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BIOSYNTHESIS OF CYANOHYDRIN GLUCOSIDES FROM UNNATURAL NITRILES IN INTACT TISSUE OF *PASSIFLORA MORIFOLIA* AND *TURNERA ANGUSTIFOLIA**

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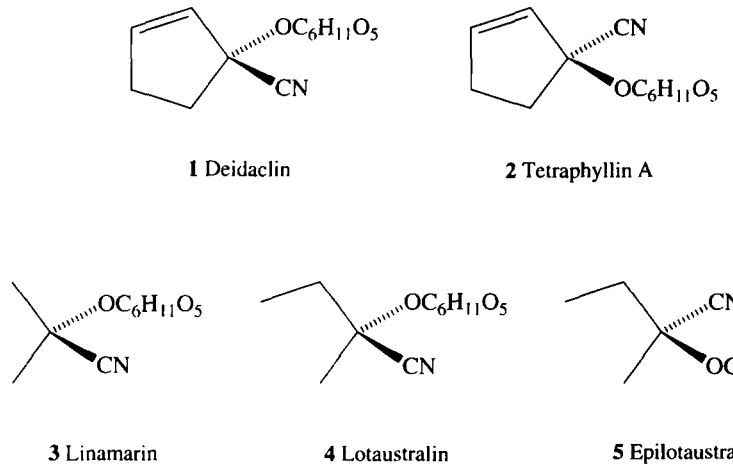
Key Word Index—Passifloraceae; Turneraceae; linamarin; lotaustralin; epilotaustralin; deidaclin; tetraphyllin A; heterodendrin; epiheterodendrin; 1-(β-D-glucopyranosyloxy)cyclopentanecarbonitrile; 2-[6-*O*-(β-D-xylopyranosyl)-β-D-glucopyranosyloxy]propane; isopropyl primeveroside; NMR.

Abstract—*Passiflora morifolia*, which under natural conditions contains cyanohydrin glucosides linamarin, lotaustralin and epilotaustralin, converted cyclopentanecarbonitrile, 2-cyclopentenecarbonitrile and 3-methylbutanenitrile into the corresponding cyanohydrin glucosides. *Turnera angustifolia*, which normally produces glucosides of cyclopentenone cyanohydrin, converted cyclopentanecarbonitrile, 2-methylpropanenitrile and 2-methylbutanenitrile, but not 3-methylbutanenitrile, into the corresponding cyanohydrin glucosides. Mixtures of epimers were produced when these glucosides contained chiral cyanohydrin carbon atoms. Feeding with cyclopentanecarbonitrile resulted in formation of 1-(β-D-glucopyranosyloxy)cyclopentanecarbonitrile, a saturated analogue of deidaclin and tetraphyllin A. Neither plant utilized cyclopropanecarbonitrile as substrate. The experiments demonstrate broad substrate specificity of nitrile hydroxylases present in these plants. A novel glycoside, 2-[6-*O*-(β-D-xylopyranosyl)-β-D-glucopyranosyloxy]propane (isopropyl primeveroside), was isolated from *P. morifolia*. The compound represents a rare example of natural isopropyl glycoside; its characterization included assignment of all ^{1}H and ^{13}C NMR signals of the primeverosyl group using two-dimensional NMR methods. Biosynthesis of the isopropyl moiety of the primeveroside is unclear, but the formation of alcohols corresponding to natural cyanohydrins may be a previously unrecognized extension of the cyanohydrin biosynthesis pathway in higher plants.

INTRODUCTION

There is now considerable support for the hypothesis that the production of glucosylated cyanohydrins from L-2-cyclopenteneglycine has evolved from cyanogenesis based on the protein amino acids valine and isoleucine [2, 3]. The cyclopentanoid cyanogens, represented by deidaclin (**1**) and tetraphyllin A (**2**) (Fig. 1.) [4] and their oxygenated counterparts [5, 6], are restricted in occurrence to a cluster of closely related plants. Most of these belong to Passifloraceae, Turneraceae and some tribes of Flacourtiaceae, and the cyclopentanoids are so far unknown outside this group; the restricted occurrence of cyclopentanoid cyanogens appears to follow from restricted occurrence of L-2-cyclopenteneglycine [2]. In contrast, the natural cyanohydrin glucosides which originate from protein amino acids, including those derived from valine and isoleucine, are widespread throughout the angiosperms, in accordance with the universal availability of these amino acids. That the enzyme specificity may have changed only slightly from the biosynthesis of **3**–**5** to that of **1** and **2** [7] is evident not only from the *a priori* apparent steric likeness between their respective precursors, valine/isoleucine and L-2-cyclopenteneglycine [3, 8], but also from the striking lack of stereospecificity in the biosynthesis of cyclopentanoid cyanogens [3, 9, 10], from the co-occurrence of the latter with **3**–**5** [9–12], and from the inhibition of the biosynthesis of **1** and **2** by the nitrile corresponding to **3** [3]. Both epimers of L-2-cyclopenteneglycine, differing in chirality of the asymmetric carbon in the ring [13], appear to be precursors of the cyanohydrins, in contrast to the fact that only the (1*R*) epimer exhibits inhibitory activity in bacteria [14, 15]. L-2-Cyclopenteneglycine is

*Part 16 in the series 'Natural Cyclopentanoid Cyanohydrin Glycosides'. For part 15 see ref. [1].



1 Deidaclin **2** Tetraphyllin A

3 Linamarin **4** Lotaustralin **5** Epilotaustralin

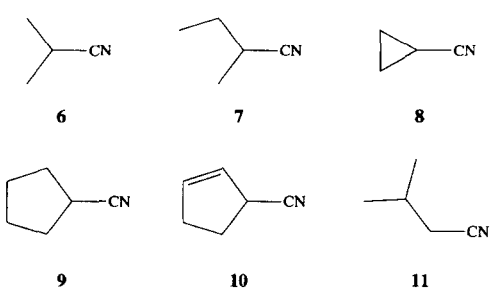
Fig. 1. Cyanohydrin glucosides of *P. morifolia* and *T. angustifolia*.

also accepted by the microsomal enzyme system of cassava, which has valine and isoleucine as the endogenous substrates [16].

RESULTS AND DISCUSSION

In the present work, the ability of *Passiflora morifolia* and *Turnera angustifolia* to metabolize a number of nitriles similar to, but non-identical with, those involved in the biosynthesis of their endogenous cyanogens was investigated. The plant species were the same as used in recent radiolabelling experiments [3]. The plants were allowed to metabolize continuously administered, unlabelled nitriles (**6–11**) (Fig. 2), and cyanogenic constituents present after three days of incubation were isolated (column chromatography and HPLC) and identified (^{1}H and ^{13}C NMR). The nitrile (**7**) was optically active with the (*S*)-configuration corresponding to isoleucine, whereas the cyclic nitrile (**10**), which so far has not been resolved, was used as the racemate. The results are summarized in Table 1.

Passiflora morifolia, although belonging to a family typically producing cyclopentanoids [10], was previously found to contain only valine- and isoleucine-derived cyanogens (**3–5**) and no cyclopentanoids [3]. This plant was therefore tested for its ability to convert the exogenous nitriles (**8–11**) into the corresponding cyanohydrin glucosides (Figs. 1 and 3). Moreover, the



6 **7** **8**

9 **10** **11**

Fig. 2. Nitriles used in feeding experiments.

(*S*)-enantiomer of **7**, which is the presumed intermediate in the biosynthesis of **4**, was also administered to *P. morifolia* in order to determine whether this resulted in a changed ratio of **4** and **5**.

Conversion of the cyclic nitriles **10** and **9** into the corresponding glucosides **1**, **2** and **12** took place in *P. morifolia* in high yield. The amounts of **1** and **2** obtained using racemic **10** were approximately equal, in contrast to natural sources of these glucosides, where one of the epimers usually predominates [4, 5, 9, 10]. Thus, both enantiomers of **10** appear to be hydroxylated in *P. morifolia*. No 4-hydroxylated derivatives were detected, although very small amounts could have passed unnoticed. The saturated nitrile **9** gave rise to **12**, isolated for the first time in this work (the glucoside was earlier reported to be produced in flax upon feeding with cyclopentanone cyanohydrin, based solely on mass spectral peaks observed upon trimethylsilylation of a lotaustralin fraction [17]). The crystalline glucoside was now characterized by spectral methods, including spectra of its tetracetate. The specific rotation of **12** was $[\alpha]_D^{19}$ −22.6°; this value is almost exactly the average of the specific rotations of **1** and **2**, which have enantiomeric aglucones (respectively $[\alpha]_D^{26}$ −25° and $[\alpha]_D^{26}$ −20° [4, 5]). Upon feeding with the (*S*)-enantiomer of **7** (optical purity >90%), *P. morifolia* produced a mixture of **4** and **5** in amounts increased by two- to four-fold relative to the control, but the ratio of the epimers was not significantly changed (see Experimental; ref. [3]). When fed with **11**, *P. morifolia* produced approximately equimolar amounts of heterodendrin (**13**) and epiheterodendrin (**14**) [18].

Turnera angustifolia was previously found to contain only cyclopentanoid cyanohydrin glucosides [3, 8, 9], which is in agreement with control experiments conducted in the present work. Consequently, the feeding experiments with this plant were designed to involve **6** as a presumed exogenous substrate, which indeed resulted in production of a large amount of linamarin (**3**) (Table 1). Interestingly, some feeding experiments with other nitriles also resulted in isolation of linamarin

Table 1. Transformation of nitriles into cyanohydrin glucosides in *P. morifolia* and *T. angustifolia*

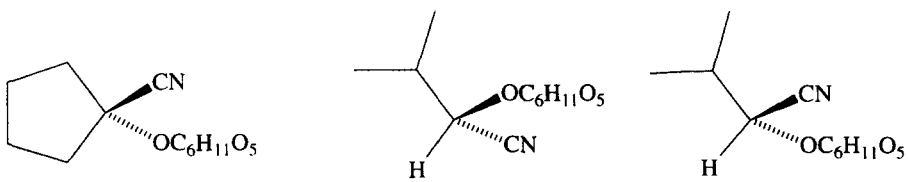
Exp. no.	Plant	Fed with	Nitrile uptake (mmol)	Glucoside produced	Yield (%)
1	*P. morifolia*	**7**	1.016	**4** and **5**, *ca* 8:1*	2.9
2	*P. morifolia*	**7**	0.942	**4** and **5**, ca 7:1*	7.6
3	*P. morifolia*	**8**	0.350	—	—
4	*P. morifolia*	**8**	0.544	—	—
5	*P. morifolia*	**9**	0.512	**12**	10.4
6	*P. morifolia*	**9**	0.400	**12**	13.3
7	*P. morifolia*	**10**	0.400	**1** and **2**, *ca* 1:1*	6.7
8	*P. morifolia*	**10**	0.410	**1** and **2**, *ca* 1:1*	5.6
9	*P. morifolia*	**11**	0.516	**13** and **14**, *ca* 1:1†	3.6
10	*P. morifolia*	**11**	0.600	**13** and **14**, *ca* 1:1†	1.2
11	*T. angustifolia*	**6**	0.372	**3**	3.9
12	*T. angustifolia*	**6**	0.230	**3**	6.8
13	*T. angustifolia*	**7**	0.670	**4** and **5**, *ca* 4:1*	0.8
14	*T. angustifolia*	**7**	0.540	**4** and **5**, *ca* 10:1*	0.8
15	*T. angustifolia*	**8**	0.340	‡	—
16	*T. angustifolia*	**8**	0.330	‡	—
17	*T. angustifolia*	**9**	0.250	**12**§	8.7
18	*T. angustifolia*	**9**	0.340	**12**§	1.1
19	*T. angustifolia*	**11**	0.280	—	—
20	*T. angustifolia*	**11**	0.284	—	—

*Ratio estimated by ^{1}H NMR.
†Ratio estimated by HPLC.
‡Compound **3** also isolated (3–12 mg per 100 g of plant material).
§Compound **3** also isolated (115–140 mg per 100 g^{-1} of plant material).

(Table 1), and it must be concluded that *T. angustifolia* is able to produce this glucoside from valine under certain conditions. Whether this is related to nitrile administration or merely represents a specific metabolic status of certain plant shoots is unknown. Similarly to *P. morifolia*, *T. angustifolia* was able to produce **12** from **9** in good yield. Formation of **4** and **5** from **7** was also observed. However, *T. angustifolia* was apparently unable to metabolize **11**.

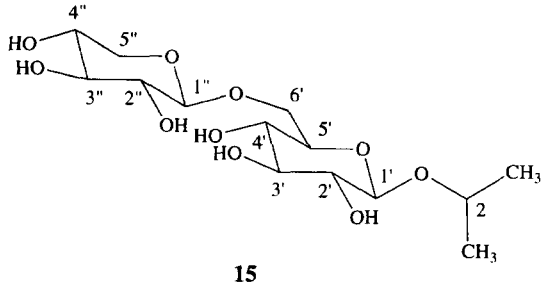
Neither *P. morifolia* nor *T. angustifolia* appeared to be able to convert **8** into the corresponding cyanohydrin glucoside. This, however, may well be the result of an energy barrier to the oxygenation of **8** [19] rather than the steric requirements of the hydroxylating enzymes [20] involved. Apart from that, *biosynthetic systems of both species were shown to be able to accept nitriles different from those involved in the biosynthesis of their endogenous cyanohydrin glucosides*, providing further evidence that the relatively broad substrate specificity of the nitrile hydroxylation step is a characteristic property of this group of plants [2, 3]. The ability of *P. morifolia* to convert **11** into a mixture of **13** and **14** is particularly noteworthy. Since these leucine metabolites are not produced naturally by this plant, it follows that the specificity of the biosynthetic pathway is controlled here at the amino acid stage.

During fractionation of numerous samples of *P. morifolia* we encountered a compound which, similarly to **13** and **14**, exhibited the characteristic ^{1}H NMR signals of an isopropyl group, and it was essential to ascertain its identity. Using one- and two-dimensional NMR techniques, this compound was shown to be the previously unknown primeveroside **15** (Fig. 4). The structural assignment rests on identification of all sugar vicinal couplings as diaxial couplings from ordinary and two-dimensional *J*-resolved ^{1}H NMR spectra, on observation of NOEs between H-2 and H-1′ and between H-1″ and H-6′, on observation of a characteristic glycosidation shift of C-6′ of glucopyranose and of C2 of the aglycone [21, 22], and on exact molecular



12 **13** Heterodendrin **14** Epiheterodendrin

Fig. 3. Cyanohydrin glucosides biosynthesized from exogenous nitriles **9** and **11**.



15

Fig. 4. Structure and numbering system of isopropyl primeveroside.

mass determination by FAB mass spectrometry. The primeverosyl moiety was previously encountered in numerous plant products [e.g. 23–33]. The ^{13}C NMR chemical shifts of **15** were assigned from a ^{1}H, ^{13}C shift correlation experiment and agreed very well with those reported for other primeverosides [28, 32].

Although this is not the first report of a natural isopropyl glycoside [34], such glycosides are quite rare. Since the glycoside (**15**) was consistently isolated in numerous independent extractions of *P. morifolia*, we assume it to be a genuine constituent of this plant. The isopropyl group is reminiscent of the aglucone of **3** minus the carbon atom originally present as the cyano group. Although the biosynthetic relationship between **3** and **15** is purely speculative at this point, we note that other cyanogenic plants were previously reported to contain alcohols (free or glycosylated) of unclear biosynthetic origin that are structurally related to the cyanohydrin glucosides present in the same plant. Thus, *Ceratiosicyos laevis* (Achariaceae) contains 4-cyclopentene-1,2,3-triol, which formally corresponds to the aglucone of gynocardin (less the cyano group) also present in this plant [35], whereas *Acacia sieberiana* yielded a glucoside of 2-methylpropane-1,2-diol, corresponding in the same manner to the aglucone of hydroxyheterodendrin [36]. A primeveroside of benzyl alcohol was isolated from a species belonging to *Prunus* [33], the genus typically producing glycosides of benzaldehyde cyanohydrin (e.g. prunasin and amygdalin). All these alcohols could arise by reduction of carbonyl compounds formed by dissociation of the cyanohydrins that accumulate (in glucosylated form) in these plants.

Free cyanohydrins are sometimes encountered in cyanogenic plants. In many cases they may represent isolation artefacts, but their occurrence as endogenous, stored products cannot be ruled out [37–42]. More importantly, free cyanohydrins may be generally formed as transient products of turnover of cyanogenic glycosides [43, 44]. Thus, the ability of plants to metabolize cyanide to asparagine via β-cyanoalanine was demonstrated by Tschiersch [45–48]. Moreover, he demonstrated a conversion of phenylalanine into β-cyanoalanine, presumably via the cyanohydrin of benzaldehyde [49]. Although the formation of β-cyanoalanine is also related to the biosynthesis of ethylene [50], Tschiersch's experiments provided the first direct evidence of the turnover of cyanohydrin glycosides in plants. We suggest that the formation of alcohols corresponding to natural cyanohydrin glycosides is a previously unrecognized extension of the cyanohydrin biosynthesis pathway in higher plants.

EXPERIMENTAL

General. NMR: Bruker AMX 400; MS: JEOL JMS-AX505W in positive mode, accurate mass measurements were performed using dual target probe with PEG-300 as ref. material; optical rotations: Perkin-Elmer 241; HPLC: Waters 590 pump, Rheodyne 7125 injector (2 ml loop), Shimadzu RID-6A RI detector, 1.6×25 cm Knauer column of LiChrosorb RP-18, 5 μm, eluted with 20% MeOH (4 ml min^{-1}); CC: silica gel 60 (Merck), 0.066–0.2 mm, eluted with EtOAc–Me_2CO–CH_2Cl_2–MeOH–H_2O (20:15:6:5:4). *P. morifolia* Mast. (Passifloraceae) and *T. angustifolia* Miller (Turneraceae) were grown as described elsewhere [3]. 2-Cyclopentenecarbonitrile was synthesized as previously reported [3]; all other chemicals were obtained from commercial sources. (*S*)-2-Methylbutanenitrile used had $[\alpha]_D^{24}$ +35.2° (*c* 6, heptane), corresponding to an optical purity >90% [51].

Feeding experiments. Freshly cut shoots (usually 20–40 g) of *P. morifolia* and *T. angustifolia* were immersed in 2 mM solns of the nitriles (Table 1) in diluted H_2O–culture nutrient soln containing the essential elements according to ref. [52]. After 3 days, during which the plants were given 12 hr illumination for each 24-hr period, the plants were extracted and the extracts fractionated on silica gel in the usual way [10]. Cyanogenic frs were further fractionated by reverse-phase HPLC and peaks eluted from the column collected. The eluates were freeze-dried and the residues examined by ^{1}H NMR. This led to identification of the glucosides as described in the text. In control experiments, which were conducted as above, but without the nitriles added to the soln, *T. morifolia* (35 g plant material) yielded 133 mg **3**, 2 mg **4** and 0.4 mg **5**. Similar control experiment with *T. angustifolia* (24 g) yielded 5 mg **1**, 0.2 mg **2** and 2.4 mg volkenin. Tetraphyllin B was not isolated in the control experiment, but trace amounts were evident in some of the feeding experiments. Amounts of endogenous glucosides isolated in feeding experiments (Table 1) were similar to those found in respective control experiments except that **3** was isolated in several feeding experiments with *T. angustifolia* (Table 1).

Identification of known compounds. The glucosides (**1–5**) plus volkenin and tetraphyllin B were identified by ^{1}H NMR in CD_3OD, which also determined the ratios between epimers (not sepd in the HPLC system used) [2–5, 10].

Heterodendrin (**13**). ^{1}H NMR (400 MHz, CD_3OD): δ 1.07, 1.11 (each 3H, *d*, $J = 6.8$ Hz, Me, 2.11 (1H, *m*, H-3), 3.22 (1H, *dd*, $J_{1',2'} = 7.7$ Hz, $J_{2',3'} = 9.2$ Hz, H-

2'), 3.30–3.37 (3H, *m*, H-3', H-4', H-5'), 3.64 (1H, *dd*, $J_{5',6'A} = 6.3$ Hz, $J_{6'A,6'B} = -12.0$ Hz, H-6'A), 3.90 (1H, *dd*, $J_{5',6'B} = 2.2$ Hz, $J_{6'A,6'B} = -12.0$ Hz, H-6'B), 4.49 (1H, *d*, $J_{1',2'} = 7.7$ Hz, H-1'), 4.75 (1H, *d*, $J =$ 5.7 Hz, H-3), in agreement with lit. [18, 53]. FAB-MS (positive ion, glycerol doped with NaOAc): *m/z* (rel. int.) 284.1112 (100) $[M + Na]^+$, calc. for $C_{11}H_{19}NO_6Na$ 284.1110.

Epiheterodendrin (**14**). ^1H NMR (400 MHz, CD_3OD): δ 1.07, 1.10 (each 3H, *d*, $J = 6.8$ Hz, Me), 2.15 (1H, *m*, H-3), 3.22 (1H, *dd*, $J_{1',2'} = 7.7$ Hz, $J_{2',3'} = 9.2$ Hz, H-2'), 3.30–3.37 (3H, *m*, H-3', H-4', H-5'), 3.71 (1H, *dd*, $J_{5',6'A} = 4.8$ Hz, $J_{6'A,6'B} = -12.0$ Hz, H-6'A), 3.89 (1H, *dd*, $J_{5',6'B} = 2.0$ Hz, $J_{6'A,6'B} = -12.0$ Hz, H-6'B), 4.40 (1H, *d*, $J_{1',2'} = 7.7$ Hz, H-1'), 4.49 (1H, *d*, $J = 5.7$ Hz, H-3), in agreement with lit. [18, 53]. FAB-MS (positive ion, glycerol doped with NaOAc): *m/z* (rel. int) 284.1112 (100) $[M + Na]^+$, calc. for $C_{11}H_{19}NO_6Na$ 284.1110.

1-(*β*-D-*Glucopyranosyloxy*)*cyclopentanecarbonitrile* (**12**). The glucoside was obtained after extraction of *P. morifolia* and *T. angustifolia* fed with **9** (Table 1); combined portions of the glucoside (total of *ca* 40 mg) were further purified by HPLC and recrystallized from EtOAc, yield 5 mg. Mp. 131–132° (uncorr.). FAB-MS (positive ion, glycerol): *m/z* (rel. int.) 547 (80) $[2M + H]^+$, 366 (80) $[M + glycerol + H]^+$, 296 (12) $[M + Na]^+$, 274 (100) $[M + H]^+$; exact mass for $[M + Na]^+$: found 296.1119, calc. for $C_{12}H_{19}NO_6Na$ 296.1110 (glycerol doped with NaOAc as matrix). $[\alpha]_D^{19}$ −22.6° (*c* 0.2, MeOH). ^1H NMR (400 MHz, CD_3OD): δ 1.74–1.98 (4H), 2.03–2.18 (2H), 2.20–2.29 (1H), 2.34–2.43 (1H) (each *m*, cyclopentane ring), 3.18 (1H, *dd*, $J_{1',2'} =$ 7.8 Hz, $J_{2',3'} = 9.0$ Hz, H-2'), 3.30–3.42 (3H, *m*, H-3', H-4', H-5'), 3.69 (1H, *dd*, $J_{5',6'A} = 5.0$ Hz, $J_{6'A,6'B} =$ −11.9 Hz, H-6'A), 3.85 (1H, *dd*, $J_{5',6'B} = 2.0$ Hz, $J_{6'A,6'B} = -11.9$ Hz, H-6'B), 4.59 (1H, *d*, $J_{1',2'} =$ 7.8 Hz, H-1'). ^{13}C NMR (100.6 MHz, CD_3OD): δ 23.93, 24.16, 40.05, 40.61 (cyclopentane ring), 62.82, 71.57, 75.05, 78.23, 78.37, 102.34 (glucosyl), 81.63 (C-1), 122.20 (CN).

The acetate was prepd by overnight treatment of **12** with pyridine–Ac_2O. ^1H NMR (400 MHz, $CDCl_3$): δ 1.943, 1.971, 2.008, 2.013 (each 3H, *s*, Ac), 1.63–1.80 (4H), 1.92–2.06 (3H), 2.19–2.23 (1H) (each *m*, cyclopentane ring), 3.71 (1H, *o*, $J_{4',5'} = 10$ Hz, $J_{5',6'A} = 2.4$ Hz, $J_{5',6'B} = 5.8$ Hz, H-5'), 4.07 (1H, *dd*, $J_{5',6'A} = 2.4$ Hz, $J_{6'A,6'B} = -12.2$ Hz, H-6'A), 4.18 (1H, *dd*, $J_{5',6'B} = 5.8$ Hz, $J_{6'A,6'B} = -12.2$ Hz, H-6'B), 4.75 (1H, *d*, $J_{1',2'} = 8.0$ Hz, H-1'), 4.93 (1H, *dd*, $J_{1',2'} = 8.0$ Hz, $J_{2',3'} = 9.6$ Hz, H-1'), 4.98 (1H, *t*, $J_{3',4'} \cong J_{4',5'} \cong 9.6$ Hz, H-4'), 5.18 (1H, *t*, $J_{2',3'} \cong J_{3',4'} \cong 9.6$ Hz, H-3'). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 19.59 (3C, Me), 19.69 (1C, Me), 21.65, 21.90, 37.74, 38.24 (cyclopentane ring), 60.89, 67.24, 69.84, 71.14, 71.63, 97.29 (glucosyl), 79.63 (C-1), 118.75 (CN), 168.30–169.56 (4C, CO).

2-[6-*O*-(*β*-D-*Xylopyranosyl*)-*β*-D-*glucopyranosyloxy*]*propane* (**15**). Portions of this glycoside (total of *ca* 25 mg crude material) were obtained during purification of *T. morifolia* cyanogens by HPLC (eluted as a peak with *k' ca* 3). The material was further purified by HPLC to give 5 mg pure, non-crystalline **15**. FAB-MS (positive ion, glycerol): *m/z* (rel. int.) 377 (32) $[M + Na]^+$, 355 (85) $[M + H]^+$, 295 (70) $[M + H -$ aglycone$]^+$, 223 (85) $[M + H -$ xylose$]^+$, 133 (100) [xylopyranosyl]$^+$; exact mass for $[M + H]^+$: found 355.1610, calc. for $C_{14}H_{27}O_{10}$ 355.1604. ^1H NMR (400 MHz, CD_3OD): δ 1.19, 1.23 (each 3H, *d*, $J = 6.2$ Hz, Me), 3.74 (1H, *dd*, $J_{6'A,6'B} = -11.5$ Hz, $J_{6'A,5'} = 5.8$ Hz, H-6'A), 3.86 (1H, *dd*, $J_{5''A,5''B} =$ −11.4 Hz, $J_{5''B,4''} = 54$ Hz, H-5''B), 4.03 (1H, *h*, $J =$ 6.2 Hz, H-2), 4.07 (1H, *dd*, $J_{6'A,6'B} = -11.5$ Hz, $J_{6'B,5'} = 2.1$ Hz, H-6'B), 4.328 (1H, *d*, $J_{1'',2''} = 7.3$ Hz, H-1''), 4.329 (1H, *d*, $J_{1',2'} = 7.9$ Hz, H-1'), remaining protons at 3.13–3.49 (complex pattern partially obscured by solvent signal). ^1H NMR (400 MHz, D_2O): δ 1.12, 1.16 (each 3H, *d*, $J = 6.2$ Hz, Me), 3.14 (1H, *dd*, $J_{1',2'} = 7.9$ Hz, $J_{2',3'} = 9.3$ Hz, H-2'), 3.22 (1H, *dd*, $J_{1'',2''} = 7.8$ Hz, $J_{2'',3''} = 9.2$ Hz, H-2''), 3.23 (1H, *dd*, $J_{5''A,5''B} = -11.6$ Hz, $J_{5''A,4''} = 9.6$ Hz, H-5''A), 3.34 (1H, *t*, $J_{3',4'} \cong J_{4',5'} \cong 9.2$ Hz, H-4'), 3.35 (1H, *t*, $J_{2'',3''} \cong J_{3'',4''} \cong 9.2$ Hz, H-3''), 3.40 (1H, *t*, $J_{2',3'} \cong J_{3',4'} \cong 9$ Hz, H-3'), 3.52 (1H, *m*, H-5'), 3.54 (1H, *dt*, $J_{3'',4''} \cong J_{4'',5''A} \cong 9.7$ Hz, $J_{4'',5''B} = 5.4$ Hz, H-4''), 3.74 (1H, *dd*, $J_{6'A,6'B} = -11.8$ Hz, $J_{6'A,5'} = 6.0$ Hz, H-6'A), 3.88 (1H, *dd*, $J_{5''A,5''B} = -11.6$ Hz, $J_{5''B,4''} = 5.4$ Hz, H-5''B), 4.03 (1H, *h*, $J = 6.2$ Hz, H-2), 4.06 (1H, *dd*, $J_{6'A,6'B} = -11.8$ Hz, $J_{6'B,5'} = 2.0$ Hz, H-6'B), 4.38 (1H, *d*, $J_{1'',2''} = 7.8$ Hz, H-1''), 4.47 (1H, *d*, $J_{1',2'} = 7.9$ Hz, H-1'); assignments supported by COSY, NOESY (600 msec mixing time) and *J*-resolved spectra. ^{13}C NMR (100.6 MHz, CD_3OD): δ 22.40, 24.17 (Me), 67.24 (C-5''), 69.97 (C-6'), 71.47 (C-4''), 71.75 (C-4'), 73.04 (C-2), 75.15 (C-2''), 75.37 (C-2'), 77.21 (C-5'), 78.00 (C-3''), 78.25 (C-3'), 102.96 (C-1'), 105.78 (C-1''). ^{13}C NMR (100.6 MHz, D_2O): δ 23.52, 24.96 (Me), 67.70 (C-5''), 71.19 (C-6'), 71.75 (C-4''), 72.01 (C-4'), 75.59 (C-2''), 75.64 (C-2, C-2'), 77.43 (C-5'), 78.16 (C-3''), 78.29 (C-3'), 102.95 (C-1'), 106.06 (C-1''); carbon spectra were assigned from 2D C,H-correlations.

The primeveroside was acetylated by overnight treatment with pyridine–Ac_2O; the product could not be recrystallized. ^1H NMR (400 MHz, $CDCl_3$): δ 1.07, 1.16 (each 3H, *d*, $J = 6.2$ Hz, Me), 1.925, 1.959, 1.964, 1.976, 1.984, 1.984 (each 3H, *s*, Ac), 3.28 (1H, *dd*, $J_{5''A,5''B} = -11.9$ Hz, $J_{5''A,4''} = 8.7$ Hz, H-5''A), 3.54 (1H, *dd*, $J_{6'A,6'B} = -10.6$ Hz, $J_{6'A,5'} = 6.9$ Hz, H-6'A), 3.59 (1H, *m*, H-5'), 3.74 (1H, *dd*, $J_{6'A,6'B} = -10.6$ Hz, $J_{6'B,5'} = 1.5$ Hz, H-6'B), 3.85 (1H, *h*, $J = 6.2$ Hz, H-2), 4.06 (1H, *dd*, $J_{5''A,5''B} = -11.9$ Hz, $J_{5''B,4''} = 5.0$ Hz, H-5''B), 4.45 (1H, *d*, $J_{1',2'} = 8.0$ Hz, H-1'), 4.49 (1H, *d*, $J_{1'',2''} = 6.7$ Hz, H-1''), 4.80–4.90 (4H, *m*, H2', H2'', H4', H4''), 5.06 (1H, *t*, $J_{2'',3''} \cong J_{3'',4''} \cong 8.4$ Hz, H-3''), 5.12 (1H, *t*, $J_{2',3'} \cong J_{3',4'} \cong 9.5$ Hz, H-3'), ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 20.72 (6C), 21.98, 23.41, 61.98, 67.79, 68.76, 69.14, 70.55, 71.26, 71.56, 72.87, 72.92, 73.28, 99.46, 100.45, 169.39, (2C), 169.68, 169.94, 170.13, 170.40, cf. refs [30, 33].

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