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Cyanogenic allosides and glucosides from *Passiflora edulis* and *Carica papaya*☆

David S. Seigler[a,*], Guido F. Pauli[b,1], Adolf Nahrstedt[b], Rosemary Leen[c]

[a]*Department of Plant Biology, University of Illinois, Urbana, IL 61801, USA*
[b]*Institut für Pharmazeutische Biologie und Phytochemie, Westfälische Wilhelms–Universität, D–48149 Münster, Germany*
[c]*USDA Forest Service, Pacific Southwest Research Station, Box 236, Volcano, HI 96785, USA*

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Abstract

Leaf and stem material of *Passiflora edulis* (Passifloraceae) contains the new cyanogenic glycosides (2*R*)–β-D-allopyranosyloxy-2-phenylacetonitrile (**1a**) and (2*S*)–β-D-allopyranosyloxy-2-phenylacetonitrile (**1b**), along with smaller amounts of (2*R*)–prunasin (**2a**), sambunigrin (**2b**), and the alloside of benzyl alcohol (**4**); the major cyanogens of the fruits are (2*R*)–prunasin (**2a**) and (2*S*)–sambunigrin (**2b**). The major cyanogenic glycoside of *Carica papaya* (Caricaceae) is **2a**; only small amounts of **2b** also are present. We were not able to confirm the presence of a cyclopentenoid cyanogenic glycoside, tetraphyllin B, in *Carica papaya* leaf and stem materials. In detailed ^{1}H NMR studies of **1a/b** and **2a/b**, differences in higher order effects in glucosides and allosides proved to be valuable for assignment of structures in this series. The diagnostic chemical shifts of cyanogenic methine and anomeric protons in **1a/b** are sensitive to anisotropic environmental effects. The assignment of C-2 stereochemistry of **1a/b** was made in analogy to previous assignments in the glucoside series and was supported by GLC analysis of the TMS ethers. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: (2*R*)–β-D-Allopyranosyloxy-2-phenylacetonitrile; (2*S*)–β-D-Allopyranosyloxy-2-phenylacetonitrile; Benzyl alcohol alloside; Cyanogenic glycosides; Prunasin; Sambunigrin; D–Allose; *Passiflora edulis*; Passifloraceae; *Carica papaya*; Caricaceae

1. Introduction

Cyanogenesis is widespread in plants, but relatively few cyanogenic compounds have been isolated and characterized (Lechtenberg and Nahrstedt, 1999). Two widely cultivated tropical plant species, the passion fruit, *Passiflora edulis* Sims (Passifloraceae), and the papaya, *Carica papaya* L. (Caricaceae), have previously been examined, but questions remain concerning the presence and identity of their cyanogenic compounds.

A variety of complex cyclopentenoid cyanogenic glycosides, as well as glycosides apparently derived from valine and isoleucine, occur in members of the Passifloraceae (Olafsdottir et al., 1989a,b). Other cyanogenic glycosides that appear to be derived from phenylalanine also occur in the family (Spencer and Seigler, 1983; Chassagne et al., 1996). One species, *P. edulis*, has considerable economic importance and is cultivated as a crop for fresh fruit and for juice (Rehm and Espig, 1976; Pittier, 1978). Nonetheless, leaves, stems and immature fruits of this species are cyanogenic, sometimes strongly so. Cyanogenic compounds in *P. edulis* were first reported by Rosenthaler (1919). The presence of prunasin was questioned by Olafsdottir et al. (1989a), but later confirmed by Chassagne et al. (1996), who found (2*R*)–prunasin (**2a**), (2*S*)–sambunigrin (**2b**), and amygdalin in the fresh fruit juice and peel. They also found compounds that were tentatively identified as mandelonitrile rhamnopyranosyl–β–D–glucopyranosides of undetermined configuration (Chassagne and Crouzet, 1998).

Several plants, primarily from the Brassicaceae (Cruciferae), have been reported to contain both cyanogenic materials and glucosinolates (Honeyman, 1956; Gibbs, 1974). Some of these reports are due to false positive results from both Feigl–Anger and picrate tests. In the instance of *C. papaya*, however, both glucosinolates and

☆ While this manuscript was under review, we became aware of another report of a cyanogenic alloside and the glucoside of benzyl alcohol from leaf and stem material of *P. edulis* (Christensen, J., Jaroszewski, J. W., 2001. Organic Letters 3, 2193–2195).

* Corresponding author. Tel.: +1-217-333-7577; fax: +1-217-244-7246.

E-mail address: d-seigler@uiuc.edu (D.S. Seigler).

[1] Current address: Institute for Tuberculosis Research, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 606012, USA.

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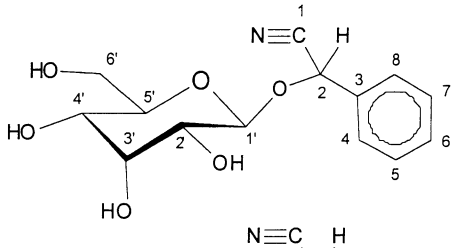
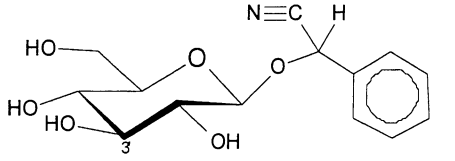
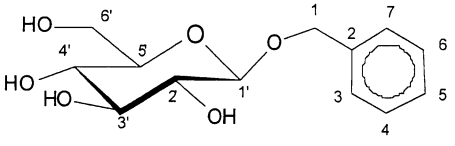
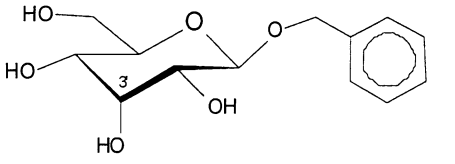
cyanogenic glycosides are found (Bennett et al., 1997). Benzylglucosinolate (glucotropaeolin) is found in all tissues of this plant, but cyanogenic compounds also occur in leaves and roots. The identity of the compounds responsible for cyanide release in this plant is open to question. Leaf material of *C. papaya* has been reported to contain (*R*)–prunasin and the cyclopentenyl cyanogen tetraphyllin B (Spencer and Seigler, 1984a; Goldstein and Spencer, 1985; Spencer, 1988). We undertook the present work to re–examine cyanogenic glycosides involved in cyanogenesis in *P. edulis* and *C. papaya*, and to clarify whether tetraphyllin B was present in the latter species or not.









2. Results and discussion

2.1. (2R)–β-D-Allopyranosyloxy-2-phenylacetonitrile (1a) and (2S)–β-D-allopyranosyloxy-2-phenylacetonitrile (1b) from P. edulis

Fractionation of an extract of leaf and stem materials indicated the presence of at least three cyanogenic compounds with most of the cyanogenic activity attributable to the compound at R_f 0.59–0.64. This material was purified by a combined series of VLC and MPLC separations to yield **1**. The DCI–NH_3 mass spectrum of **1** showed a quasi–molecular ion at *m/z* 313 corresponding to $[C_{14}H_{17}O_6N + NH_4]^+$ and a cluster ion at *m/z* 330 $[C_{14}H_{17}O_6N + NH_4 + NH_3]^+$. Considering the aromatic UV absorption and the carbon backbone and chemical shift distribution of 14 carbons (for the major component **1a**) observed in the ^{13}C NMR spectra, a starting hypothesis for the structure elucidation of **1** was the presence of a prunasin/sambunigrin–type aromatic cyanogenic glycoside. However, an initial survey of the ^1H signals drew focus to the sugar moiety as the probable point of structural difference. Whereas the typical proton signals of a monosubstituted aromatic ring (AA'BB'M or ABMNX spin system) and the methine hydrogen (5.875 ppm) were detected, the sugar proton signals lacked the characteristic all–*trans* coupling pattern of glucose (Veit and Pauli, 1999). The most prominent uncommon signal was a small 3 Hz triplet at 4.019 ppm resonating at even lower field than the hydroxymethylene AB–type double doublets of C–6 (3.893 and 3.692 ppm; 2.1/11.8 and 6.3/11.8 Hz, respectively) that typically indicate a hexopyranose sugar. Complete analysis of all resonances is possible with a magnet of moderate field (360 MHz ^1H) and, in this case, such an analysis aided by a 2D COSY map, indicated the presence of allose, not glucose, as is found in many glycosides. Because allose contains an equatorial H–3, in contrast to the axial H–3 in glucose, the signals of both H–2 and H–4 in allose are split by a large coupling (8/10 Hz) with one adjacent proton, and by a small coupling (3 Hz) with the proton H–3. Considering the β–anomeric configuration of the sugar, **1** must be the β–alloside of mandelonitrile, the allose analogue of prunasin/sambunigrin. Determination of the relative stereochemistry of allose must be based on a full complement of coupling information for the hexopyranose ring system (see Fig. 1). However, comparative literature data are in short supply because the resonances of all protons of the allose moiety are frequently not reported (Franzyk et al., 1998; Jensen, 1996; Gering and Wichtl, 1987) or only those of peracetates are measured (Toyota et al., 1996).

The NMR spectra of **1** contained two shifted sets of signals corresponding to two pairs of diastereomeric cyanogenic glycosides. Because stereochemical interconversion between prunasin and sambunigrin is known to occur on isolation of the compounds (Nahrstedt, 1981; Seigler, 1991), we cannot exclude the possibility of racemization to produce a pair of allosides (**1a/b**), although under similar conditions, pure glycosides can be isolated (Nahrstedt et al., 1983). In prunasin (**2a**), but not in sambunigrin (**2b**), the ring current anisotropism of the neighboring aromatic ring causes severe substituent chemical shift effects leading to a higher–order spin situation. This phenomenon can be recognized from the abnormally shaped anomeric doublet showing "sidebands". In contrast, due to both the wider chemical shift distribution of protons H-2 to H-5 (AMXZ) and conformational effects in allosides, the β–allose moiety

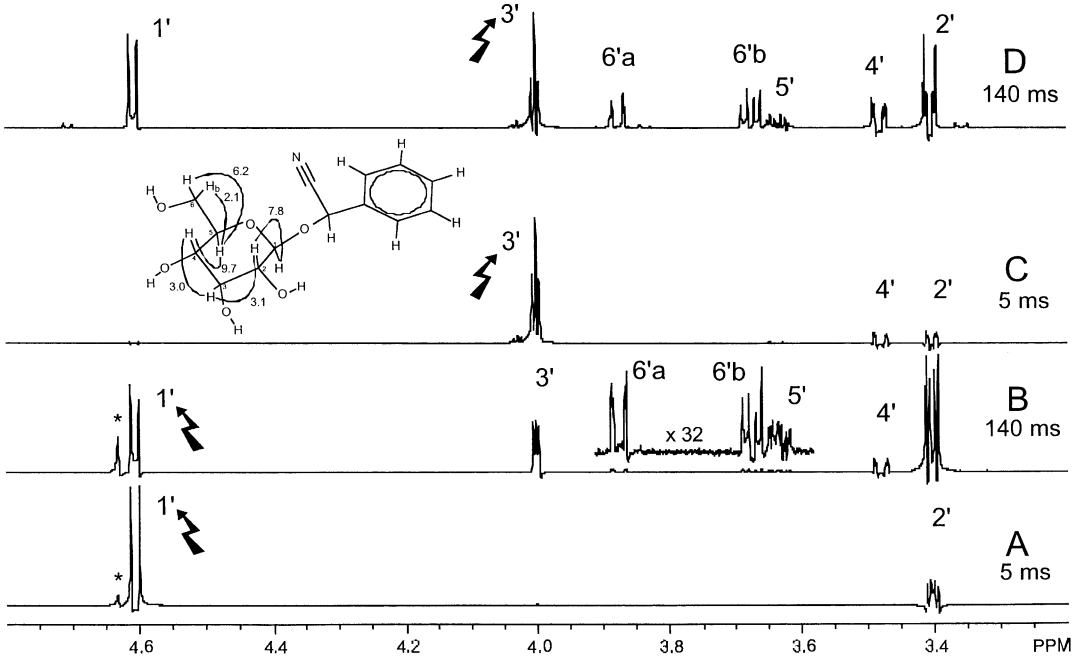


Fig. 1. The shift and coupling pattern of the β–allose moiety gives rise two a fully resolved set of first order ^{1}H NMR signals in the aromatic allosides **1a/b** and **4**. As shown for the major epimer **1a**, the two small J values arising from the equatorial position of H-3′ hinders the TOCSY magnetization transfer when exciting the anomeric proton (reduced signal intensities for H-3′ through 2H-6′, see slices A + B). Whereas in both COSY and TOCSY experiments it is generally advisable to start tracing sugar moieties from the anomeric protons, the best starting point for allosides is the downfield signal of H-3′. Moreover, choosing H-3′eq as the point of selective excitation results in advantageous signal intensities for the complete spin system (slices C + D), while still offering a well resolved point for the selective pulse. Interestingly, although H-3 is a regular sugar methine proton, its downfield resonance behavior (~4.0–4.1 ppm) comes very close to that of the anomeric methine of prunasin (**2a**) (~4.2 ppm). This relationship reflects the large impact of both configuration (equatorial/axial methine H orientation; e.g., glucose versus allose) and conformation (anisotropic (de-)shielding influence of aromatic rings) on the chemical shift behavior of sugar protons in aromatic glycosides (5 ms [A/C] and 140 ms [B/D] 1D selective TOCSY experiments of **1a** at 600 MHz; ∗residual excitation of the doublet of H-1a of compound **4,** one half of which coincides with the left hand portion of the anomeric doublet of H-1′ of **1a**).

Table 1
^{1}H and ^{13}C NMR spectral data[a] for the allosides **1a, 1b** and **4**

Position	δ_C (**1a**)[b]	δ_H (**1a**)[b]	δ_C (**1b**)[b]	δ_H (**1b**)[b]	δ_C (**4**)[b]	δ_H (**4**)[b]	Mult.[c]	J[c]	$^{2/3}J_{C,H}$ → H–atom(**1a**)[d]
1(a)	119.58	–	119.58	–	71.75	4.913	–	–	2
1b	–	–	–	–	–	4.640			
2	68.38	5.875	68.30	6.029	133.96	–	*s*	–	4, 8, 1′
3	135.01	–	134.85	–	129.23[e]	7.40∗	–	–	2, 5, 7
4	128.91	7.58∗	128.67[e]	7.58∗	129.14[e]	7.23∗	*m* [*ddd*]	~2,~2, ~8	5, 6, 8
5	130.07	7.45∗	128.60[e]	7.26∗	130.58[e]	7.40∗	*m* [*ddd*]	~2, ~8, ~8	4, 7
6	130.88	7.45∗	129.85[e]	7.45∗	129.14	7.29∗	*m* [*dddd*]	~2,~2, ~8, ~8	4, 8
7	130.07	7.45∗	128.60[e]	7.26∗	129.23	7.40∗	*m* [*ddd*]	~2, ~8, ~8	5, 8
8	128.91	7.58∗	128.67[e]	7.58∗	–	–	*m* [*ddd*]	~2, ~8	4, 6, 7
1′	99.94	4.624	99.79	5.043	100.92	4.716	*d*	7.8	2, 5′
2′	72.01	3.421	71.98	3.396	71.71	3.373	*dd*	3.1, 7.8	1
3′	72.92	4.019	72.81	4.047	72.38	4.071	*t* [*dd*]	3.0, 3.1	2′, 4′, 5′
4′	68.77	3.498	68.86	3.490	68.95	3.498	*dd*	3.0. 9.7	2, 3, 6′a, 6′b
5′	75.74	3.651	75.96	3.657	75.45	3.654	*ddd*	2.1, 6.3, 9.7	1′, 3′, 4′, 6′b
6′(a)	63.12	3.893	63.12	3.933	63.12	3.862	*dd*	2.1, 11.8	4′, 5′
6′b		3.692		3.690		3.690	*dd*	6.3, 11.8	–

[a] Observed at 600/150 MHz in CD_3OD; δ in ppm, J in Hz.

[b] Assignments are verified through APT, gHSQC, and gHMBC measurements.

[c] The aromatic protons form higher–order spin systems (∗) that could not be analyzed, even by spectral simulation, due to severe signal overlap; ^{1}H signal multiplicities (Mult.) are given under nuclei first–order assumptions in squared brackets in these cases.

[d] ^{1}H,^{13}C long–range correlations were observed in a 8.5 Hz HMBC map; analogous cross peaks could also observed for the most important long-range connectivities of **1b** (e.g., glycosidic linkage).

[e] The assignments of these aromatic nuclei maybe interchanged within one column.

Table 2
^{1}H and ^{13}C NMR spectral data[a] for the glucosides **2a**, **2b** (^{1}H), and **3**

Position	δ_C (**2a**)	δ_C (**3**)	δ_H (**2a**)	Mult. (*J*) (**2a**)	δ_H (**2b**)	Mult. (*J*) (**2b**)	δ_H (**3**)	Mult. (*J*)
1(a)	119.46	71.77[a]	–	–	–	–	4.918	*d* (11.8)
1b	–	–	–	–	–	–	4.658	*d* (11.8)
2	68.52	133.73	5.891	*s*	6.028	*s*	–	–
3	134.88	129.25	–	–	–	–	7.409	*m*
4	130.12	129.18	7.58	*m*	7.58	*m*	7.520	*m*
5	128.95	138.67	7.442	*ddd* (2, 8, 8)	7.456	*ddd* (2, 8, 8)	7.317	*m*
6	130.99	129.18	7.316	*dddd*/*m* (2, 2, 8, 8)	7.254	*dddd*/*m* (2, 2, 8, 8)	7.520	*m*
7	128.95	129.25	7.442	*ddd* (2, 8, 8)	7.456	*ddd* (2, 8, 8)	7.409	*m*
8	130.12	–	7.58[†]	*m*	7.58[†]	*m*	–	–
1′	102.11	103.32	4.253	*m* [higher order; pseudo *d*, 7.8)	4.672	*d* (7.8)	4.349	*d* (7.8)
2′	74.22	75.15	3.261	*m* [higher order]	3.25*	*dd* (7.8, 9.0)	n.d.	n.d.
3′	78.00	77.89[c]	3.29*	*m* [higher order]	3.429	*t* (9.0)	n.d.	n.d.
4′	71.50	71.73	3.318	*m* [higher order]	3.19–3.35*	*dd* (9.0/9.5)	n.d.	n.d.
5′	78.35	78.12[c]	3.117	*ddd* (2.3, 5.5, 9.7)	3.391	*ddd* (2.2, 5.9, 9.5)	n.d.	n.d.
6′(a)	62.80[b]	62.84[b]	3.907	*dd* (2.3, 12.0)	3.930	*dd* (2.2, 12.0)	3.886	*dd* (2.2, 12.0)
6′b	–	–	3.697	*dd* (5.5, 12.0)	3.697	*dd* (5.9, 12.0)	3.682	*dd* (5.5, 12.0)

[a] Observed at 360/90 MHz in CD_3OD; δ in ppm, *J* in Hz; n.d. = not determined because of severe signal overlap; [†,*]precise shift determination deterred by higher order effects and severe signal overlap.
[b,c] Assignments may be interchanged.

gives rise to a fully resolved set of first order signals in both epimers **1a/b**.

Spectral simulations augmented by a series of 1D selective TOCSY experiments (5/15/140 ms; as in Pauli, 2000) provided definitive proof for the relative stereochemistry of the hexopyranose sugar allose in **1a**. There are notable chemical shift differences in the cyanogenic methine and the anomeric protons between and within the pairs **1a/b** and **2a/b** in two instances. Within each of the pairs **1a/b** and **2a/b**, the chemical shift difference is identical (0.419 ppm, see Tables 1 and 2). However, the anomeric protons resonate farther downfield in one pair (4.624 and 5.043 ppm for **1a/b** vs. 4.253 and 4.672 ppm for **2a/b**, respectively; a difference of 0.371 ppm in each case). Previous investigators have distinguished the 2*R* and 2*S* forms by the characteristic chemical shifts of the anomeric protons (Towers et al., 1964). This relationship still holds, although each of the two anomeric protons is shifted downfield in allosides. In addition, **2a** and **2b** can be distinguished by the complexity of the anomeric "doublet", but due to differences in the order of spin systems this does not hold for allosides **1a/b**. Within the pairs **1a/b** and **2a/b**, the shift difference in the cyanogenic methine protons differs only slightly (0.154 vs. 0.137 ppm, respectively), whereas the difference between pairs is negligible.

Gas chromatographic analysis of TMS-2*R*-prunasin (TMS-**2a**) yields one peak with a retention time of 21.29 min. Under similar conditions, sample **1** had peaks with retention times of 21.28 (33%, TMS-**2a**), 20.48 (2%, TMS-2*S*-sambunigrin (TMS-**2b**)), 19.63 (59%, TMS-(2*R*)–β-D-allopyranosyloxy-2-phenylacetonitrile (TMS-**1a**)), 19.18 (6% TMS-(2*S*)–β-D-allopyranosyloxy-2-phenylacetonitrile (TMS-**1b**)), and 22.04 min (0.3%, unknown impurity). Following epimerization with 0.005 N ammonia, the ratios of signals are changed: 21.28 (15%, TMS-**2a**), 20.48 (20%, TMS-**2b**), 19.63 (29%, TMS-**1a**), and 19.20 (35%, TMS-**1b**). With a knowledge of the *R*/*S*–assignment of cyanogenic glycosides in NMR spectra (Nahrstedt, 1981; Seigler and Brinker, 1993), and assuming that the relationship of chemical shift of the methine proton (H–2) to *R*/*S*–assignment also applies to allosides differing in configuration at the C–3′ position, the major glycoside **1a** may be presumed to have 2*R*–stereochemistry. Based on gas chromatographic, epimerization data and NMR spectra, the second most abundant component is prunasin (**2a**) (compare Tables 1 and 2). The necessity for careful analysis of proton coupling patterns in the analysis of glycosides cannot be emphasized enough (Thomas, 1997). Determination of the absolute D–configuration of the β–allose moiety in **1** was achieved by capillary zone electrophoresis (CZE) using the (*S*)–phenylethylamine derivatives of authentic D– and L–allose as references (see Experimental 3.12 for details).

NMR spectra of **1** also provided evidence for the presence of small quantities of benzyl alcohol alloside (**4**) as indicated by the diagnostic signals similar of the allose portion of 1**a**/**b** and to the benzyl alcohol portion of compound **3** (see Table 2 and discussion below). Although the difference in structure between glucose and allose requires only epimerization of the 3-position, this change causes significant differences in spectral and physical properties of **1a/b** and **2a/b**. Although it is tempting to suggest that **1a** is 2*R*–β–D-allopyranosyloxy-2-phenylacetonitrile (corresponding to prunasin), cur-

rent data do not permit this *absolute* chiral assignment. Only when pure epimers (**1a** and **1b**) are available for study and chiral methods are applied will it be possible to resolve this problem. We suggest the names (2*R*)–β-D-allopyranosyloxy-2-phenylacetonitrile for compound **1a** and (2*S*)–β-D-allopyranosyloxy-2-phenylacetonitrile for compound **1b**.

At least two additional and more polar cyanogenic compounds were found in both leaf and stem materials as well as in immature fruits of this species, but these compounds were not studied further; they may correspond to the rhamnoglucosides previously reported (Chassagne et al., 1996; Chassagne and Crouzet, 1998).

2.2. *Prunasin* **2a** *and sambunigrin* **2b** *from P. edulis*

Extracts of immature fruits of *P. edulis* contain compounds that are chromatographically similar to prunasin and sambunigrin. Fractionation of the aqueous portion derived from an 80% methanol extract indicated the presence of at least three cyanogenic compounds. Most of the cyanogenic activity was attributable to a compound(s) that had R_f 0.59 in the usual TLC system employed. This mixture was further purified by MPLC. Examination of both ^{1}H and ^{13}C NMR spectral data confirms that the cyanogens are indeed prunasin (**2a**) and sambunigrin (**2b**; see Table 2). In the proton NMR spectra, the anomeric protons give rise to either a clean doublet in case of sambunigrin (**2b**), or an obscured doublet (more properly, a multiplet) in the instance of prunasin (**2a**). Both are indicative of the relative resonance behavior of the direct (^3J, attached to the vicinal α–carbon) and the adjacent proton (attached to the post–vicinal ß–carbon) in such a way that their close proximity leads to higher spin coupling and, thus, the more complex anomeric signal is observed. The methine hydrogen is observed as singlets at 5.891 (**2a**) and 6.028 ppm (**2b**), respectively.

The identification of ß–D–glucose as the sugar moiety in **2a/b** and **3** in principle is based on two precepts: (a) the relative stereochemistry of glucose can be deduced from the following H,H–coupling pattern by assuming a nuclei first–order spin framework (see Table 2 for exact *J* and multiplicity values): H–1 = *d* (8 Hz), H–2 = *dd* (8/9 Hz), H–3 = *t* (9 Hz), H–4 = *t* (10 Hz), H–5 = *ddd* (2/6/10 Hz), H–6a = *dd* (2/12 Hz), H–6b = *dd* (6/12 Hz); (b) the absolute stereochemistry was determined upon hydrolysis by CZE.

The fruit extract of *P. edulis* contains **2a** and **2b** in an 85:15 ratio as determined from the integrals of the ^{1}H NMR spectrum (see also Fig. 2). However, peaks due to benzyl alcohol glycoside (**3**) also are present as can be seen below. This glucoside has previously been reported only rarely, and was originally characterized from *Epimedium grandiflorum* Morr. var. *thunbergianum* (Miq.) Nakai (Miyase et al., 1987). Leaves and fruits of *P. edulis* have been shown to contain several cyanogenic glycosides; in addition to **1a/b**, **2a/b**, insufficiently characterized more polar glycosides also are present. Similar cyanogens have been reported from this and related species (Chassagne et al., 1996). However, peaks that are distinctive in the ^{1}H NMR of **1a/b** between δ 3.35 and 3.60 and δ 4.00 and 4.20 are completely absent in the spectra of the glucosides **2a/b**.

The cyanogenic glycosides of many other *Passiflora* species are based on a cyclopentenoid structure. Some of them, such as passicapsin, passibiflorin, and trifasciatin, bear unusual sugars. In addition to glucose, passicapsin has a boivinosyl residue, passibiflorin has an antiarosyl residue, and trifasciatin has an allomethylosyl residue

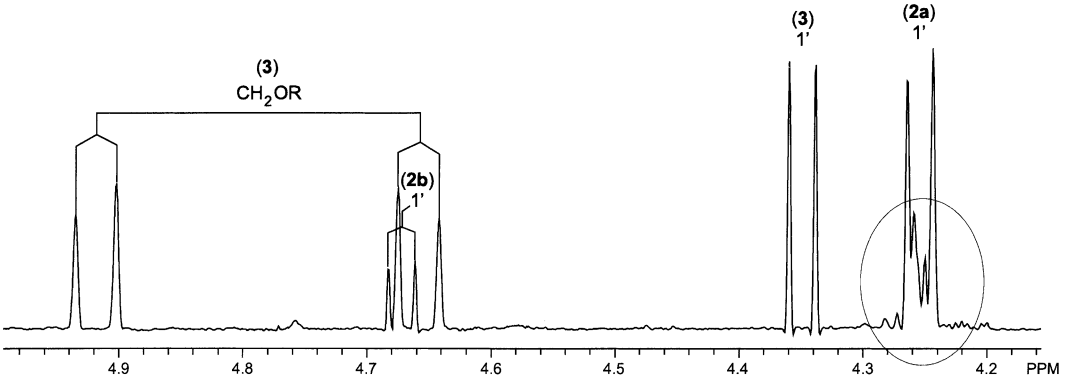


Fig. 2. Cyanogenic glycosides analogous to prunasin (2*R*, **2a**) and sambunigrin (2*S*, **2b**) exhibit an extraordinary intermolecular aromatic induced chemical shift affecting the sugar portion of the molecule. As a result, the sugar proton resonances are not only diastereotopically shifted in the two diastereomers. Higher order spin coupling of the glucose protons take place only in one of the stereoisomers (**2a**), whereas the corresponding couplings in the other isomer (**2b**) can fully be interpreted under nuclei first order assumptions. The data strongly suggest a correlation between the clean anomeric doublet and a 2*S*-configuration of prunasin–type cyanogenic glycosides on one hand, and the more complex anomeric doublet (multiplet, see encircled signal) and 2*R*-configuration on the other. Recognition of these two types of anomeric ^{1}H signals is important for the stereochemical assignment of these compounds. This figure shows a section of the prunasin (**2a**) sample isolated from fruits of *Passiflora edulis* containing epimers **2a** and **2b** in a 1:6 ratio. In addition the sample contains benzyl alcohol β–glucoside (**3**) and **2a** in a 1:1 ratio (360 MHz, in CD_3OD).

(Lechtenberg and Nahrstedt, 1999). These sugars have primarily been reported from cardiac glycosides (Reichstein and Weiss, 1962).

2.3. Prunasin and sambunigrin from Carica papaya

2a has been reported previously from extracts of *C. papaya* (Spencer and Seigler, 1984a,b). Because vegetative material of this species is only weakly cyanogenic, it was necessary to begin with relatively large amounts of extract in order to show the presence of cyanogenic compounds by TLC using the "sandwich" method for detection. Upon MPLC of re–combined fractions from different VLC separations that had cyanogenic compounds in the range of R_f 0.55 to 0.71, it was possible to confirm unequivocally the presence of **2a** and **2b** by NMR. We were unable to confirm the presence of the cyclopentenoid–derived tetraphyllin B.

The ^{1}H and ^{13}C NMR spectra of **2a** obtained from *C. papaya* were essentially identical to that of known **2a** (Hüebel et al., 1981; Nahrstedt and Rockenbach, 1993; Seigler and Brinker, 1993). However, they indicated the presence of a second compound, benzyl alcohol glucoside (**3**) that co–occurred in a 1:1.15 ratio with prunasin (**2a**) as determined from the ^{1}H NMR integrals. Accordingly, in addition to the ions corresponding to **2a**/**b**, the mass spectrum also possessed an intense quasi–molecular ion peak due to **3** at m/z 288 $[C_{13}H_{18}O_6+NH_4]^+$. Only 2% sambunigrin could be detected in the sample as determined by ^{1}H NMR. The ^{1}H and ^{13}C NMR spectral data of **3** are summarized in Table 2.

2.4. General discussion

VLC, in combination with preparative MPLC, was found to be an efficient way for both cleanup and purification of cyanogenic glucosides. Concerning the NMR characterization of cyanogenic glycosides, it has long been noted that the anomeric proton signals of cyanogenic glycosides with (*S*)–configuration are clean doublets, whereas those of glycosides with (*R*)–configuration appear as complex higher order multiplets. This effect was attributed to long–range effects due to conformational changes of the sugar residue, although with relatively constant chemical shifts of the sugar protons (Towers et al., 1964). We have demonstrated that the occurrence of higher order signals for anomeric protons is a result of significant changes in chemical shift of the neighboring protons (H–2 and H–3) in the sugar portion of the molecule, which generates conversion of an AMX or ABX to ABM or ABC type spin systems. Conversely, the observed changes are not the result of alterations in the *conformation* of the sugar, but rather reflect the influence of the anisotropic environment of the chiral aglycone. These diastereotopic effects are especially pronounced for molecules containing an aromatic ring because of the anisotropic ring current effect, but should be less pronounced for cyanogens with aliphatic groups. Analysis of such phenomena requires the application of spectral simulation in the ^{1}H domain by simulating the 7 nuclei spin systems of the hexopyranose moieties. As a result and as suggested earlier by Nahrstedt (1981) within most series of cyanogenic compounds, e.g., the aromatic prunasin/sambunigrin group, it is possible to predict the stereochemistry of the aglycone moiety by noting the complexity and chemical shifts of the anomeric proton signals. Although, this relationship holds true for chemical shift differences between 2*R* and 2*S* epimers, it does not hold true for the complexity of the corresponding anomeric signals of the newly discovered cyanogenic allosides.

The careful examination of chemical shifts, coupling constants, and especially the recognition of higher order and conformational effects, permits the unequivocal identification of the sugar in both new and known cyanogenic compounds. **1a** and **1b** are the first cyanogenic allosides to be described; **4** represents the first report of an alloside of benzyl alcohol. The D–configuration of the allose moiety was established by CZE analysis. Moreover, this is the first supportable report for the presence of a sugar other than D–glucose linked directly to the cyanohydrin hydroxyl of a cyanogenic glycoside. In contrast, the unusual sugar in the previously described 6–desoxyalloside (Olafsdottir et al., 1991) is not attached to the cyanohydrin portion of the aglycone.

Because cyanide is produced spontaneously on damage to these leaf tissues, it will be of interest to investigate the ß–glycosidase(s) responsible for hydrolysis of the substrates **1a/b** and **2a**/**b** for their specificity towards the sugar moiety of these substrates. In the laboratory, (2*R*)–β-D-allopyranosyloxy-2-phenylacetonitrile and (2*S*)–β-D-allopyranosyloxy-2-phenylacetonitrile were cleaved with both an enzyme preparation from *Aspergillus* and snail gut enzymes. It will be of interest to clarify whether an allosyl : mandelonitrile transferase or a prunasin/sambunigrin 3′–epimerase will lead to **1a/b**. Because ß–allose is found quite rarely in natural glycosides, and because the NMR and MS spectroscopic differences between the ubiquitous glucosyl moieties of cyanogenic molecules have not always been examined or reported carefully, we can only speculate whether allose actually plays a more important role in both cyanogenic and other glycosides than has generally been appreciated.

3. Experimental

3.1. Plant materials

Leaf and young stem material of *C. papaya* was cultivated at the University of Illinois, Plant Care Facility,

from seeds of papaya fruits purchased at a local grocery store. Leaf, stem and immature fruit material of *P. edulis* was collected near the Hilo Airport, Hilo, Hawaii, and maintained at the Hawaii Volcanoes National Park nursery by R. L. Leaf and stem material was air dried, and fruits were freeze dried before shipping.

3.2. Qualitative tests for HCN

Crushed plant material, extract, or purified compound was placed in a vial and moistened with distilled water or buffer [potassium phosphate, 0.1 M, pH 6.8 or citrate–phosphate (McIlvaine, pH 6.0)]. A small amount of snail gut enzyme (Sigma, St. Louis, MO) or Röhm Pr. El No. 1–77 enzyme from *Aspergillus niger* (Röhm GmbH, Darmstadt) was added when endogenous enzyme was not present. A strip of filter paper impregnated with copper ethylacetoacetate and tetra base (4,4′–tetramethyldiaminodiphenylmethane) was added to the vial and held in place with the lid of the vial (Feigl and Anger, 1966; Tantisewie et al., 1969; Brinker and Seigler, 1989, 1992). The samples were incubated at 40 °C. If they required more than 6 h for development of the color characteristic of a positive Feigl–Anger test, the test was repeated and several drops of a streptomycin sulfate solution (1.0 mM) added to inhibit bacterial contamination.

The presence of HCN–releasing compounds on thin layer plates was determined by a modified ''sandwich method'' (Brimer et al., 1983). Sheets of Polygram Ionex–25 SB–AC, 0.25 mm (Macherey Nagel, Düren) were dipped in a saturated solution of picric acid, dried with a hair dryer, then dipped in a solution of 1 M $NaHCO_3$ and again dried. Finally, the plates were sprayed with a solution of cetyl alcohol (2% in methanol). Plates to be tested were sprayed with either Röhm or snail gut enzyme in phosphate or citrate–phosphate buffer until the surface was slightly moist, a picrate–impregnated sheet placed over the freshly sprayed plate, these plates placed between two glass plates, clamped together and the total assemblage incubated at 40 °C for several hours. Positive tests resulted in a change from the yellow color of the picrate–impregnated plates to a reddish–brown color.

3.3. Extraction of plant materials

Freeze–dried immature fruits of *P. edulis* (14 g), dried leaves and stems of *P. edulis* (155 g), and dried leaf and stem material of *C. papaya* (20 kg), were ground and extracted with 80% methanol. In each case, the mixture was heated at boiling for 10 min and rinsed two times with the same solvent. Each of the combined extracts was filtered through cheesecloth, through filter paper (Whatman No. 4), and the methanol removed under vacuum. The residual aqueous mixture was partitioned between water and chloroform. Both the aqueous and chloroform phases were tested for the presence of HCN by the Feigl–Anger method; only the aqueous phase contained cyanogenic compounds. The aqueous portion of freeze–dried immature fruits of *P. edulis* was concentrated to yield 3.5 g of a solid residue, that from leaves and stems of *P. edulis* gave 20.4 g, and that from leaf and stem material of *C. papaya* resulted in 486 g of extract.

3.4. Thin layer chromatography (TLC)

Each of the extracts was examined by TLC on Merck Silica 60 F_{254} plates, 0.2 mm. The usual solvent was ethyl acetate, methanol, water (79:11:10). After development, plates were either visualized by spraying with anisaldehyde–H_2SO_4 reagent (anisaldehyde, 0.5 ml, HOAc, 10 ml, MeOH, 85 ml, and H_2SO_4, 5 ml), followed by heating at 120 °C for 10 min, or by spraying with ß–glucosidase enzyme and detection of cyanide by the ''sandwich'' method described above. The major cyanide containing spot from the crude extract of immature *P. edulis* fruits had R_f 0.62, with a smaller amount of a second cyanide releasing spot at R_f 0.31; that of *P. edulis* leaves and stems had R_f 0.64, and that of *C. papaya* had R_f 0.64. In each case, these substances gave a greenish–gray colored spot when detected with anisaldehyde–H_2SO_4 reagent.

3.5. Vacuum chromatography (VLC) of the aqueous soluble materials

Aliquots of the aqueous soluble materials from each of the above were separated by VLC (Pauli, 1993; Pelletier et al., 1986; Coll and Bowden, 1986; Millar, 1998; Pieters and Vlietinck, 1989). Typical columns consisted of silica gel (Silica 60 $PF_{254}G$, Merck, 50 g) with gypsum binder, and the entire column was washed with petroleum ether. The sample was loaded after being mixed thoroughly with a smaller amount of silica gel, and aliquots of solvent (50 ml, unless designated otherwise) were added in sequence to run the column.

3.6. Medium pressure liquid chromatography (MPLC)

Samples were chromatographed on a Büchi 26×460 mm column packed with Orpegen, RP–18, hooked up to a precolumn packed with the same material. Separations were monitored with a LKB Bromma, 2151 Variable Wavelength Monitor at 280 nm. Fractions of 10–15 ml were collected at 8 ml/min with a Büchi MPLC pump.

3.7. Fractionation of P. edulis leaf and stem extract

A portion (20.4 g) of the aqueous fraction from partition of an 80% MeOH extract with $CHCl_3$ and water after removal of MeOH was added to a column as

described above and fractionated with EtOAc, and a series of fractions with EtOAc containing increasing amounts of a 1:1 mixture of H_2O and MeOH ranging from 1 to 50% (fraction 1 (hexane–EtOAc, 1:1), 2 (EtOAc + MeOH–H_2O, 1%), 3 (2%), 4 (3%), 6 (6%), 7 (8%), 8 (10%), 9 (12%), 10 (14%), 11 (16%), 12 (18%), 13 (20%), 14 (25%), 15 (30%), 16 (40%), and 16 (50%). Analysis by TLC indicated that fractions 7–9 contained most of the cyanogenic activity (R_f 0.59). Fractions 6–11 from the above VLC of *P. edulis* leaf and stem extract (109 mg) were combined and fractionated by MPLC with a gradient from water to methanol (500 ml). TLC revealed that fractions 18–20 contained a cyanogenic mixture with R_f 0.64 (**1**, 6.0 mg).

More polar cyanogens were found in fractions 15–17 (R_f 0.31) and in fractions 18–21 (R_f 0.58 in EtOAc–MeOH–H_2O, 67:25:8; amygdalin R_f 0.79, linamarin R_f 0.59 in this solvent system).

3.8. Fractionation of P. edulis immature fruit extract

An aliquot (3.5 g) similar to that above from *P. edulis* immature fruits was fractionated by VLC as above with EtOAc containing increasing amounts of a 1:1 mixture of H_2O and MeOH from 1 to 100% [fraction 1 (hexane–EtOAc, 1:1)], 2 (EtOAc + 1% MeOH–H_2O), 3 (2%), 4 (3%), 5 (4%), 6 (5%), 7 (7%), 8 (8%), 9 (10%), 10 (12%), 11 (14%), 12 (16%), 13 (18%), 14 (20%), 15 (25%), 16 (30%), 17 (35%), 18 (40%), 19 (50%), 20 (75%), and 21 (100%)]. The cyanogenic activity of fractions 5–13 occurred mostly in fractions 7–9 (R_f 0.59). A more polar cyanogen was found in fractions 15–17 (R_f 0.31) and an even more polar compound(s) in fractions 18–21 (R_f 0.58, with EtOAc–MeOH–H_2O, 67:25:8). Fractions 7–9 were combined and purified by MPLC as above. TLC analysis revealed that fractions 28–31 contained compound **2a**/**b** with R_f 0.55 (9.2 mg).

3.9. Epimerization and gas chromatography of TMS ethers

A portion of fractions 18–20 was analyzed by gas chromatography on a non-polar column as the persilylated ether derivative. Glycoside (approximately 1 mg), was dissolved in pyridine (25 μl) with BSTFA (60 μl) and TMCS (14 μl). The derivatized mixture was analyzed on an OV225 column (30 m×0.25 mm) on an HP 6890 Gas Chromatograph by programming from 160 to 260 °C, at 5 °C per min. FID and NPD detectors were used.

Under conditions for which the TMS derivative of prunasin had a retention time of 21.29 minutes, this sample had peaks with a retention time of 21.28 (33%, TMS-2*R*-prunasin, **2a**), 20.48 (2%, TMS-2*S*-sambunigrin, **2b**), 19.63 (59%, TMS-(2*R*)–β-D-allopyranosyloxy-2-phenylacetonitrile, **1a**), 19.18 (6% TMS-(2*S*)–β-D-allopyranosyloxy-2-phenylacetonitrile), and 22.04 minutes (0.3%, unknown impurity). Following epimerization with 0.005 N ammonia, the ratios of signals are changed: 21.28 (15%, TMS-**2a**), 20.48 (20%, TMS-**2b**), 19.63 (29%, possible TMS-2*R*-**1a**), and 19.20 (35%, possible TMS-**1b**).

3.10. Fractionation of C. papaya extract

An aliquot as above (100 g) was fractionated by stepwise gradient elution using EtOAc containing increasing amounts of a 1:1 mixture of H_2O and MeOH from 1 to 100% [fraction 1 (petroleum ether), 2–4 (ethyl acetate), 5 (1% MeOH–H_2O), 6 (2%), 7 (3%), 8 (4%), 9 (5%), 10 (6%), 11 (7%), 12 (8%), 13 (10%), 14 (12%), 15 (14%), 16 (16%), 17 (18%), 18, 19 (20%), 20 (22%), 21 (25%), 22 (30%), 23 (35%), 24–26 (40%), 27 (50%), 28 (75%), and (29) 100%]. Analysis by TLC indicated that fractions before 17 contained **2a** (R_f 0.66). Fractions 20–29 were strongly cyanogenic, but also contained more polar compounds (R_f <0.60). Fractions 17–29 were combined (50 g) and re–chromatographed with EtOAc and increasing amounts of MeOH/H_2O. In this instance, a column with 100 g silica gel was used and 200 ml fractions were collected. Based on TLC, frs 3–8 contained **2a**, as well as a second cyanide releasing compound (R_f 0.71); frs 7–12 contained a third cyanogenic compound (R_f 0.47). Frs 18–24 from this second VLC contained a cyanogenic material that did not migrate from the origin; this material gave a weakly positive, slow reaction for cyanide release with the "sandwich method".

Combined frs 15–16 from the first and frs 13–17 from the second VLC were mixed with silica gel H and with 10% anhydrous $CaSO_4$, and then separated on a column of the same material. The column was eluted with EtOAc and EtOAc containing increasing amounts of a 1:1 mixture of H_2O and MeOH from 1 to 20% [fraction 1 (50:50 hexane–EtOAc), 2 (EtOAc + 1% MeOH–H_2O), 3 (2%), 4 (3%), 5 (4%), 6 (5%), 7 (7%), 8 (8%), 9 (9%), 10 (10%), 11 (12%), 12 (15%), 13 (18%), 14 (20%)]. Frs 3–9 from this chromatography were combined and purified by MPLC. The major cyanogenic material in MPLC frs 22–29 (R_f 0.62, 149 mg) was repeatedly purified by MPLC, finally with a gradient of CH_3CN and water (**2a**, 21.9 mg, R_f 0.56).

3.11. Nuclear magnetic resonance

The spectra were recorded in methanol–d_4 on Bruker AM 360 (5 mm dual probe) and Varian Unity 600 (5 mm multinuclear probe) instruments operating at 360/600 MHz for ^1H, and 90/150 MHz for ^{13}C, respectively (δ scale, 3.300 and 49.00 ppm, respectively) as follows: ^1H and ^{13}C 30 deg. pulse, delay after acquisition (D1) 1 s (^1H) and 2 s (^{13}C), processed with 0.2 (^1H) and 1.6 Hz (^{13}C) line broadening (LB) or with Lorentz–Gauss

resolution enhancement after zero–filling (^{1}H). ^{1}H–acquisition used 16 k (32 k at 600 MHz) data points in a spectral window of ca. 8 ppm yielding a digital resolution better than 0.2 Hz (or 0.0004 ppm). Water peak suppression was achieved by presaturation setting the HDO signal on resonance (O1). 2D experiments were acquired using 1 k×512 or 256 (^{1}H,^{1}H) or 1 K (^{1}H,^{13}C) increments, 90 deg. shifted sinebell–squared apodization, and zero–filling in t_1 dimension during processing. The 1D Selective TOCSY experiments were performed at 600 MHz using the eburp1/25 selective pulse shape program and acquiring 16 K data points. The delays in both regular and gradient enhanced 2D correlations were optimized for 145 ($^1J_{H,C}$) and 8.5 Hz ($^3J_{H,C}$). Offline data processing was done with the manufacturer's NMR data processing software (Varian VNMR and Bruker DISNMR) as well as the NUTS program package (Acorn NMR, Fremont, CA).

3.12. Mass spectrometry

DCI mass spectra were run on a Finnigan INCOS 50 System with ammonia as reactant gases. The emitter heating rate was 10 mA s^{-1}, calibration was done with FC–43.

3.13. Capillary zone electrophoresis (CZE)

Determination of the D/L–configuration of the sugars was performed by capillary zone electrophoresis on a Beckman P/ACE 5010 instrument using an uncoated fused silica capillary (570 mm, 50 μm i.d., 30 kV, 27 °C) and a DAD UV detector (λ 200 nm) operated with System Gold software. Conditions were chosen based on a recently published method (Noe and Freissmuth, 1995), which was optimized and allowed the analysis of small quantities (0.5–1.5 mg of glycoside). Upon hydrolysis with aqueous 23% TFA solution, the *S*–(-)–1–phenylethylamine derivatives of the free sugars were formed and reacted with $NaBH_3CN$. D/L–glucose and D/L–allose were purchased from Fluka and Aldrich. Using a 50 mM $Na_2B_4O_7$ buffer at pH 10.3 that contained 2.7 M MeCN, the migration times (t_m) of the D/L–sugar derivatives differed significantly as follows: Δt_m(allose) 0.27 min with D–allose 29.38 min, L–allose 29.11 min; Δt_m(glucose) 0.35 min with D–glucose 21.15 min, L–glucose 21.50 min.

3.14. (2R)–β-D-Allopyranosyloxy-2-phenylacetonitrile (**1a**) and (2S)–β-D-allopyranosyloxy-2-phenylacetonitrile (**1b**)

R_f 0.64; solvents as above; ^{1}H and ^{13}C NMR (600/150 MHz, CD_3OD) see Table 1. DCI–NH_3–MS (*m*/*z*): 313 (100) $[C_{14}H_{17}O_6N+NH_4]^+$, 330 (70) $[C_{14}H_{17}O_6N+NH_4+NH_3]^+$.

3.15. Prunasin (**2a**) and sambunigrin (**2b**) from P. edulis immature fruits and from C. papaya leaves and stems

R_f 0.56; solvents as above; ^{1}H and ^{13}C NMR (360/90 MHz, CD_3OD) see Table 2. These spectra also have peaks corresponding to benzyl alcohol glucoside (**3**). DCI–NH_3–MS (*m*/*z*): 313 (100) $[\mathbf{2a}/\mathbf{2b}+NH_4]^+$, 330 $[\mathbf{2a}/\mathbf{2b}+NH_3+NH_4]^+$.

3.16. Benzyl alcohol glucoside (**3**) from C. papaya leaves and stems

R_f 0.64; solvents as above; ^{1}H and ^{13}C NMR (360/90 MHz, CD_3OD) see Table 2. DCI–NH_3–MS (*m*/*z*): 288 $[\mathbf{3}+NH_4]^+$.

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References

Bennett, R., Kiddle, G., Wallsgrove, R., 1997. Biosynthesis of benzylglucosinolate, cyanogenic glucosides and phenylpropanoids in *Carica papaya*. Phytochemistry 45, 59–66.

Brimer, L., Christensen, S., Moelgaard, P., Nartey, F., 1983. A densitometric method for quantification of cyanogenic glycosides, employing enzyme preparations from *Helix pomatia* and picrate impregnated ion exchange sheets. Journal of Agricultural and Food Chemistry 31, 789–793.

Brinker, A.M., Seigler, D.S., 1989. Methods for the detection and quantitative determination of cyanide in plant materials. Phytochemical Bulletin 21, 24–31.

Brinker, A.M., Seigler, D.S., 1992. Determination of cyanide and cyanogenic glycosides from plants In: Linskens H.F., Jackson, J.F. (Eds.), Plant Toxin Analysis. Springer Verlag, Berlin, pp. 359–381.

Chassagne, D., Crouzet, J., Bayonove, C., Baumes, R., 1996. Identification and quantification of passion fruit cyanogenic glycosides. Journal of Agricultural and Food Chemistry 44, 3817–3820.

Chassagne, D., Crouzet, J., 1998. A cyanogenic glycoside from *Passiflora edulis* fruits. Phytochemistry 49, 757–759.

Coll, J., Bowden, B., 1986. The application of vacuum liquid chromatography to the separation of terpene mixtures. Journal of Natural Products 49, 934–936.

Feigl, F., Anger, V., 1966. Replacement of benzidine by copper ethylacetoacetate and tetra base as spot–test reagent for hydrogen cyanide and cyanogen. Analyst 91, 282–284.

Franzyk, H., Husum, T., Jensen, S.R., 1998. A caffeoyl phenylethanoid glycoside from *Plantago myosurus*. Phytochemistry 47, 1161–1162.

Gering, B., Wichtl, M., 1987. Phytochemical investigations on *Penstemon hirsutus*. Journal of Natural Products 50, 1048–1054.

Gibbs, R.D., 1974. Chemotaxonomy of Flowering Plants, Vol. 3. McGills–Queens University Press, Montreal.

Goldstein, W.S., Spencer, K.C., 1985. Inhibition of cyanogenesis by tannins. Journal of Chemical Ecology 7, 847–858.

Honeyman, J.M., 1956. On the occurrence of cyanogenetic glycosides in the order Rhoeadales. Taxon 5, 33–34.

Huebel, W., Nahrstedt, A., Wray, V., 1981. A structural investigation by ^{13}C–NMR of the cyanogenic glycosides. Archiv der Pharmazie 314, 609–617.

Jensen, S.R., 1996. Caffeoyl phenylethanoid glycosides in *Sanango racemosum* and in the Gesneriaceae. Phytochemistry 43, 777–783.

Lechtenberg, M., Nahrstedt, A., 1999. Cyanogenic glycosides. In: Ikan, R. (Ed.), Naturally Occurring Glycosides. Wiley, Chichester, pp. 147–191.

Millar, J.G., 1998. Liquid Chromatography. In: Millar, J., Haynes, K. (Eds.), Methods in Chemical Ecology. Chemical Methods, Vol. 1. Chapman and Hall, New York, pp. 38–83.

Miyase, T., Ueno, A., Takizawa, N., Kobayashi, H., Karasawa, H., 1987. Studies on the glycosides of *Epimedium grandiflorum* Morr. var. *thunbergianum* (Miq.) Nakai. I. Chemical and Pharmaceutical Bulletin 35, 1109–1117.

Nahrstedt, A., 1981. Isolation and structure elucidation of cyanogenic glycosides. In: Vennesland, B., Conn, E., Knowles, C., Westley, J., Wissing, F. (Eds.), Cyanide in Biology. Academic Press, London, pp. 145–181.

Nahrstedt, A., Wray, V., Grotjahn, L., Fikenscher, L., Hegnauer, R., 1983. New acylated cyanogenic diglycosides from fruits of *Anthemis cairica* and *A. altissima*. Planta Medica 49, 143–148.

Nahrstedt, A., Rockenbach, J., 1993. Occurrence of the cyanogenic glucoside prunasin and its corresponding mandelic acid amide glucoside in *Olinia* species (Oliniaceae). Phytochemistry 34, 433–436.

Noe, C., Freissmuth, J., 1995. Kapillarelektrophoretische Analytik komplexer Kohlenhydratgemische. Journal of Chromatography A 704, 503–512.

Olafsdottir, E.S., Andersen, J., Jaroszewski, J.W., 1989a. Cyclopentenoid cyanohydrin glycosides. Part 9: Cyanohydrin glycosides of Passifloraceae. Phytochemistry 28, 127–132.

Olafsdottir, E.S., Cornett, C., Jaroszewski, J.W., 1989b. Cyclopentenoid cyanohydrin glycosides with unusual sugar residues. Acta Chemica Scandinavica 43, 51–55.

Olafsdottir, E.S., Jaroszewski, J.W., Seigler, D.S., 1991. Cyanohydrin glycosides with unusual sugar residues: revised structure of passitrifasciatin. Phytochemistry 30, 867–869.

Pauli, G.F., 2000. Higher order and substituent chemical shift effects in the proton NMR of glycosides. Journal of Natural Products 63, 834–838.

Pauli, G.F., 1993. Cardenolide aus *Adonis aleppica* Boiss. Isolierung und Strukturaufklärung. PhD thesis, University of Düsseldorf, Germany.

Pelletier, W., Chokshi, H., Desai, H., 1986. Separation of diterpenoid alkaloid mixtures using vacuum liquid chromatography. Journal of Natural Products 49, 892–900.

Pieters, L., Vlietinck, A., 1989. Vacuum liquid chromatography and quantitative ^{1}H NMR spectroscopy of tumor-promoting diterpene esters. Journal of Natural Products 52, 186–190.

Pittier, H., 1978 (reprinted). Manual de las Plantas Usuales de Venezuela. Fundación Eugenio Mendoza, Caracas.

Rehm, S., Espig, G., 1976. Die Kulturpflanzen der Tropen und Subtropen. Eugen Ulmer Verlag, Stuttgart.

Reichstein, T., Weiss, E., 1962. The sugars of cardiac glycosides. Advances in Carbohydrate Chemistry 17, 65–108.

Rosenthaler, L., 1919. Distribution of hydrocyanic acid in the plant kingdom, Schweizerische Apotheker Zeitung, 57, 279–283, 295–297, 307–313, 324–329, 341–346.

Seigler, D.S., 1991. Cyanide and cyanogenic glycosides. In: Rosenthal, G., Berenbaum, B. (Eds.), Herbivores: Their Interactions with Secondary Plant Metabolites, Vol. 1. Academic Press, San Diego, pp. 35–77.

Seigler, D.S., Brinker, A.M., 1993. Characterization of cyanogenic glycosides, cyanolipids, nitroglucosides, organic nitro compounds and nitrile glucosides from plants. In: Dey, P., Harborne, J.B. (Eds.), Methods in Plant Biochemistry, Vol. 8. Academic Press, London, pp. 51–131.

Spencer, K.C., 1988. Chemical mediation of coevolution in the *Passiflora–Heliconius* interaction. In: Spencer, K.C. (Ed.), Chemical Mediation of Coevolution. Academic Press, Orlando, Florida, pp. 167–240.

Spencer, K.C., Seigler, D.S., 1983. Cyanogenesis of *Passiflora edulis*. Journal of Agricultural and Food Chemistry 31, 794–796.

Spencer, K.C., Seigler, D.S., 1984a. Cyanogenic glycosides of *Carica papaya* and its phylogenetic position with respect to the *Violales* and *Capparales*. American Journal of Botany 71, 1444–1447.

Spencer, K.C., Seigler, D.S., 1984b. Gynocardin from *Passiflora*. Planta Medica 46, 356–357.

Tantisewie, B., Ruijgrok, H., Hegnauer, R., 1969. Die Verbreitung der Blausäure bei den Kormophyten 5. Mittl. Cyanogene Verbindungen bei den *Parietales*. Pharmaceutisch Weekblad 104, 1341–1355.

Thomas, W.A., 1997. Unraveling molecular structure and conformation—the modern role of coupling constants. Progress in Nuclear Magnetic Resonance Spectroscopy 4, 183–207.

Towers, G., McInnes, A., Neish, A., 1964. The absolute configuration of the phenolic cyanogenic glucosides taxiphyllin and dhurrin. Tetrahedron 20, 71–77.

Toyota, M., Saito, T., Asakawa, Y., 1996. A phenethyl glycoside from *Conocephalum conicum*. Phytochemistry 43, 1087–1088.

Veit, M., Pauli, G.F., 1999. Major flavonoids from *Arabidopsis thaliana* leaves. Journal of Natural Products 62, 1301–1303.