacted strongly positive to the glucose oxidase test (Kusai *et al.* 1960). The ether fractions were dried with $CaCl_2$ and studied by mass spectrometry.

The cyanogen was isolated from the extract using a 600 × 25 mm glass column packed with silica gel and eluted with EtOAc–MeOH (95:5). Fractions of 10 cm^3 were collected and tested for the presence of the cyanogen. Fractions 31 to 45 with R_f 0.95 on TLC (isopropanol–H_2O–acetic acid, 20:4:1; baked at 200°C after spraying with chromic acid) were combined to yield 6.8 mg of a pale yellow solid.

Results

The cyanogenic glucoside of *Buchenroedera* was identified by mass spectrometry as prunasin (**1** in Figure 1). The mass spectrum of the material obtained after enzymatic hydrolysis clearly showed the presence of benzaldehyde (**3**), with an M^+ of m/z 106 and strong peaks at m/z 105 and 77. The absence of a peak at m/z 133 indicated that no cyanohydrin (**2**) was formed. Further proof of the identity of prunasin was found in the mass spectrum of the material obtained after acid hydrolysis. It showed, as would be expected for the cyanohydrin (*D*-mandelonitrile), an M^+ of m/z 133 and major peaks at m/z 106, 105 and 77. An attempt to study the purified glucoside directly by electron impact resulted in two large peaks at m/z 117 and 116, indicating the presence of fragment **4**, (an immediate loss of oxygen and glucose). In view of the specificity of β-glucosidase and the positive reaction to the glucose oxidase test (Kusai *et al.* 1960), there can be little doubt about glucose being the sugar moiety of the cyanogen.

Discussion

The presence of prunasin in *Buchenroedera* confirms that at least one specimen in this genus and one in *Lotononis* both follow the phenylalanine pathway for the production of cyanogenic glucosides (Fikenscher & Hegnauer 1981) rather than the more usual valine and isoleucine pathways, which lead to the production of linamarin and lotaustralin respectively. Since we have shown that cyanogenesis in the two genera is likely to be homologous, this character provides further evidence of a close affinity between *Lotononis* and *Buchenroedera*.

A summary of similarities and differences between *Buchenroedera*, some sections of *Lotononis*, and other genera of the tribe Crotalarieae is given in Table 1. The distribution of character states shows that the predictivity of the present generic and infrageneric classification can be improved by reclassifying *Buchenroedera* as a group within *Lotononis*. This was also suggested by Polhill (1976, 1981), who could find no diagnostic character for *Buchenroedera* other than the shape of the pods. Cyanogenesis can now be added to the growing body of evidence suggesting a reduction in the status of *Buchenroedera*.

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References

DÜMMER, R.A. 1913. A synopsis of the species of *Lotononis* Eckl. & Zeyh., and *Pleiospora* Harv. *Trans. R. Soc. S. Afr.* 3: 275–335.

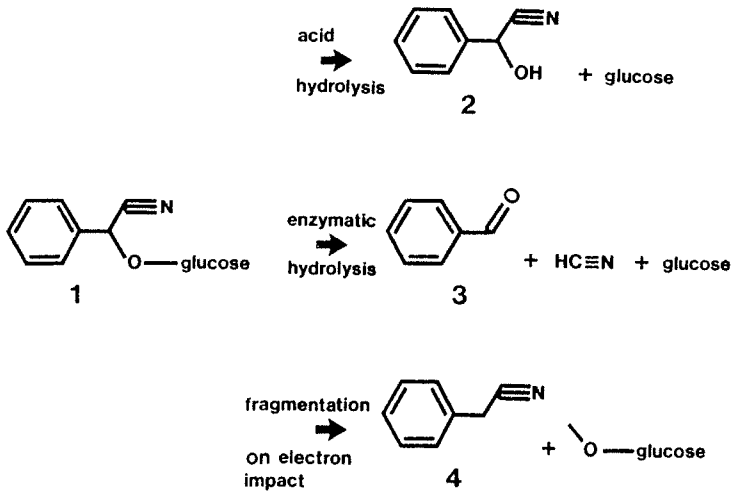

Figure 1 The degradation products of prunasin (**1**). The cyanohydrin (**2**), benzaldehyde (**3**) and the fragment (**4**) were identified by mass spectrometry in extracts from *Buchenroedera multiflora*.

Table 1 Summary of similarities and differences between some sections* of *Lotononis, Buchenroedera*, and other genera of the tribe Crotalarieae

Characters	*Lotononis* section *Aulacinthus*	*Lotononis* section *Polylobium*	*Lotononis* section *Krebsia*	*Buchenroedera*	Other genera of the Crotalarieae
Habit	woody and/or suffrutescent	suffrutescent	woody and/or suffrutescent	woody and/or suffrutescent	woody and/or suffrutescent, some annual
Stipules	solitary or absent	paired, rarely absent	paired, rarely solitary or absent	paired or absent	paired or absent, rarely solitary
Bracteoles	absent or vestigial	absent	absent or vestigial	absent	present
Flower colour	yellow, rarely pink	yellow	usually blue, rarely yellow or pinkish	usually blue, rarely yellow, white or pink	never blue, usually yellow
Standard petal	± as long as the keel	longer than the keel	± as long as the keel	longer than the keel	various
Keel petals	glabrous	glabrous	glabrous or pubescent	glabrous or pubescent	glabrous or pubescent
Fruit shape	linear to ovate; turgid	linear to ovate; turgid	linear, rarely ovate; only slightly turgid	ovate; turgid	various shapes, often turgid
Upper suture of fruit	verrucose	verrucose	smooth	smooth	smooth
Seed surface	tuberculate	tuberculate	smooth	smooth	smooth, rugose in some species of *Crotalaria*
Chromosome number ($2n$)	28	28	28, 42, 56, 84	28	never 28 except in *Dichilus*
Major alkaloid type	pyrrolizidine	pyrrolizidine	pyrrolizidine	pyrrolizidine	quinolizidine; pyrrolizidine only in *Crotalaria*
Cyanogenesis	cyanogenic	cyanogenic	mostly cyanogenic	often cyanogenic	acyanogenic

*The sections *Aulacinthus* (E. Mey.) Benth. and *Krebsia* Eckl. & Zeyh. are used here in the same sense as in Dümmer (1913), except for the exclusion of *L. hirsuta* Schinz and allies from *Krebsia*. *Polylobuium* (Eckl. & Zeyh.) Benth. refers here only to *L. involucrata* (E. Mey.) Benth. and related species.

FEIGL, F. & ANGER, V. 1966. Replacement of benzidine by copper ethylacetoacetate and tetra base as spot-test reagent for hydrogen cyanide and cyanogen. *Analyst* 21: 282–284.

FIKENSCHER, L.H. & HEGNAUER, R. 1981. Die verbreitung der Blausäure bei den Cormophyten. 18. Mitteilung. Weitere Beobachtungen bei den Leguminosae — Papilionoideae (Galegeae, Genisteae, Loteae, Phaseoleae). *Planta Med.* 43: 323–335.

KUSAI, K., SEKUZU, I., HAGIHARA, B., OKUNUKI, K., YAMAUCHI, S. & NAKAI, M. 1960. Crystallization of glucose oxidase from *Penicillium amagasakiense*. *Biochim. Biophys. Acta* 40: 555–557.

POLHILL, R.M. 1976. Genisteae (Adans.) Benth. and related tribes (Leguminosae). *Bot. Syst.* 1: 143–368.

POLHILL, R.M. 1981. Tribe 29. Crotalarieae (Benth.) Hutch. In: Advances in legume systematics, eds Polhill, R.M. & Raven, P.H., Vol. 1, pp. 399–402, Royal Botanic Gardens, Kew.

SPENCER, K.C. & SEIGLER, D.S. 1980. Deidaclin from *Turnera ulmifolia*. *Phytochem.* 19: 1863–1864.

TANTISEWIE, B., RUIJGROK, H.W.L. & HEGNAUER, R. 1969. Die verbreitung der Blausäure bei den Cormophyten. 5. Mitteilung. Über cyanogene Verbindungen bei den Parietales und bei einigen weiteren Sippen. *Pharm. Weekbl.* 104: 1341–1355.

VAN WYK, B-E. 1989. The taxonomic value of cyanogenesis in *Lotononis* and related genera. *Biochem. Syst. Ecol.* 17: 297–303.

VAN WYK, B-E. & SCHUTTE, A.L. 1988. Chromosome numbers in *Lotononis* and *Buchenroedera*. *Ann. Mo. bot. Gdn* 75: 1603–1607.

VAN WYK, B-E. & VERDOORN, G.H. 1988. Chemotaxonomic significance of integerrimine in *Buchenroedera* and *Lotononis* section *Krebsia*. *Biochem. Syst. Ecol.* 16: 287–289.

VAN WYK, B-E. & VERDOORN, G.H. 1989. A chemotaxonomic survey of major alkaloids in *Lotononis* and *Buchenroedera*. *Biochem. Syst. Ecol.* 17: 385–389.

WATT, J.M. & BREYER-BRANDWIJK, M.G. 1962. Medicinal and poisonous plants of southern and eastern Africa. 2nd edn, E. & S. Livingstone, London.