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

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Lotonois 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 Lotonois 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 Lotonois and provides further evidence that the two genera should be combined.

Lotonois en Buchenroedera is soever 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 ooreenkoms met Lotonois in morfologie, kromosoomsistologie en alkaloidie 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 prunasin, ’n verbinding wat voorheen in *L. crumanina* Burch. ex Benth. waargeneem is. Hierdie resultaat toon dat sianogenese in Buchenroedera en Lotonois homoloog is en bied verdere aanduiding dat die twee genera gekombineer behoor te word.

**Keywords:** Buchenroedera, cyanogenesis, generic relationships, Lotonois, 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 Lotonois (DC.) Eckl. & Zeyh. This similarity was interpreted as supporting evidence for the idea that Buchenroedera is indistinct from Lotonois 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 Lotonois except for the shape of the fruit. Using chromatographic methods, Fikenscher & Hegnauer (1981) reported that prunasin was the cyanogenic glucoside of Lotonois crumanina Burch. ex Benth. This is the only evidence for the source of HCN in Lotonois — 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 Lotonois.

**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 cm³ 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 ethanol extract was evaporated to dryness, dissolved in H₂O and extracted 4× with an equal volume of chloroform. The H₂O 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 Me₂CO–H₂O (5:1). Strips 1 cm wide (Spencer & Seigler 1980) were tested for the release of HCN. The cyanogenic fractions (Rₚ ca. 0.9) were combined, eluted with H₂O, and chromatographed a second time in MeCOEt–Me₂CO–H₂O (15:5:3). The cyanogenic fractions (Rₚ ca. 0.8) were again combined and eluted with H₂O. 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 CaCl₂ 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 cm³ were collected and tested for the presence of the cyanogen. Fractions 31 to 45 with Rₚ 0.95 on TLC (isopropanol–H₂O–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 cyano derivatives (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 (β-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 Lostononis 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 characteristic provides further evidence of a close affinity between Lostononis and Buchenroedera.

A summary of similarities and differences between Buchenroedera, some sections of Lostononis, 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 Lostononis. 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

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 multisulora.
### Table 1: Summary of similarities and differences between some sections of Lotiononis, Buchenroedera, and other genera of the tribe Crotalarieae

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

*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. hierata Schinz and allies from Krebsia. Polylobium (Eckl. & Zeyh.) Benth. refers here only to L. involucrata (E. Mey.) Benth. and related species.*


