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Cyanogenic glycosides and menisdaurin from *Guazuma ulmifolia*, *Ostrya virginiana*, *Tiquilia plicata*, and *Tiquilia canescens*

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Abstract

The major cyanogenic glycoside of *Guazuma ulmifolia* (Sterculiaceae) is (2*R*)-taxiphyllin (>90%), which co-occurs with (2*S*)-dhurrin. Few individuals of this species, but occasional other members of the family, have been reported to be cyanogenic. To date, cyanogenic compounds have not been characterized from the Sterculiaceae. The cyanogenic glycosides of *Ostrya virginiana* (Betulaceae) are (2*S*)-dhurrin and (2*R*)-taxiphyllin in an approximate 2:1 ratio. This marks the first report of the identification of cyanogenic compounds from the Betulaceae. Based on NMR spectroscopic and TLC data, the major cyanogenic glucoside of *Tiquilia plicata* is dhurrin, whereas the major cyanide-releasing compound of *Tiquilia canescens* is the nitrile glucoside, menisdaurin. NMR and TLC data indicate that both compounds are present in each of these species. The spectrum was examined by CI-MS, ^{1}H and ^{13}C NMR, COSY, 1D selective TOCSY, NOESY, and $^{1}J/^{2,3}J$ HETCOR experiments; all carbons and protons are assigned. The probable absolute configuration of (2*R*)-dhurrin is established by an X-ray crystal structure. The ^{1}H NMR spectrum of menisdaurin is more complex than might be anticipated, containing a planar conjugated system in which most elements are coupled to several other atoms in the molecule. The coupling of one vinyl proton to the protons on the opposite side of the ring involves a ^{6}J- and a $^{5/7}J$-coupling pathway. A biogenetic pathway for the origin of nitrile glucosides is proposed.

Keywords: Cyanogenic glycosides; *Guazuma ulmifolia*; Sterculiaceae; *Ostrya virginiana*; Betulaceae; *Tiquilia canescens*; *Tiquilia plicata*; Boraginaceae; Menisdaurin; (2*R*)-Taxiphyllin; (2*S*)-Dhurrin; Long-range coupling; Nitrile glucosides

1. Introduction

Trees of *Guazuma ulmifolia* Lam. (Sterculiaceae), commonly known as guácimo, caulote, tapaculo, or aquiche, occupy dry lowlands from Peru, north and east to Venezuela and to northern Mexico. The species is common in pastures and fencerows and its foliage and fruits are valuable cattle and horse fodder (Janzen, 1983; Schnee, 1973). Young leaves of this species are an occasional food of howling monkeys (Glander, 1975). The bark is used medicinally as a sudorific (Pittier, 1978), against malaria, and a variety of other ailments (Martínez, 1969; van Valkenburg and Bunyaphrapha, 2001). Proanthocyanidins from the bark inhibit the activity of cholera toxin (Hör et al., 1996).

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Although cyanogenesis has been reported from seeds of *Sterculia murex* Hemsl. and from leaves and stem bark of *Kleinhovia hospita* L., *Pterocymbium tinctorium* (Blanco) Merrill, *Pterospermum obliquum* Blanco, *Sterculia foetida* L., and *Theobroma cacao* L. (Hegnauer, 1964, 1973a,b), all members of the Sterculiaceae, no cyanogenic compounds appear to have been isolated and characterized from this family. Benzylcyanide has previously been reported from *T. cacao* seeds (Gill et al., 1984).

Individuals of *Ostrya virginiana* (Miller) K. Koch (Betulaceae), ironwood or hop hornbeam, are common in the eastern portion of North America (Gleason and Cronquist, 1991). Hop hornbeam has been used to treat toothache and a variety of other ailments (Moerman, 1986), but little chemical study of this species has been carried out. Although *O. virginiana* has been reported to be cyanogenic (Aikman et al., 1996), the compound(s) responsible has not been isolated and characterized.

The genus *Tiquilia* (syn. *Coldenia*) (Boraginaceae) comprises 27 species that occur in the deserts of both North and South America. A species widespread in Texas, the southwestern US, and northern Mexico, *Tiquilia canescens* (DC.) A. Richardson, has been used to bathe wounds, for sweat baths, and to prevent sleeping; other species are used to treat gonorrhea and for stomach trouble. The plants appear seldom to be grazed by goats or cattle (Richardson, 1977).

Relatively few members of the family Boraginaceae have been reported to contain cyanogenic compounds (Gibbs, 1974; Seigler, 1977; Lechtenberg and Nahrstedt, 1999), although one commonly cultivated species, *Borago officinalis* contains (2*S*)-dhurrin (Hegnauer, 1973a,b, 1989; van Valen, 1978a). Nitrile glucosides with cyclohexenylcyanomethylene structures are found in other boraginaceous species, such as *Lithospermum purpureocaeruleum* L. and *Lithospermum officinale* L. (Sosa et al., 1977) and *Ehretia philippinensis* (Simpol et al., 1994).

2. Results and discussion

2.1. Cyanogenic compounds from Guazuma ulmifolia

Cyanogenesis has not been reported previously from *G. ulmifolia* and is uncommon in that widespread species. Cyanide tests on more than 200 individuals from Costa Rica and Mexico were uniformly negative, except for two individuals at La Pacifica, near Cañas, Guanacaste, Costa Rica. Even then, this activity appears to have been transitory; three years after the beginning of the study, these trees had lost cyanogenic activity.

Thin layer chromatography (TLC) of crude extracts of leaf material collected in the first two years of the study revealed the presence of only one cyanogenic band by the "sandwich" method (Brimer et al., 1983). The problem of intractable mucilaginous compounds was circumvented by a special isolation-chromatographic procedure in which the cyanogenic compounds were both extracted and purified chromatographically in a single step. The ^{1}H NMR spectra of the active compound(s) had peaks corresponding to taxiphyllin (Nahrstedt et al., 1993; Seigler and Brinker, 1993), namely those of a *para*-substituted aromatic ring, a methine proton (5.780 ppm), an anomeric proton doublet centered at 4.165 ppm and the adjacent protons of a β-glucopyranosyl moiety. This sample consists of taxiphyllin (**1b**) (>90%) in admixture with dhurrin (**1a**) (Table 1).

Table 1
^{1}H and ^{13}C NMR spectral data[a] for (2*S*)-dhurrin (**1a**) and (2*R*)-taxiphyllin (**1b**)

Position	δ_C (**1a**)	δ_C (**1b**)	δ_H (**1a**)	Mult. (*J*) (**1a**)	δ_H (**1b**)	Mult. (*J*) (**1b**)
1	118.77	119.70	–	–	–	–
2	68.30	68.06	5.897	*s*	5.778	*s*
3	125.85	125.20	–	–	–	–
4	130.74[b]	130.86[c]	7.391[†]	*dt-like m* (~1, 2.7, 8.5)	7.385[†]	*dt-like m* (~1, 2.7, 8.5)
5	116.52[d]	116.79[e]	6.817*	*dt-like m* (~1, 2.7, 8.5)	6.836*	*dt-like m* (~1, 2.7, 8.5)
6	160.03	160.34	–	–	–	–
7	116.52[d]	116.79[e]	6.817*	*dt-like m* (~1, 2.7, 8.5)	6.836*	*dt-like m* (~1, 2.7, 8.5)
8	130.74[b]	130.86[c]	7.391[†]	*dt-like m* (~1, 2.7, 8.5)	7.385[†]	*dt-like m* (~1, 2.7, 8.5)
1′	101.87	101.16	4.666	*d* (7.6)	4.165	*m* [higher order; pseudo d, 7.8)
2′	74.75[f]	74.75[f]	3.226	*dd* (7.6, 9.2)	3.270	*m* [higher order] (7.6, 9.2)
3′	78.10	77.94	3.408	*t* (9.2)	3.244	*m* [higher order] (9.2, 9.2)
4′	71.55	71.55	3.292	*dd* (9.2/9.7)	3.233	*m* [higher order] (9.2/9.7)
5′	78.30[g]	78.30 g	3.177	*ddd* (2.2, 6.3, 9.7)	3.179	*ddd* (2.3, 6.1, 9.7)
6′a	62.82[h]	62.82[h]	3.897	*dd* (2.2, 12.0)	3.897	*dd* (2.3, 12.0)
6′b	–	–	3.708	*dd* (6.3, 12.0)	3.708	*dd* (6.1, 12.0)

[†,*] Precise shift determination revealed ca. 1 Hz anisochronism for both pairs H-4/8 and H-5/7, which exhibit AA′XX′/ABXY higher order signals (Veit and Pauli, 1999); exact shift determination precluded due to severe signal overlap.

[a] Observed at 600/90 MHz ^{1}H/^{13}C in CD3OD; δ in ppm, *J* in Hz; n.d. = not determined because of severe signal overlap.

[b–h] Assignments may be interchanged.

2.2. Cyanogenic compounds from Ostrya virginiana

In the present study, 80% of the catkins from 118 individuals of *O. virginiana* collected in east central Illinois were positive (weak to strong) for the presence of cyanogenic compounds by the Feigl–Anger test. All 30 catkins from one individual tested positive, but neither young leaf material nor pollen was cyanogenic. In general, catkins from herbarium materials taken from herbarium materials (no exogenous enzyme added) gave negative tests for the release of hydrogen cyanide.

The presence of large amounts of tannins in the 80% aqueous methanolic extract prevented effective chromatographic purification of extracts of the catkins. This solution was concentrated under vacuum and methanol was added resulting in a dense tannin-rich precipitate that was removed by centrifugation. Chromatographic purification and analysis of the supernatant from this procedure (TLC) revealed the presence of one cyanogenic band. The supernatant was additionally purified by additional vacuum chromatography (VLC) and medium performance liquid chromatography (MPLC). The ^{1}H NMR spectrum of the purified material had a *para*-substituted aromatic ring (AA′XX′/ABXY higher order spin system, pairs of *t*-like multiplets at 7.391/6.817 and 7.385/6.836 ppm; H-4,5; H-7,8), two methine protons (5.778 and 5.897 ppm, H-2), two anomeric protons centered at 4.624 (d) and 4.165 (*d*-like multiplets) (H-1′), and those of β-glucopyranosyl moiety (Table 1). The nature of the glycosidic linkage was established through 1D selective NOE measurements. The presence of two sets of six aromatic, six glucosidic, and two cyanogenic methine peaks in the ^{13}C NMR is consistent with the presence of two structurally similar diastereomers. The ^{13}C NMR had aromatic peaks consistent with a *para*-substituted aromatic ring, an anomeric carbon for dhurrin at 101.868 (C-1), and a methine carbon at 68.330 (C-2) ppm. It was ultimately established that both (2*S*)-dhurrin (**1a**) and (2*R*)-taxiphyllin (**1b**) was present in a ratio of 69:21.

2.3. Cyanide tests for Tiquilia species

Specimens from the University of Illinois Herbarium (ILL) of *T. canescens* (DC) A. Richardson (6 strongly positive, 13 negative), *Tiquilia gossypina* (Wooton and Standley) A. Richardson (one strongly positive, one negative), *Tiquilia nuttallii* (Bentham and Hooker) A. Richardson (3 strongly positive, 4 negative), *Tiquilia plicata* (Torrey) A. Richardson (9 strongly positive; see follow-up isolation described below), and *Tiquilia purpusii* (Brandeg.) A. Richardson (1 weakly positive, 1 negative) were at least in some instances positive, whereas 2 specimens of *Tiquilia cuspidata* (I. M. Johnston) A. Richardson, 3 of *Tiquilia greggii* (Torrey and Gray) A. Richardson, 12 of *Tiquilia hispidissima* (Torrey and Gray) A. Richardson, 4 of *Tiquilia mexicana* (S. Watson) A. Richardson, and 7 of *Tiquilia palmeri* (A. Gray) A. Richardson consistently tested negative for the presence of cyanogenic compounds.

2.4. Characterization of cyanogenic glycosides from Tiquilia plicata

Fractionation of an extract of *T. plicata* by vacuum chromatography (VLC) and analysis by TLC indicated the presence of two cyanogenic compounds (R_f 0.55 and 0.29). The less abundant but more polar compound was not studied farther. Based on its R_f value, this substance appeared to be menisdaurin (**2**). Subsequent purification of the more abundant and less polar compound by MPLC (two times) produced a fraction that gave only one cyanogenic band on TLC (R_f 0.55). The ^{1}H NMR spectrum of this material had signals of sugar protons corresponding to β-glucopyranosyl moiety, a *para*-substituted aromatic ring, a cyanogenic methine proton (5.88 ppm), and an anomeric proton doublet centered at 4.63 ppm, corresponding to dhurrin (**1a**) (>95% pure).

2.5. Characterization of menisdaurin (2) from Tiquilia canescens

Fractionation of an 80% aqueous methanolic extract of *T. canescens* leaf material, subsequent VLC, and analysis of the fractions by TLC, revealed the presence of two cyanogenic substances. The less abundant of these (R_f 0.60) was not studied further, but based on NMR spectra analysis and R_f value, was established to be (2*S*)-dhurrin (**1a**). Fractions containing the more polar compound (R_f 0.34) were combined and concentrated until only an aqueous solution remained. Extraneous substances were precipitated by addition of methanol and removed by centrifugation. The remaining water-soluble material was fractionated by VLC to yield an amorphous solid, which was subsequently re-chromatographed and purified further by MPLC to yield a white solid.

The CI-NH_3 mass spectrum of this substance exhibited a diagnostic ion at *m*/*z* 314 $[\text{menisdaurin} + \text{H}]^+$, a base peak at *m*/*z* 331 $[\text{menisdaurin} + \text{NH}_4]^+$, and an additional peak due to cluster ion formation at *m*/*z* 348 $[\text{menisdaurin} + \text{NH}_3 + \text{NH}_4]^+$. The presence of a nitrogen atom in the molecule is supported by the MS data (*m*/*z* 313). The ^{13}C NMR spectrum of the compound exhibits fourteen carbon atoms, all approximately equal in height; several of them have characteristic chemical shifts (Table 2). The presence of a peak at 101.608 is typical for anomeric carbons of glucosides (C-1′); additional peaks at 78.11, 78.06, 74.57, 71.78, and 63.16 ppm support the idea that the compound contains β-glucopyranose moiety (Pauli,

Table 2
^{1}H and ^{13}C NMR spectral data[a] for compound **2**

Position	δ_C (**2**)[b]	δ_H (**2**)[b]	Mult.[c]	J[c](→H-atom)	$^{2/3}J_{C,H}$ → H-atom[d]
1	118.01	–	–	–	–
2	96.96	5.485	*dddd*	1.44 (8), 0.80 (5), 0.76 (6), 0.29 (4), −0.05 (7a)	4
3	157.02	–	–	–	2, 5, 4, 7sb
4	127.69	6.275	*ddd*	9.90 (5), 1.27 (6), 0.29 (2)	2
5	140.47	6.201	*ddddd*	9.90 (4), 3.55 (6), 0.80 (2), −0.47 (7a), 0.29 (4)	7b
6	65.22	4.342	*dddddd*	6.02 (7b), 5.27 (7a), 3.55 (5), 1.27 (4), 0.76 (2), 0.27 (8)	
7a	35.95	2.228	*dddd[d]*	−13.51 (7b), 5.27 (6), 3.51 (8), −0.47 (5), −0.05 (2)	
7b		2.054	*ddd*	−13.51 (7a), 8.07 (8), 6.02 (6)	
8	72.57	4.913	*dddd*	8.07 (7b), 3.51 (7a), 1.44 (2), 0.27 (6)	2
1′	101.61	4.543	*d*	7.4 (2′)	2′, 5′
2′	71.78	3.314	*m [dd]*	7.4 (1′), n.d.	
3′	78.06[e]	3.388	*m [dd]*	∼9 (2′), ∼9 (4′)	
4′	74.565	3.282	*m [dd]*	∼9 (3′), ∼9 (5′)	
5′	78.11[e]	3.314	*m[ddd]*	2.2 (6′a), 6.1 (6′b), ∼9 (4)	
6′(a)	63.16	3.877	*dd*	12.1 (6′b), 2.2 (5′)	
6′b		3.666[f]	*dd*	12.1 (6′a), 6.1 (5′)	

[a] Observed at 600/150 MHz in CD_3OD; δ in ppm, J in Hz.
[b] The ^{13}C assignments are verified through DEPT-45/90/135 and ^{13}C,^{1}H-HETCOR (145 Hz) measurements.
[c] The glucose protons H-2 through H-5 form a higher order spin system (*) that could not be fully analyzed by spectral simulation due to solvent signal overlap; ^{1}H signal multiplicities (Mult.) are given under nuclei first-order assumptions in squared brackets in these cases; n.d. = not determined due to severe signal overlap. The aglycone spin system was solved by iteration with the PERCH software package (see text for details); thus, J values are given with signs and at increased precision.
[d] The ^{1}H,^{13}C long-range correlations were observed in a 8.5 Hz long-range ^{13}C,^{1}H-HETCOR experiment.
[e] Assignments of these two almost coinciding resonances was possible due to the high F1 resolution of the ^{13}C,^{1}H-HETCOR map.
[f] This signal shows a slight higher order effect in the lower field doublet half due to the close chemical shifts of H-4′ and H-5′.

1993). The peak at 118.00 ppm can be assigned to a nitrile carbon. In addition to these seven carbons, there are peaks at 35.95 (a methine or methylene carbon, C-7), 65.22 and 72.57 (carbons bearing oxygen, C-6, 8), and 96.96, 127.69, 140.47, and 157.02 ppm (aromatic or vinyl carbons, C-2, 4, 5, 3). A DEPT spectrum reveals that the peaks at 157.02 (C-3) and 118.00 (C-1) ppm lack protons, those at 35.95 (C-7) and 63.16 (C-6′) ppm are methylene groups, and all other carbons bear one hydrogen.

The ^{1}H NMR spectrum confirms the presence of a glucosyl group in the compound by the presence of a doublet at 4.543 ppm ($J = 7.4$ Hz, H-1′), a series of double doublet-like signals centered at 3.314, 3.388, 3.282, 3.666, 3.877 and a *ddd* centered at 3.314 ppm (Table 2) (H-5′, 3′, 4′, 6′b, 6′a, 2′). The glucose double doublets between 3.31 and 3.35 ppm largely overlap. The patterns of all other protons in the molecule are more complex. Two vinyl *ddd* and *ddddd* patterns appear at 6.28 (H-4) and 6.201 (H-5) ppm, respectively; an additional vinyl *dddd* is found at 5.49 (H-2) ppm. Two protons are on carbons bearing oxygen: a *dddd* at 4.91 and a *dddddd* at 4.34 (H-8, 6) ppm; one of these must be the site of attachment of the glucosyl group. Two geminally coupled *dddd*[*d*]/*dddd* patterns appear at 2.23 and at 2.05 (H-7a, 7b) ppm, respectively, corresponding to protons of a methylene group. Because of the long-range couplings and complex interactions, superficial inspection of a COSY spectrum reveals little definitive information about the aglycone portion of the molecule. Interestingly, the *ddddd* at 4.34 (H-6) ppm is coupled to the upfield protons at 2.23 and 2.05 (H-7a, 7b) ppm and all three vinyl protons. The vinyl *dddd* at 5.49 (H-2) ppm is coupled to the other two vinyl protons as well as to the peaks centered at 4.34 and 4.91 (H-6, 8) ppm (Jaki et al., 2003).

A ^{1}J-HETCOR experiment provides the correlations of the aglycone protons to the corresponding carbons (Table 2). Further, a long-range HETCOR experiment ($^{2/3}J$-optimized for 8.5 Hz) indicated that the proton at 5.485 (H-2) ppm interacts with the vinyl carbon at 127.69 (C-4) ppm (suggesting a *cis*-relationship of the two atoms) and with the nitrile carbon at 118.00 ppm suggesting that this proton and the nitrile group are attached to the same carbon atom. This proton (5.485 δ, H-2) also interacts weakly with the oxygen-bearing carbon at 72.57 (C-8) and the vinyl carbon at 157.02 (C-3) ppm. An interaction of this proton with the anomeric proton of the glucose moiety at 101.61 (C-1′) ppm suggests that the site of attachment of the sugar is to the carbon at 72.57 (C-8) ppm.

In selective TOCSY experiments (t_{mix} 50 and 140 ms), irradiation at 4.34 δ (H-6) leads to peaks at 6.20 and 6.28 (apparent doublets), 5.49 (apparent *s*), 4.913 (apparent *d*), and 2.23 and 2.05 ppm (each a complex pattern) (H-5, 4, 2, 8, 7a, 7b).

Finally, a NOESY spectrum indicates that the proton at 4.34 (H-6) ppm has an interaction with the vinyl pro-

ton at 6.20 (H-5) ppm, and that the proton at 4.91 (H-8) ppm has an interaction with the anomeric proton at 4.54 (H-1′) ppm. This latter proton also interacts with the upfield protons at 2.23 and 2.05 (H-7a, 7b) ppm.

Taken collectively, these data support a planar conjugated system with two double bonds, a nitrile group, and an oxygen-bearing carbon at 4.34 (H-6) ppm. Further, the proton at 5.49 (H-2) is in a *cis*-relationship to one of the vinyl carbons. The molecular formula, based on the ^{13}C-spectrum and mass spectral data, requires six sites of unsaturation, and, hence, a ring in both the sugar and aglycone portion of the molecule. Interactions between the proton at 5.49 (H-2) and the vinyl carbon at 157.02 (C-3), the carbon of the proton resonating at 4.91 (H-8), the nitrile carbon and the sugar anomeric carbon, suggest that these groups are proximal in the structure. Because the methylene group protons at 2.23 and 2.05 (H-7a, 7b) are coupled both to the proton at 4.34 (H-6) and that at 4.91 (H-8), a 6-membered ring is required.

Although the structure of menisdaurin (**2**) has been reported previously (Sosa et al., 1977; Takahashi et al., 1978; Nahrstedt and Wray, 1990) and the presence of long-range couplings noted, the nature and extent of coupling was not investigated, nor was a complete set of coupling constants, chemical shifts, and NOE interactions reported. This substance also was considered not to be cyanogenic (Nahrstedt and Wray, 1990), but was noted to liberate small amounts of cyanide under conditions of the "sandwich method" of TLC analysis in the present study and under certain other conditions (Lechtenberg and Nahrstedt, 1999). The mechanism by which cyanide is released remains unknown.

2.6. Long-range coupling in the NMR spectrum of menisdaurin (*2*)

In summary, the identification of menisdaurin (**2**) is based on extensive NMR spectroscopic studies including ^{1}H, ^{13}C, COSY, ^{1}J- and $^{2/3}J$-^{13}C, ^{1}H-HETCOR, and NOESY experiments (Table 2) leading to the unambiguous structure assignment of (*Z*)-1-cyanomethylene-4(2*R*)-hydroxy-6(*S*)-(β-glucopyranosyloxy)-2-cyclohexene (**2**). The relative stereochemistry was based on the observation of NOE interactions between H-6 (cyclohexene 4*R*) and H-8 (cyclohexene 6*S*), and H-2 and H-4 (*Z*) in agreement with Nahrstedt and Wray (1990). However, in the course of analyzing the ^{1}H,^{1}H-coupling pattern of **2** with regard to relative stereochemistry, it became apparent that the underlying spin system is much more complex than expected and recently reported (Nahrstedt and Wray, 1990). Because of its conjugated double/triple bond π electron system, the molecule exhibits a noteworthy long-range coupling pattern, which has not been carefully examined so far. Interestingly, the 7 non-exchangeable protons of the aglycone moiety. give rise to a spectrum that can be interpreted under nuclear first-order conditions. Nevertheless, all signals are very complicated manifold doublets, because a total of 15 coupling constants are involved, 8 of which are long-range (Table 2, see also Fig. 2). In addition to 5 W-type ^{4}J-relationships, there is one allylic ^{5}J-coupling between H-2 and H-5 (0.80 Hz), a homoallylic ^{6}J-coupling between H-2 and H-6 (0.76 Hz), and a $^{5/7}J$-coupling between H-2 and H-7a (0.05 Hz). The last of these can either be an allylic W-type coupling and, therefore, a ^{7}J-interaction, or understood as an allylic W-type ^{5}J-coupling (Barfield and Chakrabarti, 1969). These long-range couplings can also be observed in a semi-quantitative fashion in a normal COSY-90 experiment. Finally, a full analysis of the aglycone spin system was achieved with the PERCH iteration tool (Perch Solutions, ver. 2003) after performing an optimized FID weighting with the NUTS software package. We note that the PERCH predictor allowed reasonable prediction of chemical shift start parameters, and that a perfect match between simulated and experimental spectra was achieved only after considering a $^{5/7}J$-coupling between H-2 and H-7a in the iteration base parameters. It should also be noted that these coupling patterns can even be observed at relatively low magnetic field strength such as that of a

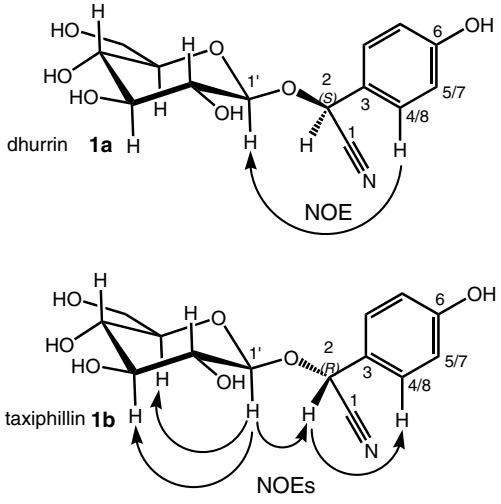

Fig. 1. Although both epimers (2*S*)-dhurrin (**1a**) and (2*R*)-taxiphyllin (**1b**) can readily form rotamers, the chiral induction of the glucose residues leads to three important differences in the NMR data sets of **1a**/**1b** that permit distinction of epimeric pairs (relative stereochemistry) such as **1a**/**1b** based on the signals patterns observed in routine 1D ^{1}H NMR: (a) anisochronism of *all* ^{1}H-chemical shifts combined with (b) marked differences in glycosidic signal shape and multiplicity, due to higher order effects in **1b**; and (c) spatial proximity of the anomeric proton and the aromatic protons H-4/ 8. Accordingly, there is only one exception in the otherwise identical nOe pattern for the epimers (**1a**) and (**1b**) as observed in 1D selective DPFG nOe, i.e., a correlation between the aromatic and the sugar core (600 MHz; see text for details and references).

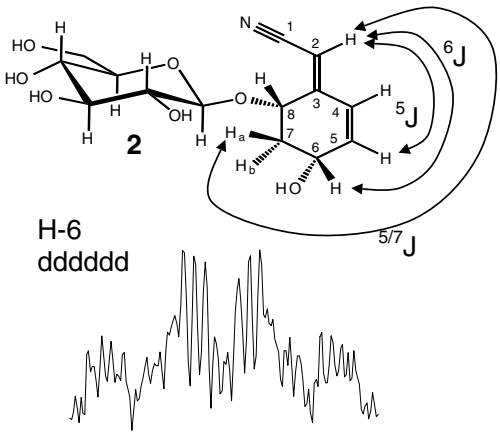

Fig. 2. Due to its extended conjugated π electron system and almost planar 3D arrangement, menisdaurin exhibits multiple long-range coupling beyond the W-type ^4J couplings that are typical for 6-membered ring systems. The particularly interesting homoallylic and farther relationships ($^{>4}J_{H,\,H}$), which give rise to fine splitting in 4 of the 7 aglycone signals, are shown as double arrows. Of particular interest is the *dddddd* signal of H-6, which based on the interpretation presented alone can serve as a fingerprint for the unambiguous identification of 2. Eight fine splitting signals due to long-range coupling were observed at only 360 MHz, underlining the importance of Lorentzian–Gaussian FID weighting for resolution enhancement.

360 MHz instrument. In order to resolve these parameters, the only requirements were careful shimming and offline data processing using Lorentzian–Gaussian resolution enhancement.

2.7. X-ray crystallography

The first X-ray structure determination of a non-derivatized cyanogenic glycoside is dominated by strong intermolecular hydrogen bonds. All five hydroxyl groups are involved, the H···O bond lengths are between 1.82 and 2.04 Å, the related O···O distances are in the range of 2.657(2) and 2.781(2) Å, the bond angles O-H···O vary between 146.1° and 176.2°. The cyano group also is involved, the hydrogen bond is weaker, indicated by a H···N distance of 2.13 Å and a O···N separation of 2.959(3) Å. Due to the absence of an anomalous scatterer, the absolute configuration can not be determined unambiguously [Flack parameter 0.4(9)], but the value of the inverted structure [1.3(9)] makes the shown enantiomer more probable (Fig. 3).

2.8. Relationship of nitrile glucosides to cyanogenic glucosides

To date, the biosynthesis of nitrile glucosides has not been explored. A series of compounds of this structural type have been reported from various dicotyledonous plants. In addition, acyclic nitrile glucosides have been reported from several families; the Rosaceae is especially important among these (Lechtenberg and Nahrstedt, 1999). Known cyclohexenylcyanomethylene structures include simmondsin (**3**) from jojoba [*Simmondsia chinensis* (Link) C. Schneider, Simmondsiaceae] (Elliger et al., 1973, 1974), bauhinin (**4**) [*Bauhinia championii* Bentham (Fabaceae)] (Chen et al., 1985) and lithospermoside (**5**) [*Griffonia simplicifolia* Baill., Fabaceae] (Dwuma-Badu et al., 1976), [*Thalictrum rugosum* Aiton, *Thalictrum revolutum* DC. (Ranunculaceae), *L. purpureocaeruleum*, and *L. officinale* (Boraginaceae)] (Sosa et al., 1977; Wu et al., 1979). More recently, additional series of nitrile glucosides from *Lophira alata* Banks (Ochnaceae) (Murakami et al., 1993; Tih et al., 1994) and *E. philippinensis* A. DC. (Boraginaceae) (Simpol et al., 1994) have been isolated. An unnamed glucoside (**6**) from *Ilex*

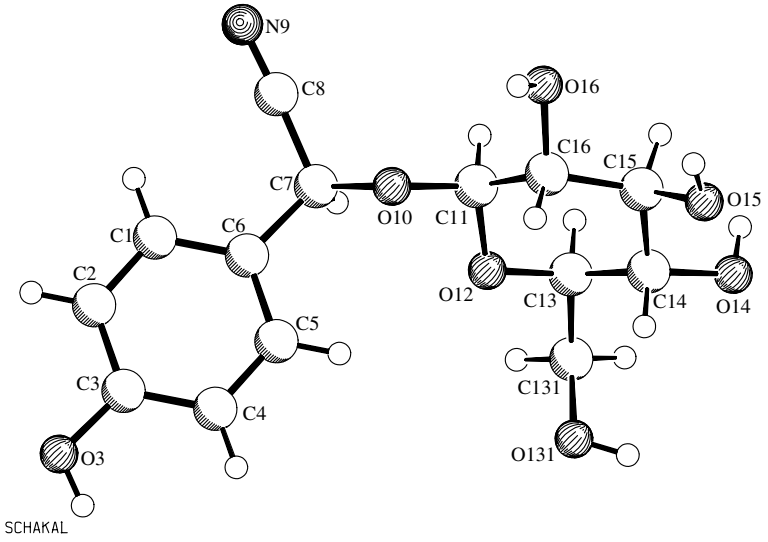

Fig. 3. ORTEP plot of the X-ray crystal structure of (2*S*)-dhurrin (**1a**) from *Tiquilia plicata*.

warburgii Loes (Ueda et al., 1983) and 2,4-dihydroxyphenylacetonitrile (**7**) from *Erica scoparia* L. (Ballester et al., 1975) also have been described (structures of all compounds are given in Lechtenberg and Nahrstedt, 1999). Menisdaurin (**2**) has previously been reported from *Menispermum dauricum* DC. (Menispermaceae) (Takahashi et al., 1978), *Ilex aquifolium* L. (Willems, 1988; Nahrstedt and Wray, 1990) and *I. warburgii* (Ueda et al., 1983) (Aquifoliaceae). Dhurrin (**1a**) and menisdaurin (**2**) co-occur in *T. canescens* and *T. plicata* (Boraginaceae); previous studies (Mao and Anderson, 1965), indicate that these compounds degrade rather than epimerize, especially under alkaline conditions. Further, taxiphyllin (**1b**) and triglochinin (**8**) are found in *Thalictrum aquilegifolium* L. (Ranunculaceae) (Sharples et al., 1972), and as noted above structurally similar nitrile glucosides are found in other *Thalictrum* species. Taxiphyllin (**1b**), dhurrin (**1a**), and triglochinin (**8**) all derive from tyrosine (Hegnauer, 1973a,b). It seems probable that nitrile glucosides with cyclohexenylcyanomethylene structures also derive from tyrosine, although this remains to be established (Simpol et al., 1994; Lechtenberg and Nahrstedt, 1999; Møller and Seigler, 1999). We propose the following biogenetic scheme for the origin of these compounds (Fig. 4) including dasycarponin (**9**), lophiroside B2 (**10**) and ehretioside (**11**).

2.9. Chemotaxonomy

Taxiphyllin (**1a**), dhurrin (**1b**), triglochinin (**8**), and similar cyanogens found in *Goodia* (Fabaceae), *Nandina* (Berberidaceae), *Macadamia* (Proteaceae), and *Sorghum* (Poaceae), all appear to arise from tyrosine. The fact that relatively pure taxiphyllin (**1b**) and dhurrin (**1a**) were isolated in the preceding steps (for *G. ulmifolia* and for *T. plicata*) suggests that mixtures of the epimeric pairs occur in the plants themselves, as was previously observed for prunasin and sambunigrin (Seigler et al., 2002).

The distribution of dhurrin (**1a**) and taxiphyllin (**1b**) and its chemotaxonomic importance has previously been reviewed (Hegnauer, 1973b, 1977; Seigler, 1977; Lechtenberg and Nahrstedt, 1999). However, recent advances in our understanding of phylogenetic relationships of plants and new reports of the distribution of these compounds require some re-evaluation of the data.

(2*R*)-Taxiphyllin (**1b**) occurs in the gymnospermous genera *Taxus*, *Metasequoia*, and *Juniperus*. This compound also has been reported from members of the Magnoliid Complex (Judd et al., 2002) [Magnoliales: *Liriodendron*, *Magnolia* (Magnoliaceae) (Fikenscher and Hegnauer, 1981; van Valen, 1978c), Laurales: *Calycanthus*, *Chimonanthus* (Calycanthaceae) (van Valen, 1978c)], monocots [Alismatales: *Lilaea, Triglochin* (Juncaginaceae) (Hegnauer and Ruijgrok, 1971; Nahrstedt et al., 1979, 1984); Commelinoid clade, Commelinales: *Tinantia* (Commelinaceae) (Tjon Sie Fat, 1978a); Poales: several grasses (Poaceae) (Tjon Sie Fat, 1978c,d; Tjon Sie Fat and van Valen, 1978); *Juncus* (Juncaceae) (Hegnauer and Ruijgrok, 1971)] and Eudicots. Among the Eudicots, taxiphyllin (**1b**) has been reported from the Basal Tricolpates [*Dicentra* (Papaveraceae including Fumariaceae) (van Valen, 1978e), *Nandina* (Berberidaceae)], the Core Eudicots [Caryophyllid clade: *Girgensohnia* (Amaranthaceae, including Chenopodiaceae) (Nahrstedt et al., 1993); the Rosid clade, Eurosids I, Malpighiales: *Phyllanthus* (Phyllanthaceae); Rosales: *Cercocarpus* (Nahrstedt and Limmer, 1982) and *Chamaebatia* (Nahrstedt et al., 1987) (Rosaceae); Fagales: *Ostrya* (Betulaceae) (this study); and Eurosids II, Malvales: *Guazuma* (Malvaceae including Sterculiaceae) (the present study).

The enantiomeric compound (2*S*)-dhurrin (**1a**) has been reported from monocots [Commelinoid clade, Poales: several species of grasses, notably *Sorghum* (Poaceae) (Tjon Sie Fat and van Valen, 1978), the basal Tricolpates [Ranunculales: *Ranunculus* (Ranunculaceae), *Papaver* (Papaveraceae) (van Valen, 1978e); Proteales: *Platanus* (Platanaceae) (Fikenscher and Ruijgrok, 1977; van Valen, 1978a), *Macadamia*, *Stenocarpus* (Proteaceae) (Seigler, 1977); *Trochodendron* (Trochodendraceae) (van Valen, 1978b)]; the core Eudicots [Caryophyllid clade: *Suckleya* (Amaranthaceae, including Chenopodiaceae) (Nahrstedt et al., 1993); the Rosid clade, Eurosids I, Malpighiales: *Bridelia* (Phyllanthaceae) (van Valen, 1978d); Fagales: *Ostrya* (Betulaceae) (this study); Eurosids II, Malvales: *Guazuma* (Malvaceae including Sterculiaceae) (the present study); and the Asterid clade, Solanales: *Borago*, *Tiquilia* (Boraginaceae) (van Valen, 1978a, this study)].

Another tyrosine-derived cyanogenic compound, triglochinin (**8**), is found in plants of the Magnoliid complex (Magnoliales: *Liriodendron*, *Magnolia* (Magnoliaceae) (Fikenscher and Hegnauer, 1981; van Valen, 1978c), Monocots [Alismatales: *Alocasia*, *Anthurium*, *Arum*, *Dieffenbachia*, *Lasia*, *Pinellia* (Araceae) (Nahrstedt, 1975); *Lilaea*, *Triglochin* (Juncaginaceae) (Nahrstedt et al., 1984); Poales: several grasses (Poaceae) (Tjon Sie Fat, 1978c,d; Tjon Sie Fat and van Valen, 1978)] and Eudicots (Basal Tricolpates, Ranunculales: *Aquilegia*, *Leptopyrum*, *Ranunculus*, *Thalictrum* (Ranunculaceae), *Eschscholtzia*, *Papaver* (Papaveraceae) (Tjon Sie Fat, 1979; van Valen, 1978e) and *Platanus* (Platanaceae) (Fikenscher and Ruijgrok, 1977); the core Eudicots [Rosid clade, Eurosids I, Malpighiales: *Andrachne*, *Bridelia*, *Poranthera*, *Securinega* (Phyllanthaceae); (van Valen, 1978d); Asterid clade, Euasterids II, Asterales: *Campanula* (Campanulaceae) (Tjon Sie Fat, 1978b).

Although these three compounds primarily are found in the basal groups of the Magnoliid Complex, they also appear in some highly derived taxa. Further, all three glycosides appear in several groups of closely related

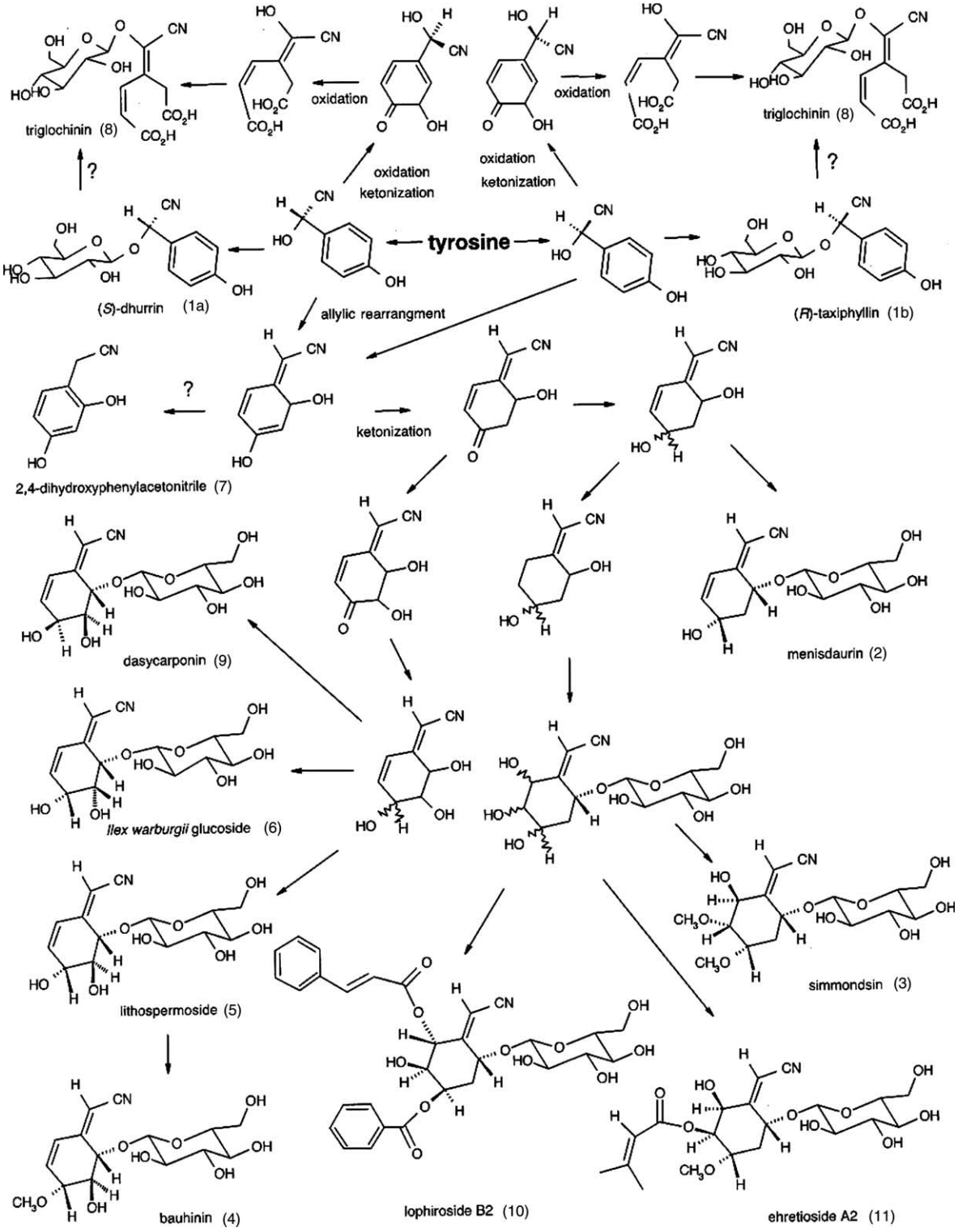

Fig. 4. Proposed biosynthesis of nitrile glucosides.

plants within certain families (e.g., Papaveraceae, Phyllanthaceae, Poaceae). The presence of nitrile glucosides and these three glucosides in some of the same families (Boraginaceae, Poaceae, Ranunculaceae), genera (e.g., *Thalictrum*); and, in particular, in the same species of *Tiquilia*, suggests a close biosynthetic relationship of these compounds (Lechtenberg and Nahrstedt, 1999; this study).

3. Experimental

3.1. Instrumentation

For NMR spectroscopy, samples were analyzed in methanol-d_4 with an isotopic purity of 99.8% D (Aldrich, Milwaukee, no. 15194).

The ^{1}H NMR spectra were recorded on Bruker AM360 (5 mm dual probe) and Varian Unity 600 (5 mm multinuclear probe) instruments operating at 360/600 MHz for ^{1}H, and 90/150 MHz for ^{13}C, respectively. Chemical shifts are reported in ppm on the scale with the solvent as internal standard (3.300 and 49.00 ppm, resp.), the coupling constants (J) are given in Hz; s = singlet, d = doublet, dd = double doublet etc., t = triplet. The spectra were obtained under the following conditions: ^{1}H 30° pulse, delay after acquisition (D1) 1 s, processed with 0.2 Hz line broadening (LB) or with Lorentz–Gauss resolution enhancement after one level of zero-filling. Acquisition used 16k (SI) (32k 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). In small samples, water peak suppression was achieved by presaturation (presat.au) with the HDO signal on resonance (O1). ^{1}H 2D COSY, D 1 s, 1k × 512 or 256 increments, 90° shifted sinebell-squared apodization, zero-filled in t1 dimension during processing. The 1D Selective TOCSY experiments were performed at 600 MHz using the eburp1/25 selective pulse shape program (pws = 200, trimpwr = 54) and acquiring 16K data points (sw = 3900 Hz, aq = 1.9 s).

For off-line NMR spectral processing, the NUTS software package from AcornNMR, CA, was used. ^{1}H NMR spectral iteration, simulation and prediction was performed with PERCH 2003 from PERCH Solutions, Kuopio (Finland) on PC. A complete simulation of the aglycone spin system was achieved in D-mode using J value starting values obtained from energy-minimized molecular models (MMS), which subsequently were refined by experimental observations, iteration, and by taking into account the fitted lineshape of the resolution enhanced spectrum (JCAMP-DX exchange format).

DCI mass spectra were run on a Finnigan INCOS 50 System with ammonia as reactant gas. The emitter heating rate was 10 mA s^{-1}, calibration was done with FC43.

3.2. Plant materials

Leaf material of *G. ulmifolia* was collected at La Pacifica, near Cañas, Guanacaste Prov., Costa Rica, 30 July 1986. A voucher collection (DS-12728) is deposited at the University of Illinois Herbarium (ILL). The cyanogenic individual from which leaf material was taken was marked (tree M) and sampled in 1985, 1986, and 1988. This tree was part of a plot previously studied by one of us (KEG) in earlier feeding studies of howling monkeys (Glander, 1975). Only this tree and one other (tree H) proved to be cyanogenic. When retested in 1988, neither tree was still cyanogenic. Catkins of *Ostrya canadensis* were collected near Charleston, Coles Co., Illinois, 6 July 1991 [*J.E. Ebinger 25295*]. A voucher specimen for each site is deposited at the Stover-Ebinger Herbarium of Eastern Illinois University (EIU). Leaf material of *T. plicata* was collected at Painted Rock Camp Ground, near Gila Bend, Maricopa Co., Arizona, 26 May 1986 *[J.E. Ebinger 23457]*. A voucher specimen is deposited at the Stover-Ebinger Herbarium Eastern Illinois University Herbarium (EIU). Leaf material of *T. canescens* was collected 10 miles se of Laredo, Webb Co., Texas, 30 June 1986 [*D.S. Seigler and B. R. Maslin, DS 12671*]; Brammer Engineering Road, s. of Tilden, McMullen Co., Texas, on Hwy. 16, 11 May 1991 [*D.S. Seigler, H.D. Clarke, K. Readel, and J.E. Ebinger, DS 13268*]; and 2.1 miles n of China, Nuevo León, Mexico, on Hwy. 40, 5 June 1991 [*D.S. Seigler, H.D. Clarke, K. Readel*, and *J.E. Ebinger, DS 13753*]. A voucher specimen for each collection is deposited at the University of Illinois Herbarium (ILL).

3.3. 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 McIlvaine citrate–phosphate buffer, pH 6.0). A small amount of snail gut enzyme (Sigma, St. Louis, MO) or Röhm Pr. El No. 1-77 enzyme (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 a previously unused cork or 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 the samples required more than 6 h for development of the color characteristic of a positive test of the Feigl–Anger test, the test was repeated after several drops of a streptomycin sulfate solution (1.0 mM) were added to inhibit bacterial contamination.

3.4. Cyanide tests for specimens of Guazuma ulmifolia

Cyanide tests on more than 100 individuals from La Pacifica, near Cañas, Guanacaste Prov., Costa Rica, July 1986, were uniformly negative except for the individuals labeled Tree M and Tree H. When retested in 1988, neither tree was still cyanogenic. Tests of other individuals from several locations in Mexico and more than 100 individuals at Gómez Farías, Tamaulipas, Mexico, were all negative for cyanide production.

3.5. Cyanide tests for specimens of Ostrya virginiana

The catkins of 56 individuals of *O. virginiana* collected at Lincoln Log Cabin State Park, Coles Co., Illinois, 45 from Walker Ford Road, near Kickapoo Creek, Coles Co., 15 from Rocky Branch Nature Preserve, Clark Co., Illinois, and two collected at Charleston, Coles Co., Illinois were tested for release of cyanide. Of those from Lincoln Log Cabin State Park, 8 were negative, and of those of Walker Ford Road, 15 were negative; all others gave positive tests. The amount of cyanide released, based on the Feigl–Anger test, ranged from weak to strong. However, when catkin material from herbarium materials collected in Illinois (24) and other states (9) was tested for the presence of cyanide-releasing compounds (Feigl–Anger), only 1 of the 33 specimens tested tested positive. All of 30 catkins from one individual collected at Charleston, Coles Co., Illinois, tested positive. The leaves of this plant have never been found positive. The pollen from the individual with cyanogenic catkins was not cyanogenic.

3.6. Cyanide tests for Tiquilia species

Of herbarium materials from the University of Illinois Herbarium (ILL), specimens of *T. canescens* (6 strongly positive, 13 negative), *T. gossypina* (one strongly positive, one negative), *T. nuttallii* (3 strongly positive, 4 negative), *T. plicata* (9 strongly positive), and *T. purpusii* (one weakly positive, one negative) were at least in some instances positive, but two specimens of *T. cuspidata*, one of *Tiquilia flava*, three of *T. greggii*, 12 of *T. hispidissima*, four of *T. mexicana*, two of *Tiquilia procumbens*, and 7 of *T. palmeri* consistently tested negative for the presence of cyanogenic compounds.

3.7. Extraction of plant materials

Dried leaf and stem material of *G. ulmifolia* (87.5 g), dried catkins of *O. virginiana* (20.4 g), dried leaf material of *T. plicata* (100 g), and dried leaf material of *T. canescens* (671 g) were ground and extracted with MeOH–H_2O (4:1). In each case, the suspension was heated until boiling began, this being held for 10 min, after which the residue was rinsed two times with the same solvent system. The combined extract for each species was filtered sequentially through cheesecloth and filter paper (Whatman 4), with the methanol removed under vacuum. The residual aqueous mixture was partitioned between water and chloroform, with both aqueous and chloroform phases tested for the presence of HCN by the Feigl–Anger method. Only the aqueous phases contained cyanogenic compounds. The aqueous portion from leaf and stem material of the four species was concentrated to yield a solid residue [*G. ulmifolia*, *O. virginiana* (20.4 g), *T. plicata* (8.56 g), and *T. canescens* (39.8 g)].

Another sample of dried leaf material (10 g) of *G. ulmifolia* was extracted and chromatographed simultaneously. The sample was ground finely and mixed with microcrystalline cellulose (50 g, Macherey Nagel 300, without binder). For other details of the fractionation, see below.

3.8. Thin layer chromatography

Each of the aqueous extracts prepared as above was examined by thin layer chromatography on Kieselgel 60-F254 plates, 0.2 mm (Merck 642, Darmstadt), using EtOAc:MeOH:H_2O (79:11:10) as eluant. After development, plates were visualized by spraying with anisaldehyde–H_2SO_4 spray reagent (anisaldehyde, 0.5 ml, HOAc, 10 ml, MeOH, 85 ml, and H_2SO_4, 5 ml) (Wagner et al., 1984), followed by heating for 120 °C for 10 min, or by spraying with β-glucosidase solution and detection of cyanide by the "sandwich" method described below.

The major cyanide containing spot from the crude extract of *G. ulmifolia*, *O. virginiana* and *T. plicata* and the minor spot from *T. canescens* (R_f 0.52–0.63) gave a blue-gray colored spot when visualized with anisaldehyde–H_2SO_4 reagent. The second, minor spot of the crude extract of *T. plicata* and the major, although less strongly cyanogenic, spot of *T. canescens* (R_f 0.32–0.36) also produced a blue-gray colored spot when developed with anisaldehyde–H_2SO_4 reagent.

3.9. HCN determination by the "sandwich" method

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, Germany) 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) until the surface of the plates was moistened. Plates to be tested were sprayed with enzyme (either Röhm or snail gut as above) 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 with large clips 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.

3.10. Vacuum liquid chromatography of the aqueous soluble materials

Aliquots of the aqueous soluble materials from each of these species were separated by vacuum liquid chromatography (VLC) (Pelletier et al., 1986; Coll and Bowden, 1986; Pieters and Vlietinck, 1989; Millar, 1998;

Petereit et al., 1998). The columns consisted of silica gel (Kieselgel 60, with gypsum binder, Merck, Art. 7749, PF_{254}) (50 g) unless otherwise indicated. When dry and friable, the sample-silica gel mixture was added to the top of the silica gel in the column, and then the entire column washed with petroleum ether (100 ml) while evacuated. In one instance, with *G. ulmifolia*, cyanogenic compounds were isolated directly from ground dried leaf material by isolation-extraction chromatography on microcrystalline cellulose (see below).

3.11. Medium performance liquid chromatography

Samples were chromatographed on a column (26×460 mm, Büchi) packed with Orpegen, R.P. 18 with a precolumn packed with the same material. Separations were monitored with an LKB Bromma, 2151 Variable Wavelength Monitor at 280 nm. Fractions (10–15 ml) were collected with a Pharmacia LKB fraction collector at pumped at 8 ml/min.

3.12. Fractionation of Guazuma ulmifolia extract

Attempts to work directly with the aqueous portion from partition of an 80% methanol extract with chloroform and water after removal of methanol were not satisfactory with this species; for that reason, a combined isolation-chromatography procedure was used. Leaf material (10 g) was ground finely and mixed with microcrystalline cellulose (50 g, Macherey Nagel 300, without binder). This mixture was placed on a vacuum column packed with microcrystalline cellulose (50 g). The column was run with fractions (50 ml) of hexane, EtOAc, and EtOAc with 1–75% MeOH, and MeOH with 5–80% water, and water. Cyanogenic fractions 15–18 from the isolation-chromatography of *G. ulmifolia* with microcrystalline cellulose were combined, concentrated and purified by vacuum chromatography with EtOAc (50 ml fractions), and a series of fractions with EtOAc containing increasing amounts of a 1:1 mixture of H_2O and MeOH (1–100%). Analysis by TLC indicated that fractions 2–11 all contained cyanogenic compound(s) (R_f 0.60). These fractions were combined and re-chromatographed with EtOAc and increasing amounts of MeOH–H_2O (1:1) (1–40%). Fractions 12–17 (33.8 mg) were strongly cyanogenic. They were combined (33.8 mg) and separated further by MPLC. The column was equilibrated with H_2O and fractions were collected with a gradient from H_2O to MeOH (500 ml). Analysis by TLC indicated that MPLC fractions 21–25 contained a cyanogenic glycoside with R_f 0.69.

3.13. Fractionation of Ostrya virginiana leaf extract

An aliquot of the extract of *O. virginiana* (13.4 g) was dissolved in H_2O (50 ml) and diluted with MeOH (250 ml). The dense cyanide-negative precipitate that resulted was removed by centrifugation and discarded. The supernatant was concentrated to yield a sticky, water-soluble solid material (2.5 g) that was added to a vacuum column and fractionated with EtOAc (5 fractions, 50 ml each), and a series of fractions with EtOAc containing increasing amounts of a 1:1 mixture of H_2O and MeOH (1 to 50%). Analysis by TLC indicated that fractions 6–16 all contained a cyanogenic compound(s) (R_f 0.61). These fractions were combined and re-chromatographed with chloroform and increasing amounts of MeOH (0.5–100%) and MeOH with 20% H_2O. Fractions 14–19 from the vacuum chromatography above contained most of the cyanogenic materials and were further fractionated by MPLC. The column was equilibrated with H_2O and fractions were collected with H_2O (1 L), H_2O–MeOH (95:5) with (1 L), H_2O–MeOH (l:1, 1 L), and MeOH (500 ml). In total, 140 fractions were collected. Cyanide-releasing fractions 22–27 were combined and rerun by MPLC as before. NMR spectroscopic analysis of fraction 23 from this second MPLC revealed the presence of a mixture of dhurrin (**1a**) and taxiphyllin (**1b**) (Table 1) in a (69:31) ratio.

3.14. Fractionation of Tiquilia plicata leaf extract

Methanol was removed from an MeOH–H_2O (4:1) extract of *T. plicata* leaves and the remaining aqueous portion partitioned between chloroform and water. An aliquot of this aqueous fraction (8.56 g) was added to a vacuum column as described above and fractionated with EtOAc (50 ml), and a series of fractions with EtOAc containing increasing amounts of a 1:1 mixture of H_2O and MeOH (1–100%). Analysis by TLC indicated that fractions 10–13 all contained cyanogenic compound(s). Fractions 11–18 contained a more polar compound that only weakly released cyanide (R_f 0.29). Fractions 9–13 were combined and purified by MPLC. The column was equilibrated with water and fractions collected with a gradient from H_2O to MeOH (500 ml). TLC analysis of the fractions from MPLC revealed that fractions 27–32 contained a compound with R_f 0.55. Fractions 29–32 contained a more polar compound with R_f 0.29. An additional MPLC of fractions 27–32 gave fractions 23–29. NMR analysis of fraction CPC2 from this MPLC revealed the presence of a mixture of dhurrin (**1a**) and taxiphyllin (**1b**) (Table 1).

3.15. Fractionation of Tiquilia canescens leaf extract

Methanol was removed from a MeOH–H_2O (4:1) extract of *T. canescens* and the remaining aqueous portion partitioned between chloroform and water. An aliquot of this aqueous fraction (39.8 g) was added to a vacuum column as described above and fractionated with EtOAc (50 ml), and a series of fractions with EtOAc containing

increasing amounts of a 1:1 mixture of H_2O and MeOH (2–100%) was collected. Fractions 11–13 and fractions 19–25 were positive for cyanide when analyzed by the Feigl–Anger method.

Fractions 13–18 from the second vacuum chromatography of *T. canescens* (1.40 g) were further fractionated by MPLC. The column was equilibrated with water and fractions were collected with a gradient from H_2O to MeOH (500 ml). Fractions 25–27 contained a weakly cyanogenic compound (R_f 0.41). Fractions 25–27 from the first MPLC of *T. canescens* (168 mg) were further fractionated by MPLC. The column was equilibrated with water and fractions were collected with a gradient from water to methanol (500 ml). Fractions 33–40 contained a compound that was weakly cyanogenic by the "sandwich method" (R_f 0.26). The spectral data for this compound correspond to those previously published for menisdaurin (**2**) (Table 2).

3.16. X-ray crystallography of dhurrin (*1a*)

A data set was collected from crystals of (2*S*)-dhurrin (**1a**) isolated from *T. plicata*. A Nonius KappaCCD diffractometer, equipped with a rotating anode generator Nonius FR591, was used. Programs used were as follows: data collection COLLECT (Nonius, 1998), data reduction Denzo-SMN (Otwinowski, 1997; Otwinowski and Minor, 1997), absorption correction data SORTAV (Blessing, 1989, 1995, 1997), structure solution SHELXS-97 (Sheldrick, 1990), structure refinement SHELXL-97 (Sheldrick, 1997), graphics SCHAKAL (Keller, 1997).

Details of the X-ray crystal structure analysis: formula $C_{14}H_{17}NO_7$, $M = 311.29$, colorless crystal $0.45 \times 0.25 \times 0.20$ mm, $a = 5.342(1)$, $b = 9.183(1)$, $c = 14.460(1)$ Å, $\beta = 91.04(1)°$, $V = 709.2(2)$ Å^3, $\rho_{calc} = 1.458$ g cm^{-3}, $\mu = 1.18$ cm^{-1}, empirical absorption correction ($0.949 \leqslant T \leqslant 0.977$), $Z = 2$, monoclinic, space group $P2_1$ (No. 4), $\lambda = 0.71073$ Å, $T = 198$ K, ω and ϕ scans, 4802 reflections collected ($\pm h$, $\pm k$, $\pm l$), $[(\sin\theta)/\lambda] = 0.65$ Å^{-1}, 2741 independent ($R_{int} = 0.028$) and 2414 observed reflections [$I \geqslant 2\,\sigma(I)$], 201 refined parameters, $R = 0.035$, $wR^2 = 0.080$, max. residual electron density 0.16 (-0.20) e Å^{-3}, Flack parameter $-0.4(9)$, hydrogens calculated and refined as riding atoms.

Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication CCDC-235811.

3.17. Dhurrin (*1a*) and Taxiphyllin (*1b*)

The NMR spectral data of the two epimers **1a** and **1b** are compiled in Table 1. Due to their diastereomeric relationship, two completely anisochronic sets of proton signals were observed. In particular, the two sets of glucose signals differ significantly from each other, and almost appear as being due to different sugars. However, spin simulation revealed the higher order nature of the proton sugar signals in **1b**, while the signals of **1a** were of first order, which explains the apparent discrepancy. In addition, the aromatic protons form an AA′XX′/ABXY spin system of a *para*-substituted aromatic ring, and thus are analogous to the B-ring protons of a *para*-hydroxylated flavonoid skeleton, the spin system of which has been only recently elucidated (Veit and Pauli, 1999). All sugar spin systems were elucidated by 1D selective TOCSY measurements, and are in full agreement with previous observations made for two analogous cyanogenic glucosides prunasin and sambunigrin (Seigler et al., 2002). Furthermore, the spatial arrangement of the epimers was investigated by means of 1D selective NOE (see Fig. 1). This technique also led to the determination of a notable exception of the structural congruence of the epimeric pair **1a** and **1**b. The fact that only in (2*S*)-dhurrin a spatial proximity exists between the anomeric glucose proton and the aromatic protons facing the cyanogenic site is a result of the different configuration at the cyanohydrin carbon (see also Fig. 1).

3.18. Menisdaurin (*2*)

The NMR spectral data for **2** (76 mg) (R_f 0.29) from *T. canescens* are given in Table 2.

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