Phytochemistry 69 (2008) 1870–1874

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Frequency and distribution of cyanogenic glycosides in *Eucalyptus* L'Hérit

Roslyn M. Gleadow [a,*], J. Haburjak [b], J.E. Dunn [b], M.E. Conn [b], Eric E. Conn [b]

[a] *School of Biological Sciences, Monash University, Victoria 3800, Australia*
[b] *Section of Molecular and Cellular Biology, University of California, Davis, CA 95616, USA*

ARTICLE INFO

Article history:
Received 13 November 2007
Received in revised form 2 March 2008
Available online 10 May 2008

Keywords:
Eucalyptus
Myrtaceae
Eucalypt
Prunasin
Cyanide
Cyanogenic glucoside
Herbivore
Taxonomy
Defence

ABSTRACT

In this study approximately 420 of the described species of *Eucalyptus* were examined for cyanogenesis. Our work has identified an additional 18 cyanogenic species, 12 from living tissues and a further six from herbarium samples. This brings the total of known cyanogenic species to 23, representing approximately 4% of the genus. The taxonomic distribution of the species within the genus is restricted to the subgenus *Symphyomyrtus*, with only two exceptions. Within *Symphyomyrtus*, the species are in three closely related sections. The cyanogenic glycoside was found to be predominantly prunasin (**1**) in the 11 species where this was examined. We conclude that cyanogenesis is plesiomorphic in *Symphyomyrtus* (i.e. a common basal trait) but has probably arisen independently in the other two subgenera, consistent with recent phylogenetic treatments of the genus. The results of this study have important implications for the selection of trees for plantations to support wildlife, and to preserve genetic diversity.

1. Introduction

Eucalyptus is a large genus, with over 500 species (Pryor and Johnson, 1971; Chippnedale, 1988), although recent revisions would put that figure higher at around 700–800 (Brooker, 2000). The genus is indigenous to Australia and some neighbouring islands, but is used extensively in plantations around the world. The replacement of indigenous forests can have a significant effect on the local fauna and the choice of species is vital in order to limit the negative effects (Hartley, 2002). A number of physical and chemical characteristics of *Eucalyptus* foliage make them less palatable to herbivores such as toughness, low leaf protein, phenolics, sideroxylonals and various monoterpenes (Moore et al., 2004; Loney et al., 2006). In addition, a number of species have been reported to be cyanogenic, capable of producing toxic cyanide when the leaf tissue is disrupted by chewing (Goodger et al., 2006). There have been numerous cases of livestock poisoning from eating *E. cladocalyx* F. Muell. (Everist, 1981) and there are reports of koalas dying after eating cyanogenic *E. viminalis* Labill. (Morris, 1944).

Cyanogenic glycosides are found in a wide range of taxa including ferns, palms, woody and herbaceous plants, as well as some bacteria and insects (Conn, 1980; Lechtenberg and Nahrstedt, 1999; Zagrobelny et al., 2004). It has been estimated that 11% of all plant species contain cyanogenic individuals (Jones, 1998), although this figure seems high in the view of recent extensive testing of non-commercial species (Miller et al., 2006). All of the six species of *Eucalyptus* from which cyanogenic compounds have so far been identified are members of the same subgenus, *Symphyomyrtus* (see Table 1). Moreover, the aromatic-derived glycoside (*R*)-prunasin (**1**) is the only cyanogen in five species, and the primary one in *E. camphora* subsp. *humeana* Johnson & Hill (Neilson et al., 2006; see Fig. 1). The existence of other cyanogenic species of *Eucalyptus* seemed likely, and therefore we initiated an extensive survey of living plants and herbarium specimens. Few of these species had been tested before. Our aim was to (1) determine the prevalence of cyanogenesis in *Eucalyptus* and its taxonomic associations; and (2) to ascertain whether prunasin (**1**) was the primary cyanogenic glycoside. In order to do this leaf samples were collected from extant individuals growing in arboretums in Australia and California and the cyanogenic glycosides identified. This was supplemented by extensive testing of herbarium specimens.

2. Results and discussion

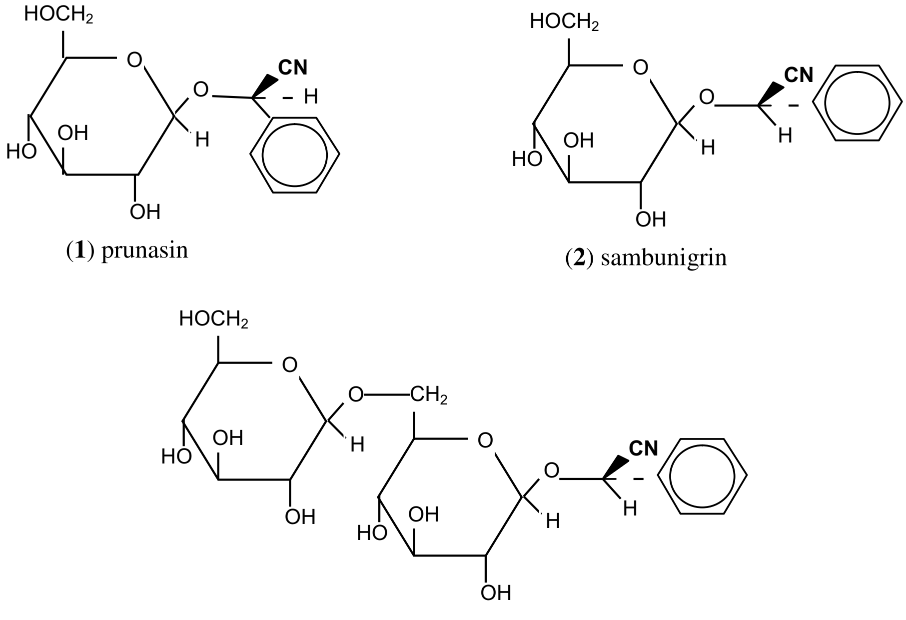
2.1. Frequency of cyanogenesis in Eucalyptus

Approximately 2200 individual plants representing nearly 400 species were tested for the presence and absence of cyanogenic glycosides using the qualitative Feigl–Anger procedure (Brinker and Seigler, 1992). We identified 18 new cyanogenic species and one subspecies (see Tables 2 and 3). In addition we confirmed

---

* Corresponding author. Tel.: +61 (0) 3 9905 1667; fax: +61 (0) 3 9905 1450.
E-mail address: ros.gleadow@sci.monash.edu.au (R.M. Gleadow).

0031-9422/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2008.03.018


(**1**) prunasin

(**2**) sambunigrin

(**3**) amygdalin

Fig. 1. Structures of three cyanogenic glycosides found in *Eucalyptus*. All are derived from phenylalanine. Prunasin (**1**) is ubiquitous, the epimer sambunigrin (**2**) is rare and the diglycoside amygdalin (**3**) has only been detected in one species to date.

Table 1
Species of *Eucalyptus* known to be cyanogenic. In all species prunasin (**1**) was identified as the primary cyanogenic agent

Species	Section	Series
E. camphora subsp. *humeana* Johnson & Hill[a]	*Maidenaria*	*Foveolatae*
E. cladocalyx F. Muell.[b]	*Sejunctae*	n/a
E. cladocalyx var. *nana*[c]		
E. nobilis Johnson & Hill[d]	*Maidenaria*	*Viminales*
E. polyanthemos Schauer subsp. *vestita* Johnson & Hill[e]	*Adnataria*	*Heterophloiae*
E. viminalis Labill.[f]	*Maidenaria*	*Viminales*
E. yarraensis Maiden & Cambage[g]	*Maidenaria*	*Foveolatae*

All were confirmed as cyanogenic in this study (see Table 2). Accession numbers can be found in Appendix I (supplementary data).

[a] Neilson et al., 2006.
[b] Finnemore and Cox, 1928; Gleadow et al., 1998.
[c] Burns et al., 2002.
[d] Gleadow et al., 2003.
[e] Goodger and Woodrow, 2002.
[f] Finnemore et al., 1935.
[g] Goodger et al., 2002.

the cyanogenic status of four of the six species known to be cyanogenic (Tables 1–3). This brings the total known number of cyanogenic *Eucalyptus* to 23 and represents approx 4% of the species tested. Jones (1998) estimated that approximately 11% of all plants were cyanogenic. This is likely to be an overestimate, as much of the testing until this century has been on crop plants, and two-thirds of crop plants appear to have at least some cyanogenic parts (Jones, 1998). A figure of 5% is perhaps more realistic in natural communities (Adsersen et al., 1988; Adsersen and Adsersen, 1993; Thomsen and Brimer, 1997; Miller et al., 2006) and is similar to the approximately 5% of *Acacia* species were found to be cyanogenic in an extensive survey by Conn and co-workers (Conn et al., 1989).

2.2. Identification of cyanogen

We identified (*R*)-prunasin (**1**) as the cyanogen in 12 of the 18 species found to be cyanogenic (Table 4). The NMR spectroscopic data presented here shows in most cases that the plants contained only prunasin (**1**), although sambunigrin (**2**) was detected in some (see Fig. 1). Traces of sambunigrin (**2**) have been found in *Eucalyptus* before, but this was attributed to racemisation during extraction (Conn, 1980; Gleadow et al., 2003). The presence of >10% sambunigrin (**2**) found in our study raised the question of whether mixtures of prunasin (**1**) and sambunigrin (**2**) occur naturally in *Eucalyptus*, as Maslin et al. (1988) found in some species of *Acacia*. In that paper it was concluded that non-enzymatic racemization could not account for the mixtures of the enantiomers observed. Recent work by Neilson et al. (2006) found that *E. camphora* contained several cyanogenic glycosides in addition to (*R*)-prunasin (**1**), including its epimer (sambunigrin (**2**)) and the diglycoside (amygdalin (**3**)). In two of the species for which we present data here (*E. leucoxylon* and *E. orgadophila*), the proportion of sambunigrin (**2**) differed depending on the analytical method (NMR and GLC). This was almost certainly the result of racemisation of one of the sample preparations (see Table 4).

2.3. Taxonomic considerations

Aromatic cyanogenic glycosides appear to have evolved earlier than the aliphatic forms (Bak et al., 2006). Moreover, the only occasions when aliphatic and aromatic cyanogenic glycosides occur in the same species is the result of alloploidy. Our finding that eucalypts appear to have only phenylalanine-derived cyanogenic glycosides is consistent with the position of *Eucalyptus* at the base of the Rosids clade within higher plants (Ladiges and Udovicic, 2000; Bak et al., 2006).

Extensive sampling meant that the survey of the genus was comprehensive. Tables 2 and 3 list the cyanogenic species together with the taxonomic groups to which they are assigned. The proportion of species tested in each taxonomic grouping was estimated, although exact numbers in each taxon are currently under revision (cf. Pryor and Johnson, 1971; Brooker, 2000; Ladiges and Udovicic, 2000; Bohte and Drinnan, 2005). Overall, we estimate that we sampled 60% of all species from 10 of the 13 currently recognised subgenera. Within the two largest subgenera (*Symphyomyrtus*,