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Phytochemistry 67 (2006) 43–51
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Cyanogenic glycosides from the rare Australian endemic rainforest tree *Clerodendrum grayi* (Lamiaceae)

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Received 15 July 2005; received in revised form 28 September 2005
Available online 22 November 2005

Abstract

The cyanogenic diglycoside lucumin ((*R*)-mandelonitrile-β-D-primeveroside) and monoglucoside prunasin ((*R*)-mandelonitrile-β-D-glucoside) were isolated from the foliage of the rare Australian rainforest tree species *Clerodendrum grayi* (Lamiaceae). This is the first reported isolation of the diglycoside lucumin from vegetative tissue (foliage), and the first reported co-occurrence of lucumin and prunasin. Furthermore, unusually, the diglycoside lucumin was the most abundant cyanogen accounting for approximately 60% of total cyanide in a leaf tissue.

Keywords: *Clerodendrum grayi*; Lamiaceae, Verbenaceae; Cyanogenesis; Cyanogenic glycoside; Lucumin, prunasin; Tropical rainforest; Chemotaxonomy

1. Introduction

Clerodendrum grayi Munir (Lamiaceae syn. Verbenaceae) is a subcanopy rainforest tree (3–5 m tall) endemic to the northern part of Queensland, Australia. It has a very limited distribution on the Atherton Tableland: it is only known south south east of Mareeba, between latitudes 17° and 18°S, and longitudes 145° and 146°E (Munir, 1989).

Recent revisions of the division between Lamiaceae and Verbenaceae families (Cantino, 1992; Wagstaff et al., 1997, 1998) have transferred the genus *Clerodendrum* to the Lamiaceae or "mint" family. The Lamiaceae is a large cosmopolitan family (>250 genera, 6700 spp. worldwide) and includes many economically important species such as medicinal and culinary herbs [e.g., *Lavandula* (lavender); *Mentha* (mint)], cultivated ornamentals [e.g., *Salvia* (sage)], and also tropical hardwood species (e.g., teak), formerly in the Verbenaceae. In terms of secondary metabolites, typical constituents are monoterpenoids, diterpenes or triterpenes [e.g., *Thymus* (thyme); *Ocimum* (basil)], as well as flavonoids and iridoid glycosides (Gibbs, 1974; Hegnauer, 1989; Taskova et al., 1997). By contrast, cyanogenesis in Lamiaceae, and also Verbenaceae, has rarely been reported. Even within the order Lamiales, cyanogenesis is considered rare, with only a few reports, mostly from early workers (e.g., Juliano, 1923; see also Gibbs, 1974).

As part of a large study of cyanogenesis in Australian tropical rainforests, *C. grayi* was found to be very highly cyanogenic, with foliar concentrations of cyanogenic compounds ranging from 1.8 to 4.8 mg CN g^{-1} dry wt in mature field-grown tree leaves (Miller, 2004). These concentrations are among the highest reported for tree leaves. Several species of *Clerodendrum* are known to be toxic (e.g., Pammel, 1911; Hurst, 1942; Webb, 1948; CFSAN, 2003) although the poisons have not always been detailed, and while a few species of *Clerodendrum* have been found to be cyanogenic – *C. intermedium* Berthold Thomas (Gibbs, 1974), *C. molle* var. *molle* (Adsersen et al., 1988)

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0031-9422/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2005.09.038

and *C.* sp (Tjon Sie Fat, 1979) – no cyanogenic constituents have previously been identified in the genus. Indeed, cyanogenic glycosides have only been identified in one species in the Lamiaceae family: prunasin and an isomer of amygdalin ((*R*)-mandelonitrile 2-*O*-β-D-glucoside-β-D-glucoside) were isolated from the leaves of the medicinal annual herb *Perilla frutescens* Britt. var. *acuta* Kudo (Aritomi et al., 1985). In addition, no cyanogenic glycosides have been identified from species formerly or currently in the Verbenaceae family. Therefore, the aim of this study was to identify the cyanogenic constituents in leaves of *C. grayi* Munir.

2. Results

2.1. Concentration of cyanogenic glycosides in C. grayi

Cyanogenic glycosides were distributed throughout all tissues sampled in *C. grayi*: leaves, woody stems, floral buds and flowers. Foliar concentrations of cyanogenic glycosides ranged from 1.1 to 4.9 mg CN g^{-1} dry wt. In floral buds and flowers, the concentrations of cyanogenic glycosides were 0.60–0.73 mg CN g^{-1} dry wt and 0.54–0.96 mg CN g^{-1} dry wt, respectively.

2.2. Cyanogenic glycosides in C. grayi

Fractionation of a leaf MeOH extract of *C. grayi* by HPLC indicated the presence of two main cyanogenic compounds (Fig. 1). Similarly, only two cyanogenic compounds were detected following fractionation of non-polar cyanogens by HP-TLC (see Section 4). The two pairs of compounds proved to be the same (TLC, ESI-MS, GC–MS) and shall henceforth be referred to as compounds **1** and **2**. Based on HPLC analysis of the crude MeOH extract, the two main cyanogens accounted for 92% of total cyanide. The balance of cyanide (approximately 8%) indicated the presence of a third minor cyanogenic compound at HPLC RT 12–14 min, which was not detected in the extract fractionated by HP-TLC. The minor cyanogen was not purified in this study.

Compound **2** (HPLC RT 22 min; HPTLC $R_f = 0.4$ in solvent A) had a retention time coincident with authentic prunasin using HPLC and HP-TLC, under the same conditions. GC–MS compositional analysis following acid methanolysis and TMS-derivitisation identified glucose as the sole sugar. The molecular weight was determined by ESI-MS (positive: *m/z* 318 $[M+Na]^+$; negative: *m/z* 294 $[M-H]^-$) to be 295 amu, isobaric with prunasin/sambunigrin ($C_{14}H_{17}NO_6$). GC–MS analysis of TMS-derivatised

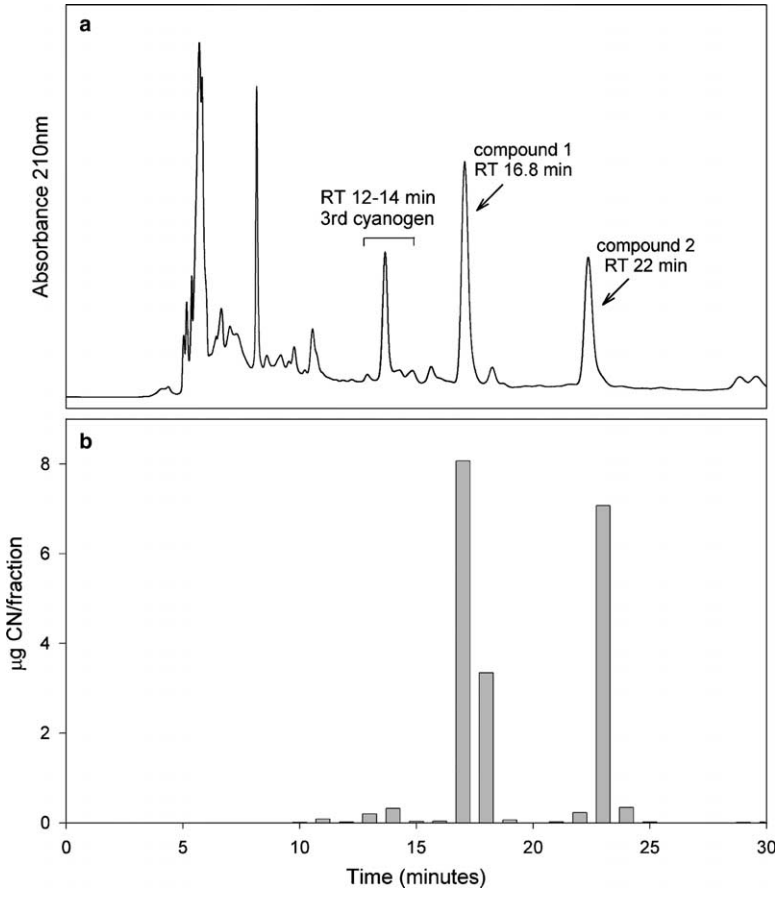

Fig. 1. (a) Fractionation of *Clerodendrum grayi* leaf MeOH extract by RP-HPLC (18% MeCN at 2 mL min^{-1}) analysed at λ 210 nm, and (b) cyanide content of fractions across the profile. The total CN detected is equivalent to that from approximately 4 mg dry wt leaf tissue.