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CYANOHYDRIN GLYCOSIDES OF PASSIFLORACEAE*

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Key Word Index—*Adenia* sp.; *Passiflora* sp.; *Smeathmannia* sp.; Passifloraceae; barterin; cyclopentenoid cyanohydrin glycosides; linamarin; lotaustralin; epilotaustralin; alloisoleucine.

Abstract—Barterin, a classical cyclopentenoid cyanohydrin glucoside, was shown to be (1*S*, 4*S*)-1-(β-D-glucopyranosyloxy)-4-hydroxy-2-cyclopentene-1-carbonitrile, being thus identical with tetraphyllin B, contrary to previous statements in the literature. Cyanohydrin glycosides from *Adenia dinklagei*, *A. epigea*, *A. firingalavensis*, *A. frutescens*, *A. hastata*, *A. letouzeyi*, *A. spinosa*, *Passiflora coriacea*, *P. subpeltata*, *P. warmingii* and *Smeathmannia pubescens* were isolated and identified. A summary of the present knowledge of distribution of cyanohydrin glycosides in Passifloraceae shows clear differences between the two chief genera, *Adenia* and *Passiflora*. Thus, the former genus appears to be dominated by β-D-glucopyranosides of 2-cyclopenten-1-one and 4-hydroxy-2-cyclopenten-1-one cyanohydrin; the glycosides generally occur as pairs having enantiomeric aglycones and the cyclopentene ring is usually *trans*-1,4-dioxygenated. By contrast, the pattern of cyanohydrin glycosides of *Passiflora* appears to be highly diversified, comprising valine or isoleucine-derived glycosides as well as cyclopentenoid glycosides, including more elaborate forms than those found in *Adenia*. The origin of epilotaustralin, possibly arising from the (3*R*)-epimer of L-isoleucine, is briefly discussed.

INTRODUCTION

Members of the Passifloraceae [2–4] have long been recognized as cyanogenic [5], but the metabolites responsible for this property were not purified before the late 1960's. Thus in 1969 barterin [6] and deidaclin [7] were isolated in crystalline form from *Barteria fistulosa* [8] and *Deidamia clematoides* (*Efulensia clematoides* [9]), their structures with the exception of the chirality of the cyanohydrin centers being established soon afterwards [10]. The gross structures of tetraphyllin A and B isolated from *Tetrapathaea tetrandra* [11] were reported in 1971 [12]. The full stereochemistry of tetraphyllin B and deidaclin was established only recently by single-crystal X-ray diffractometry [13, 14]; the aglucone chirality of tetraphyllin A is opposite to that of deidaclin [14].

At present, the list of unambiguously identified cyclopentenoid cyanohydrin glycosides from the Passifloraceae and allied families includes deidaclin (**1**) and tetraphyllin A (**2**), volkenin (**3**) [15] and tetraphyllin B (**4**), epivolkenin (**5**) and taraktophyllin (**6**) [16], their 6′-*O*-α-L-rhamnopyranosyl derivatives [17], and gynocardin [18], as well as passicapsin and passibiflorin [1].

In this paper we critically assess present knowledge of the distribution of structural types of cyanogenic compounds in the Passifloraceae, report structures of cyanogens from some new sources, and close the discussion on the structure of barterin.

RESULTS AND DISCUSSION

Barterin was formulated in 1970 as (4*S*)-1-(β-D-glucopyranosyloxy)-4-hydroxy-2-cyclopentene-1-carbonitrile, the configuration of the cyanohydrin centre being left unspecified [10]. Barterin was thus the first monohydroxylated cyclopentenoid cyanohydrin glucoside to be isolated and identified in considerable detail. The elucidation of the relationship between barterin and tetraphyllin B was delayed by the circumstance that the optical rotation originally reported for the latter [12, 13] was low by a factor of two [15, 19]. Thus, the rotation reported for tetraphyllin B not only suggested its non-identity with barterin, but was also discordant with the expected strong change of the rotation relative to **1** and **2** caused by the introduction of the allylic hydroxy group [19]. The physical properties of all four stereoisomeric 4-monohydroxylated cyclopentenoid cyanohydrin β-D-glucopyranosides (**3–6**), including a redetermination of the optical rotation of tetraphyllin B, have been reported only recently [15, 16], affording an opportunity to elucidate the stereochemistry of barterin.

Spencer and Seigler [20] claimed the identity of barterin with volkenin (**3**), a glucoside originally detected in *Adenia volkensii* [21].† The conclusion was based on a ^{1}H NMR spectroscopic study of non-crystalline material isolated from *B. fistulosa* [20]. However, barterin cannot be identical with volkenin, because the latter is dextrorotatory [15, 19], whereas barterin was laevorotatory [6]. Thus, taking the optical rotations into account, barterin ($[\alpha]_D$-78° in ethanol [6]) could only be identical with tetraphyllin B (**4**) or taraktophyllin (**6**), both having $[\alpha]_D$ −75° (in methanol) [15, 16, 19].

We have now recorded ^{1}H and ^{13}C NMR spectra of the four isomers **3–6** in D_2O under standardized conditions (Tables 1 and 2). The ^{1}H NMR spectra of the isomers

*Part 9 in the series on cyclopentenoid cyanohydrin glycosides. For part 8 see ref. [1].

†In refs [20, 21] and in the following articles of this group volkenin is designated as epitetraphyllin B. Recent structural revision [15] rendered the latter name untenable.

Table 1. ^{1}H NMR spectra (250 MHz) of isomeric 1-(β-D-glucopyranosyloxy)-4-hydroxy-2-cyclopentene-1-carbonitriles in D_2O*

Proton	Volkenin (**3**) (1*R*, 4*R*)	Tetraphyllin B (**4**) (1*S*, 4*S*)	Epivolkenin (**5**) (1*S*, 4*R*)	Taraktophyllin (**6**) (1*R*, 4*S*)
H-2, H-3	6.21, 6.38	6.25, 6.44	6.20, 6.40	6.15, 6.35
H-4	5.07	5.08	4.94	4.94
H-5	2.46, 2.79	2.38, 2.89	2.25, 3.15	2.17, 3.18
H-1′	4.81	4.70	4.82	4.86
H-2′	3.28	3.28	3.32	3.31
H-3′	3.53	3.51	3.55	3.54
H-4′	3.39	3.41	3.43	3.42
H-5′	3.51	3.50	3.51	3.51
H-6′	3.71, 3.90	3.74, 3.92	3.75, 3.91	3.73, 3.91

*0.05 M solutions at 27°, with DSS (δ 0.0) as internal standard. The resonances of sugar residues were assigned from COSY spectra. Coupling constants are similar to those reported for the solutions in CD_3OD [16, 19].

Table 2. ^{13}C NMR spectra (125.7 MHz) of isomeric 1-(β-D-glucopyranosyloxy)-4-hydroxy-2-cyclopentene-1-carbonitriles in D_2O*

Carbon	Volkenin (**3**) (1*R*, 4*R*)	Tetraphyllin B (**4**) (1*S*, 4*S*)	Epivokenin (**5**) (1*S*, 4*R*)	Taraktophyllin (**6**) (1*R*, 4*S*)
C-1	82.9	82.0	81.7	82.2
C-2, C-3	132.4, 143.0	131.2, 144.0	130.9, 143.1	131.8, 142.2
C-4	74.9	75.1	74.0	73.8
C-5	47.0	47.1	46.8	46.4
CN	120.1	120.5	120.3	120.0
C-1′	100.5	100.1	99.9	100.3
C-2′	73.6	73.6	73.6	73.6
C-3′, C-5′	76.3, 77.0	76.3, 77.0	76.3, 77.0	76.3, 77.0
C-4′	70.2	70.2	70.2	70.2
C-6′	61.3	61.3	61.3	61.3

*0.12 M solutions at 27°, with McOH (δ 49.3) as internal standard. The resonances of sugar residues were assigned using available data for model compounds. The resonance of C-4 was assigned assuming constancy of the chemical shift of C-2′ throughout the series.

are distinguishable also in this solvent, previous suggestions to the contrary [20, 22, 23] notwithstanding. Through the kindness of Dr Martin G. Ettlinger (University of Copenhagen), we have obtained a copy of a 60 MHz ^{1}H NMR spectrum of barterin in D_2O, recorded by R. Paris and coworkers in 1969 (*cf.* [10]). The spectrum shows resonances at δ 2.35 and 2.85 (H-5), 4.67 (H-1′), 5.05 (H-4), and 6.21 and 6.40 (H-2 and H-3).† The values correspond very well to those of tetraphyllin B (Table 1), with an off-set of 0.03–0.04 ppm. In particular, the possible identity of barterin with taraktophyllin (**6**) is ruled out (Table 1). Barterin is thus identical with tetraphyllin B (**4**).

†The ^{1}H NMR spectrum of a sample of barterin sent by Prof. R. Paris was also measured in 1970 at the U.S. Army Natick Laboratories [M. G. Ettlinger, personal communication]. The ^{1}H NMR data given in the original paper of Paris *et al.* [6] (δ 2.50, 2.75, 6.05 and 6.30), which do not match any of the possible isomers (Table 1), are in error.

The remaining issue is the nature of the material isolated by Spencer and Seigler [20] from *B. fistulosa*, which they assumed to be identical with the barterin obtained by Paris [6]. The former isolate was obtained from a 1.5 g sample of dried aerial parts of the plant [20], whereas barterin was originally isolated in 0.5% yield from root bark [6]. Since the glucosides **3** and **4** do occur together in plants, the predominance of the former in leaves and of the latter in roots of *B. fistulosa* is likely to be the clue.

We have isolated cyanogenic constituents from several Passifloraceae species which have not been studied thus far. The compounds were identified by 500 MHz ^{1}H NMR spectra, in each case recorded before and after acetylation of the purified materials. The results are collected in Table 3, together with data reported prior to this work [1, 6, 10, 12–15, 19–37]. Some of the earlier reports warrant reinvestigation; thus the structures of the cyclopentenoids from *P. coccinea* [34] and *P. trifasciata* [33] have yet to be determined [1, 17, 38], and only gross structures of the glucosides from *P. caerulea* [26] and *P. suberosa* [37] are known.

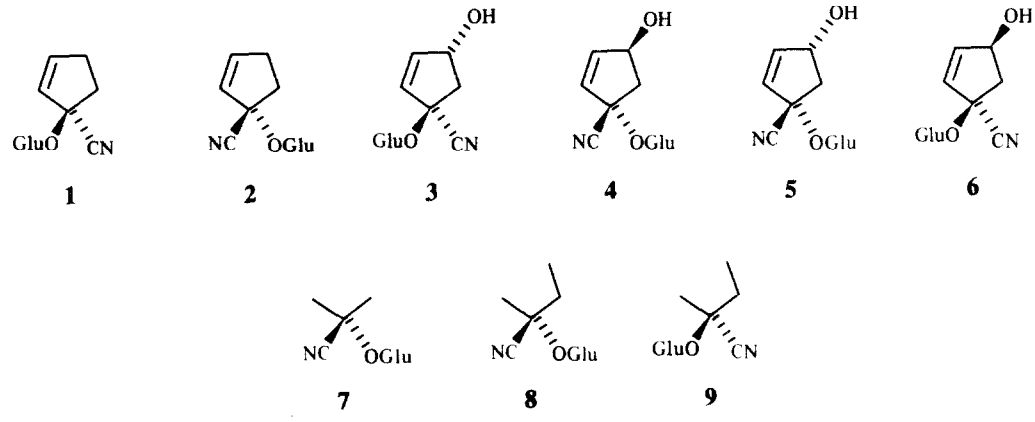
Table 3. Summary of distribution of cyanohydrin glycosides in Passifloraceae.

Species	Compounds	References
Smeathmannia pubescens	volkenin and tetraphyllin B	this work
Barteria fistulosa	volkenin and tetraphyllin B	this work [6, 10, 20]
Passiflora coriacea	deidaclin, tetraphyllin A, tetraphyllin B, epivolkenin, taraktophyllin, possibly volkenin	this work [36]
P. suberosa	cyclopentenoid epoxides, stereochemistry unknown	[37]
P. warmingii	linamarin, lotaustralin, epilotaustralin, possibly linustatin	this work [24, 35]
P. pendens	linamarin, lotaustralin, linustatin, neolinustatin	[35]
P. lutea	linamarin, lotaustralin, and a cyclopentenoid	[32]
P. trifasciata	unknown cyclopentenoid	[33]
P. talamancensis	unknown cyclopentenoid	[33]
P. biflora	passibiflorin	[1, 33]
P. capsularis	epivolkenin, taraktophyllin, passicapsin	[1, 24]
P. adenopoda	linamarin, lotaustralin	[35]
P. perfoliata	unknown cyclopentenoid	[24]
P. coccinea	unknown sulphated cyclopentenoid	[34]
P. incarnata	possibly gynocardin	[31]
P. edulis	possibly prunasin	[29]
P. caerulea	cyclopentenoid sulphate esters, stereochemistry unknown	[26]
P. violacea	linamarin	[24]
P. subpeltata	linamarin	this work [24]
Tetrapathaea tetrandra (*P. tetrandra*)	deidaclin, tetraphyllin A, volkenin, tetraphyllin B	[12, 19, 22]
Efulensia clematoides (*Deidamia clematoides*)	deidaclin, tetraphyllin A	[7, 10, 14]
Adenia fruticosa	deidaclin, tetraphyllin A, epivolkenin and taraktophyllin	this work
A. glauca	volkenin and tetraphyllin B	[27]
A. spinosa	deidaclin, tetraphyllin A, volkenin and tetraphyllin B	this work
A. firingalavensis	volkenin and tetraphyllin B	this work
A. perrieri	volkenin and tetraphyllin B	[30]
A. epigea	volkenin and tetraphyllin B	this work
A. globosa	deidaclin and tetraphyllin A	[14]
A. letouzeyi	volkenin and tetraphyllin B	this work
A. hastata	volkenin and tetraphyllin B	this work
A. volkensii	volkenin and tetraphyllin B	[15, 19, 21]
A. digitata	tetraphyllin B*	[25]
A. cissampeloides	tetraphyllin B*	[28]
A. dinklagei	epivolkenin and taraktophyllin	this work
A. gracilis	volkenin and tetraphyllin B	[36]

*Co-occurrence of volkenin was not excluded.

Although we hardly expect the distribution pattern of cyanohydrin glycosides of Passifloraceae to be taxonomically useful at the subgenus level, the two big genera *Adenia* [4] and *Passiflora* [3] show, taken as a whole, strikingly different patterns of the glycosides. Thus the species of *Adenia*, about 15% of which have now been scrutinized, have a quite uniform pattern of cyclopentenoid glucosides, usually comprising mixtures of pairs of monohydroxylated glucosides with enantiomeric aglucones (usually **3** and **4**, in two cases **5** and **6**), sometimes accompanied (and in *A. globosa* replaced) by the non-hydroxylated counterparts (**1** and **2**). By contrast, the species of *Passiflora* (about 3.5% of which have been investigated) show a highly diversified pattern of glycosides. *Smeathmannia pubescens*, the first representative of this genus to be investigated, fits well into the general pattern of the family; the genus can, like *Barteria*, be considered intermediate between the Passifloraceae and the Flacourtiaceae [39].

Passiflora contains valine and isoleucine-derived cyanogens (**7–9**) as well as cyanogens originating from 2-cyclopentenylglycine or its hydroxylated counterparts. Steric likeness between these two types of amino acids and the hypothesis of biogenetic relationship between enzyme sets that convert them to the respective cyanohydrins have been discussed elsewhere [40]. In *P. warmingii* we detected epilotaustralin (**9**), only recently found in nature [41], along with lotaustralin (**8**). Since there is no immediate, chemically acceptable mechanism for epimerization of **8** to **9**, the question arises as to the biosynthesis of 2-methylbutanenitrile, which must be a precursor [42, 43] of **9**. It would appear either that the



1 2 3 4 5 6 7 8 9

Glu = β-D-glucopyranosyl

nitrile is hydroxylated in the usual way, i.e. with retention of configuration [42, 43], when the nitrile must possess the (*R*)-configuration corresponding to the unnatural amino acid alloisoleucine [44], or that the nitrile is (*S*) and originates from isoleucine, but the hydroxylation is unusual and inverts the configuration at C-2. The problem of epilotaustralin biosynthesis thus deserves further attention.

New data on the distribution of **1**–**6** reinforce previous expectations [14–16, 19, 40] that within this group of plants the pairs of glucosides having enantiomeric aglucones generally co-occur. This, however, appears only to apply to **1**–**6**, which for the present purpose may be designated as a primitive cyclopentenoid group. Insofar as further sophistication of the cyclopentenoid structures consists in direct elaboration of the primitive ones, stereospecificity is likely to be encountered in these late biosynthetic steps, leading to accumulation of single stereoisomeric representatives within the more advanced group. Accordingly, gynocardin [40], passicapsin [1, 24] and passibiflorin [1] have until now only been found as single isomers. Investigation of as yet unidentified, more complex (advanced) cyclopentenoids of *Passiflora* (Table 3) is therefore of interest to test this possible trend. As previously noted [45], the predominance of *trans*-1,4-dioxygenation of the cyclopentene ring in the Passifloraceae at large is still strong. Interestingly, *P. coriacea* contains both structural types of monohydroxylated cyanogens at once.*

EXPERIMENTAL

Plant material. Fresh material of *Adenia epigea* Perr., *A. firingalavensis* var. *firingalavensis* (Drake *ex* Jumelle) Harms, *A. fruticosa* Burtt Davy, *A. hastata* Harv. Schinz, *A. letouzeyi* de Wilde, *A. spinosa* Burtt Davy, *Passiflora coriacea* Juss., and *P. warmingii* Mast. were obtained from the Botanical Garden, University of Copenhagen, Copenhagen. *P. subpeltata* Ortega (*P. alba* Link & Otto) was grown at this laboratory from seeds obtained from the Botanical Garden, Szeged, Hungary. Dried aerial parts of *A. dinklagei* Hutch. & Dalz. and an extract of leaves of *Smeathmannia pubescens* Soland. were obtained from Dr F. C. Fischer (University of Utrecht). Leaves of *A. gummifera* (Harv.) Harms obtained from South Africa and of *A. lanceolata* ssp. *scheffleri* (Engl. & Harms) de Wilde, obtained from The Royal Botanical Gardens, Kew, were not cyanogenic.

General isolation procedure. Fresh plant material was boiled for 5 min with 80% aq. MeOH, homogenized, boiled again, filtered, and the liquid evapd. The residue was adsorbed on silica gel in MeOH, and chromotographed on a column of silica gel (Merck silica gel 60, 0.066–0.2 nm), using EtOAc–Me_2CO–CH_2Cl_2–MeOH–H_2O (20:15:6:5:4). The fractions (25 ml) were monitored by TLC (silica gel) with the same solvent, using naphthoresorcinol [46] or the cyanide-specific picrate sandwich assay [47]. Appropriate fractions were combined, evapd, and purified further by prep. HPLC on Lichrosorb RP-18, 5 μm (1.6 × 25 cm column), using 4 ml/min of H_2O–MeOH (9:1 or 4:1 for fractions containing 4-hydroxylated and non-hydroxylated cyclopentenoids, respectively). The separations were monitored with a differential refractometer and the individual components collected and tested by TLC [47] for cyanogenesis. Under these conditions the pairs of glucosides of enantiomeric cyanohydrins (**1** and **2**, **3** and **4**, **5** and **6**) were eluted as single peaks [14–16]. The individual glucosides were usually not separated but were identified and their ratio measured by means of 500 MHz ^{1}H NMR spectra in CD_3OD. The identification was in each case confirmed by 500 MHz ^{1}H NMR spectra in $CDCl_3$ of acetates obtained by overnight treatment (room temp.) with pyridine–Ac_2O.

A. dinklagei. Dry material (120 g) yielded 118 mg of a mixture of epivolkenin and taraktophyllin (13:10).

A. epigea. Fresh leaves (21 g) collected in October yielded 35 mg of a mixture of volkenin and tetraphyllin B (2:9).

A. firingalavensis. Fresh leaves (13.5 g) collected in October gave about 0.2 g of a mixture of volkenin and tetraphyllin B (1:40).

A. fruticosa. Fresh leaves (28 g) collected in October yielded *ca* 0.4 g of a deidaclin and tetraphyllin A mixture (1:16), and about 0.2 g of a mixture of epivolkenin and taraktophyllin (9:1).

A. hastata. Leaves were collected in July and freeze-dried; 10.3 g of dry material yielded 19 mg of a mixture of volkenin and tetraphyllin B (1:1).

A. letouzeyi. Fresh leaves (77 g) collected in October yielded *ca* 0.5 g of a mixture of volkenin and tetraphyllin B (1:25).

A. spinosa. Leaves were collected in July and freeze-dried; 7.8 g of dry material yielded *ca* 2 mg of a mixture of deidaclin and tetraphyllin A (2:1) and 42 mg of a mixture of volkenin and tetraphyllin B (1:1).

*We note that the structural and spectral assignments reported for *P. coriacea* glucosides in another recent study [36] are regrettably all incorrect.

P. coriacea. Fresh leaves (388 g) harvested in September yielded 3 mg of a mixture of deidaclin and tetraphyllin A (1:19), 135 mg of tetraphyllin B, and 93 mg of a mixture of epivolkenin and taraktophyllin (25:1). No volkenin could be detected in the sample of tetraphyllin B by ^{1}H NMR (detection limit about 0.3%). Analysis of leaves collected from the same specimen in June gave similar results.

P. subpeltata. Leaves were collected in January and freeze-dried; 1.6 of dry material yielded 13.5 mg of linamarin, isolated and identified as described for *P. warmingii.*

P. warmingii. Two unripe fruits (13 g) picked in October were extracted and the extract fractionated as described above, using 20% aq. MeOH for HPLC. This yielded 20 mg of linamarin (R_t 13.5 min) and 1 mg of a mixture of lotaustralin and epilotaustralin in a ratio of 10:1 (eluted as a single peak with R_t 21.5 min). There was no evidence for any appreciable amount of linustatin or neolinustatin [35]. Linamarin. ^{1}H NMR (500 MHz, CD_3OD): δ1.67 and 1.70 (Me), 3.19 (H-2′), 4.68 and 4.85 (H-6′, $^2J_{AB} = -12.0$ Hz, $^3J_{AX} = 5.0$ Hz, $^3J_{BX} = 2.5$ Hz), 4.62 (H-1′, $^3J_{1,2} = 7.75$ Hz), 3.30–3.40 (H-3′, H-4′, H-5′), identical with the spectrum of authentic (Carlbiochem) material and in agreement with lit. [48] data. *Linamarin tetraacetate.* ^{1}H NMR (500 MHz, $CDCl_3$): δ1.61 and 1.65 (Me), 2.01, 2.04, 2.07 and 2.075 (AcO), 3.78 (H-5′), 4.14 and 4.25 (H-6′, $^2J_{AB} = -12.2$ Hz, $^3J_{AX} = 2.3$ Hz, $^3J_{BX} = 5.7$ Hz), 4.87 (H-1′, $^3J_{1,2} = 7.9$ Hz), 5.01, 5.05 and 5.26 (H-2′, H-3′, H-4′, $^3J_{2,3} \approx {}^3J_{3,4} \approx {}^3J_{4,5} \approx 9.6$ Hz), identical with the spectrum of authentic linamarin acetate and in agreement with lit. [48] data. Lotaustralin. ^{1}H NMR spectrum (500 MHz, CD_3OD) identical to that previously reported [40]; the presence of epilotaustralin (*ca* 10%) was indicated by the presence of a singlet at δ1.65 (Me). Lotaustralin tetraacetate. ^{1}H NMR spectrum (250 MHz, CCl_4) identical to that previously reported [40]; the Me resonance of epilotaustralin tetraacetate appeared at δ1.60, in agreement with lit. [49]. The epimeric acetates were further identified and their ratio confirmed by GLC on ECNSS-M by Prof. A. Nahrstedt [41].

Smeathmannia pubescens. Leaf extract obtained from Dr F. C. Fischer contained a mixture of volkenin and tetraphyllin B (7:1).

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