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LEUCINE-DERIVED NITRILE GLUCOSIDES IN THE ROSACEAE AND THEIR SYSTEMATIC SIGNIFICANCE*

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Key Word Index—Rosaceae-Prunoideae; *Osmaronia cerasiformis*; *Prinsepia uniflora*; *Exochorda serratifolia*; *E. giraldii*; Rosaceae-Kerrieae; *Rhodotypos scandens*; *Neviusia alabamensis*; *Kerria japonica*; nitrile glucosides; chemotaxonomy.

Abstract—Six nitrile glucosides and one cyanogenic glucoside (epiheterodendrin) have been isolated from the leaves of members of the Rosaceae-Prunoideae and Rosaceae-Kerrieae. Two of these, 4-β-D-glucopyranosyloxy-2*R*,3*R*-epoxy-3-hydroxymethyl-butyronitrile (sutherlandin epoxide) and 4-β-D-glucopyranosyloxy-3*S*-methyl-butyronitrile (dihydro-osmaronin), are new compounds. The aglycones of all the nitrile glucosides are apparently derived from L-leucine. The distribution and taxonomic significance of these glucosides in the species investigated is discussed briefly.

INTRODUCTION

Recently, we reported on the occurrence of the cyanoglucosides osmaronin (**1**), osmaronin epoxide (**2**) and sutherlandin (**3**) in *Osmaronia cerasiformis* (Torr. et A. Gray) Greene (Prunoideae) [1]. During our investigations, we observed the presence of additional nitrile glucosides in leaves and flowers of this species at lower concentrations than those of the above mentioned compounds. Investigation of additional members of the Prunoideae and some species of the Kerrieae has now led to the isolation of a total of six non-cyanogenic nitrile glucosides (**1**–**6**), in addition to the cyanogenic glucoside, epiheterodendrin (**7**). The carbon skeletons of all these compounds suggest that they are biogenetically derived from L-leucine. We report herein on the isolation and structural elucidation of sutherlandin epoxide (**4**), dihydro-osmaronin (**5**), epidermin (**6**), epiheterodendrin (**7**) and the cyanoglucosides **1**–**3** mentioned above, as well as their distribution in members of the subfamily Prunoideae (*O. cerasiformis*, *Prinsepia uniflora* Batal., *Exochorda serratifolia* S. Moore and *E. giraldii* Hesse) and members of the tribe Kerrieae (*Rhodotypos scandens* [Thunb.] Mak., *Neviusia alabamensis* A. Gray and *Kerria japonica* [L.] DC.).

RESULTS AND DISCUSSION

Methanolic extracts from freeze-dried leaves of the above mentioned species were chromatographed on TLC plates using the picrate-sandwich method [2] or anisaldehyde–H_2SO_4 for detection. They showed different numbers of weakly cyanogenic zones with R_f values between amygdalin and prunasin (see Experimental). Crude methanolic extracts were also monitored by GC after trimethylsilyl (TMSi) derivatization using a N-selective detector; chromatograms showed as many as seven N-positive signals, e.g. in *R. scandens*.

Crude extracts obtained from the leaves of *O. cerasiformis* (nitrile glucosides **1**–**3** and **5**), *P. uniflora* (**2**, **3**), *E. serratifolia* (**1**, **2**, **4**), and *R. scandens* (**1**–**7**) were purified by column chromatography on silica gel and MCI gel, and fractions were monitored by TLC and GC. Chromatographically pure (TLC, GC as TMSi derivatives) compounds were obtained by final purification on LPLC (RP-18) or MPLC (RP-18). All compounds yielded glucose (TLC or GC) when hydrolysed with a non-specific enzyme preparation. Coupling constants of 7.5–7.7 Hz in the ^{1}H NMR of the anomeric protons indicated a β-configuration for the glycosidic linkages (Table 1) [3]. IR signals in the region 2225–2250 cm^{-1} for **1**–**6** confirmed the presence of a nitrile group; in **7**, the nitrile band was quenched, as is typical for glucosylated cyanohydrins [3].

In the case of **1**–**3**, all spectroscopic data (^{1}H/^{13}C NMR, NOE experiments, mass spectra and IR) were consistent with published data [1, 4]. Table 1 shows the NMR data for **1**–**3** measured in methanol-d_4, in order to allow a direct comparison with the nitrile glucosides, **4**–**7**.

Underivatized **4** had a M_r of 291 (D/CI mass spectra: $[M + 18]^+ = 309$). After peracetylation, the M_r was 501 (D/CI mass spectra: $[M + 18]^+ = 519$), indicating one additional free hydroxyl group, in addition to the four of glucose. The ^{13}C APT spectrum showed 11 carbon resonances, six of them belonging to glucose (Table 1). The

*Part of the projected PhD thesis of Matthias Lechtenberg.
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Table 1. ^1H and ^{13}C NMR data (all spectra measured in CD_3OD; for numbering see Fig. 1). Data for compounds **1–3**, **6** and **7** are listed here for comparison in the same solvent as that used for compounds **4** and **5**

	1	**2**	**3**	**4**	**5**	**6**	**7**
	(Osmaronin)	(Osmaronin epoxide)	(Sutherlandin)	(Sutherlandin epoxide)	(Dihydro-osmaronin)	(Epidermin)	(Epi-heterodendrin)
H-2	5.46 (*d*, 1.5)	3.65 (*s*)	5.72 (*br s*)	3.78–3.86*	2A: 2.65 (*dd*, 16.9/5.3) 2B: 2.48 (*dd*, 16.9/7.2)	2A: 2.78 2B: 2.77	4.48 (*d*, 5.7)
H-3	—	—	—	—	2.17 (*m*)	—	2.14 (*m*)
H-4	4A: 4.57 (*d*, 13.2) 4B: 4.45 (*d*, 13.2)	3.91 (*d*, 1.7)	4A: 4.64 (*d*, 13.4) 4B: 4.52 (*d*, 13.4)	4A: 4.15 (*d*, 12.3) 4B: 3.78–3.86*	3.6–3.8 (*m*)	1.43 (*s*) 1.41 (*s*)	1.08 (*pseudo-t*)
H-5	2.03 (*d*, 1.5)	1.48 (*s*)	4.34 (*d*, 2.0)	3.78–3.86*	1.07 (*d*, 6.8)		
H-1′	4.29 (*d*, 7.6)	4.36 (*d*, 7.5)	4.29 (*d*, 7.6)	4.39 (*d*, 7.6)	4.26 (*d*, 7.6)	4.50 (*d*, 7.7)	4.39 (*d*, 7.5)
H-2′–H-5′	3.15–3.40	3.15–3.40	3.15–3.40	3.15–3.40	3.15–3.40	3.15–3.40	3.15–3.40
H-6′	6′A: 3.88 (*dd*) 6′B: 3.70 (*dd*)	6′A: 3.85–3.95 (*dd*) 6′B: 3.7 (*dd*)	6′A: 3.89 (*dd*, 13.5/1.9) 6′B: 3.68 (*dd*, 13.5/4.9)	6′A: 3.96 (*dd*, 12.5/3.9) 6′B: 3.70 (*dd*, 12.5/3.4)	6′A: 3.87 (*dd*) 6′B: 3.56 (*dd*, 10.2/4.9)	6′A: 3.85 (*dd*) 6′B: 3.64 (*dd*)	6′A: 3.9 (*dd*) 6′B: 3.7 (*dd*)
C-1	117.10	117.53	117.20	117.31	120.32	119.49	119.61
C-2	97.51	47.94	95.68	44.94	21.50	31.46	74.36
C-3	162.94	63.03	165.61	65.33	32.15	76.10	33.01
C-4	70.68	72.72	68.18	69.39	73.49	27.06‡	18.10‡
C-5	20.82	18.40	62.94	60.39	16.20	25.91‡	17.52‡
C-1′	104.02	105.09	104.12	104.94	104.19	98.87	105.07
C-2′	75.01	74.98	74.93	74.71	75.02	75.03	75.00
C-3′	78.08	77.96	78.05	77.67	78.02†	78.13†	78.05†
C-4′	71.49	71.49	71.48	71.22	71.61	71.66	71.45
C-5′	78.08	77.96	78.05	77.67	77.92†	77.74†	78.26†
C-6′	62.60	62.69	62.60	62.47	62.74	62.84	62.77

*Complex pattern of signals.
†‡Assignments are interchangeable in vertical columns.

remaining five aglycone signals were assigned to a CN group (117.3 ppm), a >CH-OR group (44.9 ppm), two $-CH_2-OR$ groups (60.4 and 69.4 ppm) and one quaternary carbon, with a large downfield shift (65.3 ppm). The ^1H NMR of **4** showed a complex pattern of downfield signals at 3.15 ppm (several $-CH_x-OR$ groups). Complete analysis of the ^1H NMR of the **4** pentaacetate showed four doublets of two independent AB systems, due to the two diastereotopic couplets H-4A/H-4B and H-5A/H-5B, one singlet (H-2), typical glucose signals and five resonances between 2.0 and 2.1 ppm, representing the $-COCH_3$ residues. COSY and HETCOR ($^1H/^{13}C$-correlation, ^1J) spectra facilitated these assignments. These data, in comparison with those obtained for osmaronin epoxide [1] and sutherlandin [1, 4], suggest an aglycone moiety with an oxirane grouping and a hydroxylated C-5 position. The absolute stereochemistry of the oxirane carbons as 2*R*,3*R*, was deduced from an X-ray crystallographic study of **4** pentaacetate (Fig. 1). Thus, **4** is 4-β-D-glucopyranosyloxy-2*R*,3*R*-epoxy-3-hydroxymethyl-butyronitrile (sutherlandin epoxide), identified and described here for the first time.

The ^{13}C APT NMR of **5** exhibited five aglycone signals, a CN group (120.3 ppm), one $-CH_2-OR$ group (73.5 ppm) and three signals in the upfield region due to a $-CH_2-$ group (21.5 ppm), a >CH– group (32.2 ppm) and a methyl group (16.2 ppm). The ^1H NMR spectrum emphasized these results (Table 1) and HETCOR confirmed all assignments unambiguously. Acid hydrolysis of **5** gave the lactone **8** [5–7], which was identical in its GC and ^1H NMR data with authentic material, and D-glucose. Compound **8** showed a negative optical rotation almost identical to that measured for authentic (−)-(*S*)-3-methyl-γ-butyrolactone. These data reveal that **5** is the (*S*)-epimer of 4-β-D-glucopyranosyloxy-3*S*-methyl-butyronitrile (dihydroosmaronin), identified and described here for the first time.

Compound **6** showed a M_r of 261 (D/CI mass spectra $[M + 18]^+ = 279$); its NMR spectroscopic data were consistent with those published for 3-β-D-glucopyranosyloxy-3-methyl-butyronitrile (epidermin), recently isolated from the epidermal layer of barley leaves (*Hordeum vulgare*) and identified as its TMSi derivative [8].

Compound **7** afforded NMR spectra identical to those of the cyanogenic glucoside, epiheterodendrin, isolated from *H. vulgare* [9, 10]. A (*R*) configuration at the chiral centre in **7** was confirmed by comparison of the NMR spectra with published data. Treatment with 0.005 N

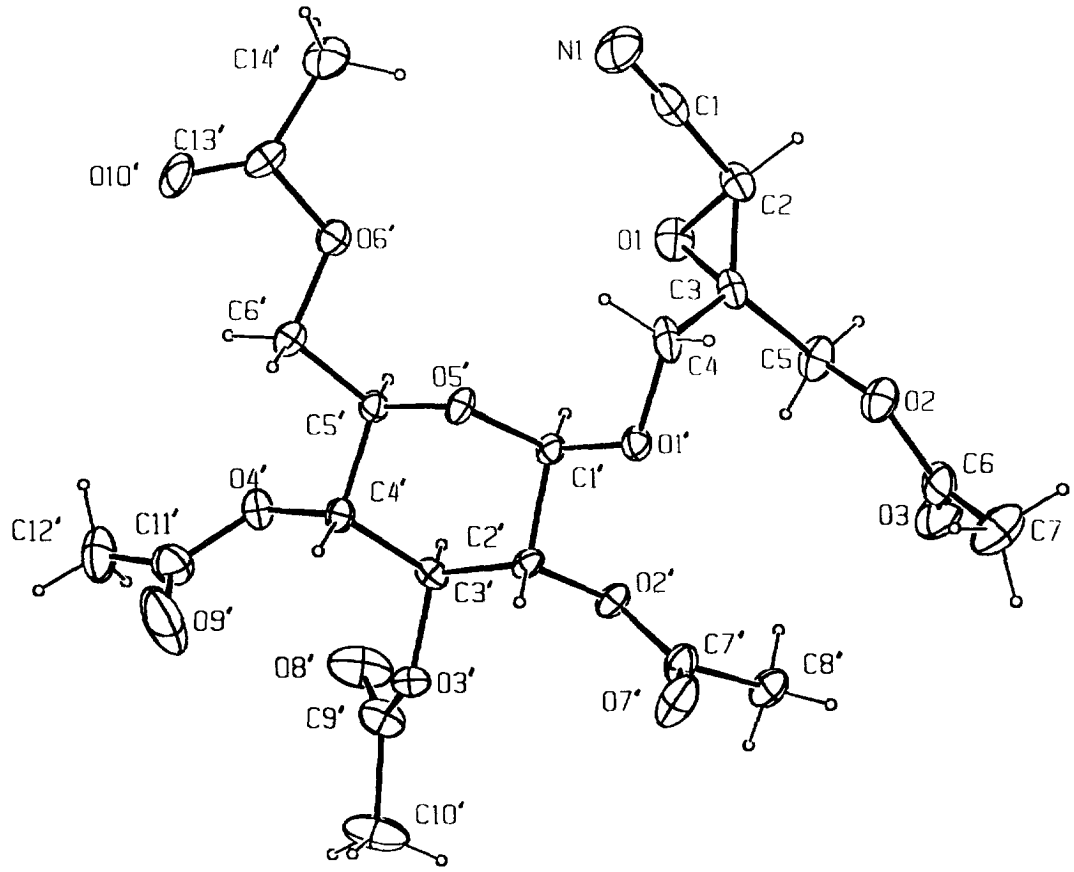
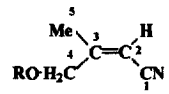
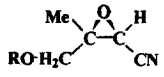


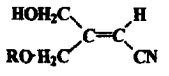
Fig. 1. Crystal structure of pentaacetate **4**, with 20% ellipsoids.



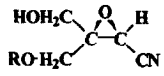
1 Osmaronin



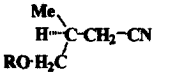
2 Osmaronin epoxide



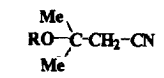
3 Sutherlandin



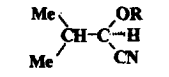
4 Sutherlandin epoxide



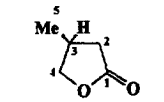
5 Dihydroosmaronin



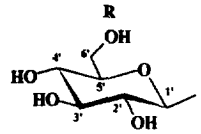
6 Epidermin



7 epi-Heterodendrin



8 (-)(*S*)-3-Methyl-γ-butyrolactone



NH_3 caused epimerization at C-2, resulting in a doubling of the signals for H-1′ and H-2, with new signals at lower field and with a ratio of $(R)/(S) = 11:9$ [3]. Thus, **7** is 2-β-D-glucopyranosyloxy-3-methyl-2*R*-butyronitrile (epiheterodendrin).

The distribution of **1**–**7** in members of the Prunoideae and Kerrieae was measured by GC with N-selective detection; the results are shown in Table 2. All seven nitriles, including the cyanohydrin derivative **7**, were detected in *E. serratifolia*, *E. giraldii* and *R. scandens*, the others lacking either **4** (*O. serratifolia* and *P. uniflora*) or the epoxides **2** and **4** (*N. alabamensis* and *K. japonica*). The major constituents were either **2**, **3** or **4**. Cyanohydrin derivative **7** was present in all species at low or very low concentrations. The occurrence of small amounts of the cyanogenic glucoside, epiheterodendrin, in all species examined and the possibility of HCN liberation from **2** (or **4**) via hydrolysis of the oxirane moiety [1], can explain the weak cyanogenesis detected in all species investigated. Although no biogenetic studies have been performed, the biogenetic precursor of **1**–**7** is most likely leucine. Compounds **1**–**7** are thus the first leucine-derived nitrile glucosides which have been detected in the Prunoideae and Kerrieae [1].

The natural classification of the Rosaceae is still a matter of debate. This family is usually separated into four subfamilies (Spiraeoideae, Rosoideae, Maloideae and

Table 2. Distribution of compounds **1**–**7** in the species investigated

	Harvest	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Osmaronia cerasiformis	lv, 3'95	0.4	2.9	0.8	n.d.	0.1	*	*
Osmaronia cerasiformis	fl, 3'95	0.5	3.5	1.5	n.d.	0.2	*	0.2
Prinsepia uniflora	lv, 4'95	*	1.3	1.1	n.d.	*	*	*
Exochorda serratifolia	lv, 4'95	0.2	3.3	0.1	0.6	*	*	*
Exochorda giraldii	lv, 5'95	0.1	0.8	*	0.5	0.1	*	*
Rhodotypos scandens	lv, 4'95	*	0.9	0.2	1.0	0.2	*	0.1
Neviusia alabamensis	lv, 4'95	*	n.d.	0.7	n.d.	0.1	*	*
Kerria japonica	lv, 5'95	*	n.d.	0.4	n.d.	*	*	*

g $100\ g^{-1}$ freeze-dried material. * = < 0.1 g, $100\ g^{-1}$. n.d. = Not detected.
lv = Leaves; fl = flowers.

Prunoideae [11]), two of which, the Spiraeoideae and Prunoideae, are obviously not natural units. Hutchinson [12] did not mention these subfamilies when he grouped the genera of this family into 20 units (tribes) with the same taxonomic value. In this paper, the classification of Hegnauer [13], in which nine main groups and a number of subgroups in the Spiraeoideae *sensu restricto*, Maloideae and Rosoideae *sensu restricto* are combined, is accepted as the basis for discussion.

The situation with respect to cyanogenesis within the Rosaceae is complex. Both leucine- and phenylalanine-derived cyanogenic glycosides are found in the Spiraeoideae [13]. Cyanogenic glycosides derived from phenylalanine are widespread and typical for the Maloideae and the genus *Prunus*, including *Pygeum* within the Prunoideae. Species of the Dryadeae-Cercocarpinae and Dryadeae-Purshiinae contain dhurrin as the cyanogenic glucoside, as exemplified by *Cercocarpus ledifolius* [14] and *Chamaebatia foliolosa*, respectively [15]; one cannot, however, exclude the possibility in these species that phenylalanine, instead of tyrosine, is the actual precursor, followed by an intermediate 4-hydroxylation step.

Some authors have argued in favour for the separation of the three genera, *Osmaronia*, *Prinsepia* and *Exochorda*, from the Prunoideae, for morphological reasons [16–19]. In 1976, Hegnauer [20] assumed that the unknown type of cyanogenesis present in *Osmaronia* and *Prinsepia*, two presumably ancient genera of the Prunoideae, may be the same as that in the primitive woody Rosaceae with a chromosome number $x = 9$. Hegnauer considered this type of cyanogenesis to be 'primitive' and found it "not unlikely that present-day Prunoideae derived from ... ancient Spiraeoideae-like plants" [20]. On the other hand, in 1981, Hegnauer considered prunasin (phenylalanine type) as the phylogenetically oldest cyanogenic glucoside of the Rosaceae [21].

The present results supplement our understanding of the distribution of different biogenetic types of nitrile glucosides in the Rosaceae considerably (for a classification, see Ref. [13]). It seems clear that the primitive Rosaceae were able to produce nitrile glucosides of the phenylalanine and the leucine type, some of which are cyanogenic, although others are not. The Rosoideae, Neuradoideae and Chrysobalanoideae, which are regarded as advanced, apparently have lost this property. *Exochorda*, *Osmaronia* and *Prinsepia*, with leucine-type nitrile glucosides, clearly differ phytochemically and morphologically [16–19] from the genus *Prunus* s.l., and should be segregated from the Prunoideae. Our results are also covered by the phylogenetic free presented by Morgan *et al.* [30] based on *rbc*L gene analysis, fruit types and chromosome numbers: *Osmaronia* and *Exochorda* are placed in one group closely related to *Prinsepia*; *Neviusia* is the closest relative of *Rhodotypos* (*Kerria* was not examined).

The Rosaceae are biochemically reminiscent of the Fabales, in which both phenylalanine- and leucine-type nitrile glucosides also have been detected [22]. Compound **3** was first discovered in *Acacia sutherlandii* (Mimosaceae) [4]. Those Rosaceae with leucine-type nitriles are similar to members of the Sapindaceae with leucine-type cyanogenic glycosides and cyanogenic and non-cyanogenic lipids [23].

It will be of interest to investigate the biogenetic relationship of the pathways leading to cyanogenic and to non-cyanogenic nitrile glucosides. Following the biogenetic scheme for the cyanogenic glycosides [24], the present data suggest a common pathway for both types of nitrile glucosides starting from leucine to give the corresponding 3-methylbutyronitrile as a branch point. Genera of the Rosaceae that are regarded as more primitive favour the branch leading to the non-cyanogenic type, in that they accumulate compounds such as **1**–**6**, although they definitely possess the second branch and accumulate small amounts of cyanogenic compounds, such as **7**. More data on acyanogenic nitrile glucosides are necessary to substantiate this hypothesis.

Many of the non-cyanogenic nitrile glucosides identified so far have been detected more or less by chance because no simple method can be used for their detection, such as is possible for cyanogenesis from cyanogenic glycosides. Thus, our current knowledge about these compounds is limited. The occurrence of non-cyanogenic nitrile glucosides in several genera of the Rosaceae may indicate that their distribution may be wider than was hitherto believed.

EXPERIMENTAL

General. D/CI MS were recorded with NH_3. Reference compounds originated from the collection of A.N. ^1H and ^{13}C spectra were recorded at 200 and 50 MHz, respectively. Chemical shifts are given in ppm relative to TMS, coupling constants in Hz.

Plant material. All species (with the two exceptions mentioned below) came originally from the collection of Prof. R. Hegnauer, Leiden. They were cultivated in the experimental garden of the Institut für Pharmazeutische Biologie und Phytochemie at Münster, where vouchers (PBMS-No.) are deposited: *O. cerasiformis* (PBMS-88), *P. uniflora* (PBMS-109), *E. serratifolia* (PBMS-110), *E. giraldii* (PBMS-120), *R. scandens* (PBMS-106), *N. alabamensis* (PBMS-108) and *K. japonica* (PBMS-121a). Specimens of *E. giraldii* and *K. japonica* were collected in the Botanical Garden at the University of Münster.

Isolation. Leaves were ground in liquid N_2. After lyophilization and extraction with cold MeOH, the crude extracts were evapd to dryness and suspended in H_2O. Chlorophyll was removed by chilling (4°, 24 hr) followed by filtration. The concd filtrate was fractionated by silica gel CC (4 × 80 cm, 2 ml min^{-1}) with EtOAc–MeOH–H_2O (79:11:10) as mobile phase. N-positive compounds (GC with FID/PND) were found in frs A (1.0–2.9 l), B (2.9–3.4 l) and C (3.4–4.3 l). Fr. A (containing **7**) was concd and further purified on a MCI gel column (2.5 ml min^{-1}; MeOH–H_2O gradient). The cyanogenic frs (380–565 ml) were concd. Final purification was achieved by RP18-LPLC (8.5 ml min^{-1}, MeOH–H_2O, 1:19). Frs 600–800 yielded chromatographically (TLC, GC) pure (*R*)-epiheterodendrin (for comparison: elution vol. of (*S*)-heterodendrin: 850–1000 ml). For isolation of **2**, concd fr. B was chromatographed on a MCI gel column (2.5 ml min^{-1}; MeOH–H_2O gradient; elution vol. 130–210 ml). Final purification was carried out on RP18-MPLC. Fr. C (containing **1** and **3**–**6**) was concd and chromatographed on a MCI gel column (3.1 ml min^{-1}, MeOH–H_2O gradient) and yielded the nitrile glucoside-containing frs (100–400 ml). After concn, a RP18-MPLC step provided 4 frs: C_I (pure **4**), C_{II} (**3** plus **4**), C_{III} (**1**, **5** and **6**) and C_{IV} (pure **5**). Fr. C_{III} was rechromatographed on a RP18-LPLC column (5 ml min^{-1}, MeOH–H_2O, 1:49) elution vol.: **6** (400–440 ml), **1** (460–520 ml) and **5** (530–1300 ml). Lyophilized **1**–**7** were used for structural elucidation.

Sutherlandin epoxide peracetate. ^1H NMR ($CDCl_3$): δ5.26–4.97 (*m*, H-2′, H-4′), 4.67 (*d*, *J* = 7.8 Hz, H-1′), 4.51 (*dd*, *J* = 12.5, 2.3 Hz, H-6′A), 4.44 (*d*, *J* = 12.7 Hz, H-5A), 4.24 (*d*, *J* = 12.9 Hz, H-4A), 4.16 (*d*, *J* = 12.7 Hz, H-5B), 4.00 (*dd*, *J* = 12.5, 4.1 Hz, H-6′B), 3.80 (*d*, *J* = 12.9 Hz, H-4B), 3.70 (*s*, H-2), 3.81–3.73 (*m*, H-5′), 2.11, 2.09, 2.07, 2.05, 2.01 (–$COCH_3$). ^{13}C NMR: δ 115.28 (C-1), 101.79 (C-1′), 72.68, 72.26 (C-3′, C-5′), 71.04 (C-2′), 68.74 (C-4), 68.03 (C-4′), 61.58 (C-3), 61.10, 61.02 (C-6′, C-5), 45.11 (C-2), 20.57–20.73, 169.37–170.54 (–$COCH_3$).

(S)-3-*Methyl-γ-butyrolactone.* ^1H NMR ($CDCl_3$): δ4.42 (*dd*, *J* = 8.9, 7.5 Hz, H-4A), 3.88 (*dd*, *J* = 8.9, 6.4 Hz, H-4B), 2.5–2.8 (*m*, H-2), 2.14 (*m*, H-3), 1.17 (*d*, *J* = 6.6 Hz, H-5).

Absolute configuration of **5**. Compound **5** (20 mg) was dissolved in 0.5 ml HCl (37%) and kept at 60° for 4 hr. After cooling, the resulting mixt. was extracted with $CHCl_3$. The $CHCl_3$ layer contained the lactone **8**, identical with (−)(*S*)-3-methyl-γ-butyrolactone (Merck); $[\alpha]_D^{20}$; −23.5° ($CHCl_3$; *c* 7.8). The aq. layer contained D-glucose. Compound **8** had a negative optical rotation, $[\alpha]_D^{20}$; −21.7° ($CHCl_3$, *c* 0.203).

X-Ray analysis. A needle **4** pentaacetate of dimensions 0.06 × 0.08 × 0.68 mm was used for data collection on an Enraf–Nonius CAD4 diffractometer equipped with Cu K_α radiation (λ = 1.54184 Å) and a graphite monochromator. Crystal data are: $C_{21}H_{27}NO_{13}$, M_r = 501.4, orthorhombic space group $P2_12_12_1$, *a* = 7.575(1), *b* = 14.905(6), *c* = 22.3704(4) Å, *V* = 2526(2) Å^3, *Z* = 4, d_c = 1.322 g cm^{-3}, *T* = 22°. While the crystal was only of fair quality and not entirely single, it yielded diffraction data that were sufficient to determine the structure. Attempts to grow better crystals were unsuccessful. Intensity data were measured by ω − 2θ scans of variable rate. An octant of data was collected within the limits 2 < θ < 75°. Data reduction included corrections for background, Lorentz polarization, 'decay' (5.0% increase, linear) and absorption effects. Absorption corrections (μ = 9.2 cm^{-1}) were based on Ψ-scans with a minimum relative transmission coefficient of 80.4%. Of 2959 unique data, 2035 had $I > 1\ \sigma(I)$ and were used for refinement. The structure was solved by direct methods using program SHELXS [25] and refined by full-matrix least squares, treating non-hydrogen atoms anisotropically, using the Enraf–Nonius MolEN programs [26]. Absolute configuration was assumed to be known from the glucoside unit. Hydrogen atoms were placed in calculated positions, guided by difference maps, and were not refined. Convergence was achieved with *R* = 0.099, R_w = 0.093 and GOF = 2.753 for 317 variables. Maximum residual electron density was 0.44 eÅ^{-3}. Coordinates, bond-distances and bond-angles have been deposited at the Cambridge Crystallographic Data Centre.

TLC. Precoated TLC plates (silica gel, Merck 5554) were used with EtOAc–MeOH–H_2O (79:11:10) as mobile phase. Detection was with the sandwich-picrate test [2] or with anisaldehyde–H_2SO_4. R_f values were for **1**: 0.29, **2**: 0.35, **3**: 0.21, **4**: 0.24, **5**: 0.27, **6**: 0.23, **7**: 0.48, heterodendrin: 0.50, prunasin: 0.56 and amygdalin: 0.18.

MPLC. A Orpegen HD Sil RP-18; 26 × 460 mm column (Büchi-No, 17982 and 17988) was used with MeOH–H_2O (1:49) (elution vol. of purified compounds: 300–350 ml **4**; 460–660 ml **3**; 550–825 ml **6**; 600–1000 ml **2**; 825–1250 ml **5**; 1100–1500 ml **1**), 5.5 ml min^{-1}; 15–20 bar. UV detection at 210 nm.

LPLC. A RP18 silica gel [Europrep C-18 (Besta Technik) GmbH, Wilhelmsfeld], 60 Å, 20–45 μm, 35 × 300 mm column was used at 1–5 bar.

MCI gel system. A MCI CHP 20P gel (Mitsubishi Casei) 75–150 μm, 25 × 250 mm column was used at 1–2 bar in the LPLC mode.

Derivatization. TMSi ethers: Compounds were dissolved in pyridine–BSTFA–TMCS (1:3:1) and used for GC after 2 hr at room temp. *Peracetates*: These were prepd by dissolving 20 mg of pure compound in 1 ml pyridine–Ac_2O (1:1) for 24 hr. After adding ice and H_2O, the ppt. was isolated.

Purification and recrystallization of **4** *peracetate.* The peracetate of **4** was put at the head of a Bond Elut column (Varian 1210–2028, RP-18) and the column eluted successively with H_2O, H_2O–MeOH (9:1), H_2O–MeOH (1:1) and MeOH. Fr. 3 was evapd to dryness, suspended in H_2O and MeOH added until the substance was just dissolved. Solvent was then slowly evapd at room temp. under normal press. The crystalline residue was freeze-dried under high vacuum and used for X-ray crystallographic study.

Hydrolysis. A non-specific enzyme prepn with β-glucosidase, β-glucuronidase and esterase activity (Röhm EL 1-77) and β-glucosidase from *Hevea* [27] were used in citrate–Pi buffer (0.15 M, pH 6) at room temp. for 12 hr.

Measurement of HCN liberation. Liberation of HCN from plant material and isolated compounds was evaluated using the Feigl–Anger [28, 29] and Guignard–Mirande tests [29]. All species investigated (Table 2) showed only weak cyanogenesis (< 10 mg HCN $100\ g^{-1}$ freeze-dried leaves) when incubated in buffer (see Hydrolysis) on a semi-quantitative basis.

GC. He (1.5 ml/min^{-1} at 50°); injector 180°; FID/PND 330°; DB-5 and OV-225 capillary columns, 30 m × 0.25 mm × 0.25 μm. DB-5 160–280°, 8° min^{-1}, 6 min isothermal, 280–320°, 10° min^{-1}, 20 min isothermal. OV-225: 160–260°, 5° min^{-1}, 15 min isothermal. Linamarin was used as int. standard. ΔR_t values in min ($\Delta R_t = R_{t(\text{substance})} - R_{t(\text{int. standard})}$) for DB-5 (OV-225) were TMSi-**1**: 2.9 (5.6), TMSi-**2**: 2.6 (5.4) TMSi-**3**: 5.6 (7.7), TMSi-**4**: 5.2 (7.2) TMSi-**5**: 2.9 (5.8), TMSi-**6**: 1.9 (4.0) and TMSi-**7**: 1.2 (1.9). Lactone **8**: DB-225 (25 m × 0.25 mm × 0.25 μm), 80–150°, 5° min^{-1}, one peak with $R_t = 12.2$ min which co-chromatographed with the authentic substance.

Quantitative determination of nitrile glucosides. Lyophilized plant material (500 mg, see Table 2) was exhaustively extracted with MeOH (Ultra-Turrax). The filtered and combined extracts were made up to 100 ml and 0.5 ml then transferred to a 2-ml vial. After evapn of solvent *in vacuo*, 25 μl of a soln containing the int. standard (6.34 mg linamarin in 5 ml dry pyridine), 60 μl BSTFA and 1.5 μl TMCS were added. The vial was closed and kept for 2 hr at room temp. Aliquots of this soln (2 μl) were used for GC analysis. Linearity was established for compounds **2** and **3** in the range 0.1–3.3 μg substance (per μg linamarin; $r > 0.998$). Compound identity was checked on two columns (see GC) with N-selective detection and co-chromatography with authentic substances (see Isolation). All results were calculated using response factors for **1**–**5**; the uncorrected areas (PND, OV-225) were used for **6** and **7**; they are given in g $100\ g^{-1}$ of lyophilized plant material.

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