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DETECTION AND IDENTIFICATION OF CYANOGENIC GLUCOSIDES IN SIX SPECIES OF *ACACIA*

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Abstract—The presence of prunasin in *Acacia deanei* ssp. *paucijuga*, sambunigrin in *Acacia cunninghamii*, and acacipetalin in *Acacia giraffae* has been established by both chromatographic and NMR spectral evidence. Mandelonitrile glucosides of unknown configuration in *Acacia parramattensis* and *Acacia pulchella* and (an) as yet unidentified glycoside(s) in *Acacia farnesiana* are also reported.

INTRODUCTION

We wish to report the presence of cyanogenic compounds in six species of the genus *Acacia*. We have identified prunasin in *A. paucijuga*, sambunigrin in *A. cunninghamii*, and acacipetalin in *A. giraffae*. In addition a mandelonitrile glucoside of unknown configuration has been detected in *A. parramattensis* and *A. pulchella*, and an unidentified glycoside is found in *A. farnesiana*. *A. cunninghamii*, *A. parramattensis*, *A. deani* ssp. *paucijuga* and *A. pulchella* are Australian species while *A. giraffae* is an African member of the genus. *A. farnesiana* is widely distributed in the subtropical and tropical areas of the world but is probably of American origin.

A. cunninghamii has been reported to contain a cyanogenic glucoside [1] but the nature of the glucoside was not determined. Australian specimens of *A. farnesiana* and *A. pulchella* were also reported by these workers not to contain cyanogenic glycosides [1] but plants of these two species growing in the arboretum of the University of California at Davis are cyanogenic. Rehr *et al.* [2] reported that Costa Rican plants of *A. farnesiana* contain cyanogenic compounds. There are no literature reports pertaining to the cyanogenic nature of *A. parramattensis* and *A. deani* ssp. *paucijuga*.

Other investigations of cyanogenic glucosides occurring in this genus have established that sambunigrin is present in the Australian species *A. glaucescens* and *A. cheelii* [3]. Acacipetalin was isolated from the South African species *A. hebeclada* (= *A. lasiopetala*) and *A. sieberiana* var. woodii (= *A. stolonifera*) [4,5]. Its occurrence in *A. sieberiana* has recently been confirmed [6] and the existence of a second cyanogenic glycoside dihydroacacipetalin in that species demonstrated [7]. Acacipetalin also occurs in the American species, *Acacia constricta* [8].

RESULTS AND DISCUSSION

Chemical identification. Purification and analysis by NMR spectroscopy of the TMS-ethers of the cyanogenic material from *A. cunninghamii* and *A. deanei* ssp. *paucijuga* clearly showed that the former species contains sambunigrin while *A. deanii* ssp. *paucijuga* contains prunasin. The NMR spectra (Fig. 1) allow one to distinguish the two epimers by the positions of the anomeric glucose protons (a doublet centered at 4.58δ vs 3.95δ) and the cyanohydrin methane proton (5.65 vs 5.46δ). Analysis of the TMS-ethers by GLC [6] also showed that each of the two samples was composed entirely of either sambunigrin or prunasin. There was no evidence for epimerization of either compound during isolation. On the other hand, epimerization could be performed [13] on each isolated sample to yield a mixture of nearly equal amounts of the two epimers.

Similar spectroscopic analysis of the material isolated from *A. giraffae* showed that this species contains acacipetalin. There was no indication (by GLC) for any significant quantity of dihydroacacipetalin in *A. giraffae*. Analysis of fresh leaves of *A. giraffae* indicated the concentration to be 30 μmol/gm fr.wt.

It was not possible to isolate enough of the glucosides from *A. pulchella* and *A. parramattensis* to determine their structure by NMR spectroscopy. However, enzymatic studies (see below) suggest that these species contain mandelonitrile glucosides. In the case of *A. parramattensis* enough material purified by PC was available to silylate and examine by GLC [6]. When this was done, the compound exhibited the same characteristics as prunasin.

Enzymatic studies. Fresh leaves, phyllodes or other tissues of the Australian species and *A. farnesiana* were examined for the presence of endogenous β-glucosidases that could hydrolyze the cyanogens in the tissues (Table 1). The results show that little or no HCN was released from the Australian species until almond emulsin was added to the homogenates. On the other hand, some HCN was released initially from the homogenate of *A. farnesiana* and the amount was increased 3-fold when flax linamarase was subsequently added (after emulsin). These results suggest that of these five species only *A. farnesiana* contains significant amounts of an endogenous enzyme capable of hydrolyzing cyanogenic glycosides. The release of HCN from these extracts in the absence

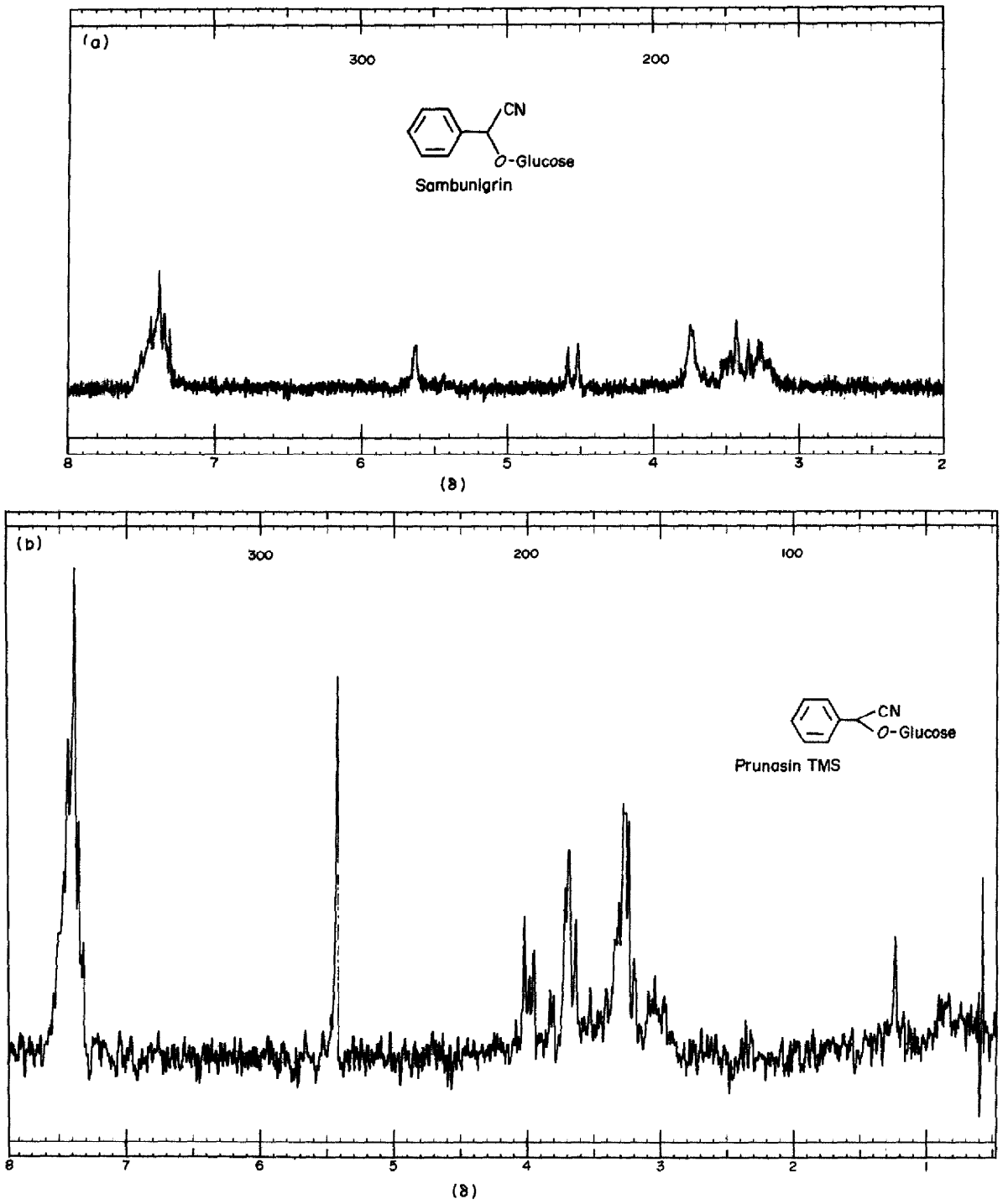

Fig. 1. NMR spectra of the TMS ethers of sambunigrin (top) and prunasin (bottom) from *Acacia cunninghamii* and *Acacia deanei* ssp. *paucijuga* respectively.

of added hydrolytic enzyme could also occur in the presence of a labile cyanohydrin. Finnemore and Gledhill [1] have previously noted the absence of a glycosidase in *A. glaucescens*, *A. cheeli*, *A. doratoxylon*, and *A. cunninghamii* although those species do contain a cyanogenic compound. The results obtained clearly show the need for adding exogenous glucosidases to plant homogenates in order to insure complete hydrolysis of cyanogenic material. The data in Table 1 may also be taken as a measure of the amount of cyanogenic glycoside in these specimens.

Additional information on the chemical nature of the cyanogenic material in these species is provided by Table 2. In these experiments the cyanogens were first extracted from the plant tissue, taken up in isopropanol (see Experimental) and then the action of almond emulsin and flax

Table 1. Production of HCN from fresh *Acacia* species by endogenous enzyme(s) and after addition of almond emulsin and flax linamarase

Species	Tissue	HCN produced (μmol/g fr.wt.)		
		Endogenous enzyme	Emulsin added	Linamarase added
Acacia cunninghamii	phyllodes	0.12	9.60	0.63
A. pulchella	leaves, stems, spines	0.04	6.33	0.30
A. parramattensis	leaves	0.07	0.37	0.04
A. deanei ssp. *paucijuga*	leaves	0.00	0.16	0.04
A. farnesiana	leaves	0.74	0.25	2.18

The procedure used is described under Experimental.

linamarase on these specimens observed. In Expt. 1 (Table 2), material from the Australian species was hydrolyzed when almond emulsin was added and no further hydrolysis occurred when flax linamarase was added. However, less than 10% of the sample from *A. farnesiana* was hydrolyzed in Expt. 1 and to obtain HCN from this species, flax linamarase had to be added.

In the reciprocal experiment (Table 2, Expt. 2), performed only with *A. farnesiana* and *A. cunninghamii* because of a lack of material, the addition of linamarase first to the isopropanol extracts released more than 90% of the HCN that was ultimately obtained from the *A. farnesiana*, and the subsequent addition of emulsin released only a small amount of HCN. In the case of *A. cunninghamii* (which is known to contain sambunigrin) the addition first of linamarase did hydrolyze about 35% of the total available HCN. However, emulsin had to be added in order to release the rest of the cyanogenic material that was released in the time periods employed when emulsin was added first (Expt. 1).

The results described in Table 2 demonstrate a distinct difference in the chemical nature of the cyanogenic compound(s) in *A. farnesiana* and those in the Australian acacias. The action of commercial almond emulsin preparations, which readily hydrolyze prunasin, amygdalin, sambunigrin and acacipetalin but not linamarin (J. E. Dunn and E. E. Conn, unpublished results), on the extracts from *A. cunninghamii* and *A. deanei* ssp. *paucijuga* is consistent with our finding that these species contain sambunigrin and prunasin respectively. The data also suggest that the cyanogenic material in the other two Australian species either has an aromatic aglycone or is acacipetalin. The rapid release by linamarase of nearly all the HCN bound, presumably in glycosidic linkage, in the sample from *A. farnesiana* indicates that this plant may contain linamarin or lotaustralin. It presumably does not contain acacipetalin or this would have been released in Expt. 1, Table 2 by the action of emulsin. The limited amounts of material available from this plant has not yet permitted identification of the cyanogen(s) it contains.

EXPERIMENTAL

Materials. Young and mature plants of the following species growing in the arboretum of the University of California at Davis were used in these studies: *Acacia cunninghamii* Hook (Canberra, Australia); *Acacia farnesiana* Willd. (Santa Barbara Botanic Garden, Santa Barbara, Calif.); *Acacia parramattensis* Tindale (Canberra, Australia); *Acacia deanei* (R. T. Bak.) Welch, Coombs and McGlynn ssp. *paucijuga* (F. Muell. ex. Wakefield) Tindale (Canberra, Australia); and *Acacia pulchella* R. Br. (Kings Park, East Navvogiov, Australia). Determinations of *A. farnesiana*, *A. parramattensis*, *A. deanei* ssp. *paucijuga* and *A. pulchella* were confirmed by Dr. Mary D. Tindale, Royal Botanic Gardens and National Herbarium, Sydney, N.S.W.; her assistance is gratefully acknowledged. Leaf material of *Acacia giraffae* was obtained from specimens in the Los Angeles County Arboretum, Monrovia, Calif.

Procedures. Samples for analysis of glycoside content of the different species were prepared as follows: fresh leaves and phyllodes (in some cases stems and spines) were ground in

Table 2. Production of HCN from isopropanol concentrates of different *Acacia* species

Species	Tissue	HCN produced (μmol/g fr.wt.)	
		1st treatment	2nd treatment
Expt. 1.			
A. farnesiana	leaves	0.31	4.73
A. cunninghamii	phyllodes	8.45	0.10
A. pulchella	leaves, stems, spines	3.70	0.00
A. parramattensis	leaves	0.43	0.00
A. deanei ssp. *paucijuga*	leaves	0.34	0.00
Expt. 2.			
A. farnesiana	leaves	4.79	0.36
A. cunninghamii	phyllodes	3.02	5.15

In Expt. 1, almond emulsin was first added to the concentrate and HCN production measured before linamarase was added in the second treatment. In Expt. 2, the order of addition of the enzymes was reversed.

liquid N_2, and placed in boiling 80% EtOH (4 vols per wt of plant material) for approximately 3 min. The extract was then filtered, evaporated to dryness, residue taken up in 10% *i*-PrOH (2.5–5.0 ml), and centrifuged for 3–4 min. Quantitative analysis was performed on aliquots of the supernatant. The following procedure was used to study the effect of the 2 enzymes almond emulsin (Sigma grade) and flax linamarase [8] upon these concentrates: An aliquot (0.25–0.5 ml) of the *i*-PrOH concentrate was added to the main compartment of a small flask (25 ml) that contained a center well along with 4 ml Pi buffer (0.1 M, pH 6.4), a soln of either emulsin or linamarase, and 0.375 mg chloramphenicol to retard microbial activity. 0.5 ml 1.0 N NaOH was placed in the center well, the flask sealed, and the sample placed on a shaker–incubator (35°) for 18–24 hr. At that time the NaOH from the center well was removed and analyzed for cyanide by the colorimetric methods of Aldridge [9] or Epstein [10]. The other enzyme, either linamarase or emulsin, was then added to the same flask, more NaOH soln. was added to the center well, and incubation and cyanide analysis repeated. In order to determine presence of hydrolytic enzymes (β-glucosidases) capable of hydrolyzing cyanogens in fresh plant materials of these species, ~1.0 g fresh leaves and phyllodes (in some cases stems and spines) were ground in liquid N_2, immediately placed in center well flasks containing Pi buffer (0.1 M, pH 6.4), 0.375 mg chloramphenicol in the main compartment and 0.5 ml 1.0 N NaOH in the center well, sealed, and placed on the shaker–incubator (35°) for 18–24 hr. At that time the NaOH was removed and analyzed for the presence of cyanide; new NaOH soln. was then placed in the center well, emulsin added to the main compartment, and incubation and cyanide analysis repeated. New NaOH soln. was then placed in the center well, linamarase added to the main compartment, and the procedure repeated.

Chromatography and purification. Cyanogenic compounds were isolated by methods similar to those previously described [6,7] but in *i*-PrOH (10%) instead of EtOH. Phenolic compounds and ionic constituents were removed by passing the concentrates over a mixed-bed ion exchange resin. The eluate was then concentrated, taken up in 10% *i*-PrOH and streaked on Whatman 3MM paper and further purified in MeCOEt–Me_2CO–H_2O (30:10:6), and *n*-BuOH–HOAc–H_2O (12:3:5. The isolates run on these chromatographs were compared with standards of amygdalin, linamarin, lotaustralin, prunasin, acacipetalin and dihydroacacipetalin. The chromatograms were then analyzed using either the method of ref. [11] or that of ref. [6].

NMR analysis. Purified cyanogenic compounds were analyzed by conversion to their TMS derivatives and measurement of their NMR spectra by previously described techniques [12].

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