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Cyanohydrin glycosides of *Passiflora*: distribution pattern, a saturated cyclopentane derivative from *P. guatemalensis*, and formation of pseudocyanogenic α-hydroxyamides as isolation artefacts☆

Jerzy W. Jaroszewski[a], Elin S. Olafsdottir[b], Petrine Wellendorph[a], Jette Christensen[a,*], Henrik Franzyk[a], Brinda Somanadhan[a], Bogdan A. Budnik[c], Lise Bolt Jørgensen[d], Vicki Clausen[a]

[a]*Department of Medicinal Chemistry, Royal Danish School of Pharmacy, Universitetsparken 2, DK-2100 Copenhagen, Denmark*
[b]*Faculty of Pharmacy, University of Iceland, Hagi, Hofsvallagata 53, IS-107 Reykjavik, Iceland*
[c]*Department of Chemistry, University of Southern Denmark, Campusvej 55, DK-5230 Odense, Denmark*
[d]*Botanical Institute, University of Copenhagen, Gothersgade 140, DK-1123 Copenhagen, Denmark*

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Abstract

Nineteen species of *Passiflora* (Passifloraceae) were examined for the presence of cyanogenic glycosides. Passibiflorin, a bisglycoside containing the 6-deoxy-β-D-gulopyranosyl residue, was isolated from *P. apetala*, *P. biflora*, *P. cuneata*, *P. indecora*, *P. murucuja* and *P. perfoliata*. In some cases this glycoside co-occurs with simple β-D-glucopyranosides: tetraphyllin A, deidaclin, tetraphyllin B, volkenin, epivolkenin and taraktophyllin. *P. citrina* contains passicapsin, a rare glycoside with the 2,6-dideoxy-β-D-*xylo*-hexopyranosyl moiety, while *P. herbertiana* contains tetraphyllin A, deidaclin, epivolkenin and taraktophyllin, *P. discophora* tetraphyllin B and volkenin, and *P.* ×*violacea* tetraphyllin B sulfate. The remaining species were noncyanogenic. The glycosides were identified by ^{1}H and ^{13}C NMR spectroscopy following isolation by reversed-phase preparative HPLC. From *P. guatemalensis*, a new glucoside named passiguatemalin was isolated and identified as a 1-(β-D-glucopyranosyloxy)-2,3-dihydroxycyclopentane-1-carbonitrile. An isomeric glycoside was prepared by catalytic hydrogenation of gynocardin. α-Hydroxyamides corresponding to the cyanogenic glycosides were isolated from several *Passiflora* species. These α-hydroxyamides, presumably formed during processing of the plant material, behave as cyanogenic compounds when treated with commercial *Helix pomatia* crude enzyme preparation. Thus, the enzyme preparation appears to contain an amide dehydratase, which converts α-hydroxyamides to cyanohydrins that liberate cyanide; this finding is of interest in connection with analysis of plant tissues and extracts using *Helix pomatia* enzymes.

Keywords: Cyanogenesis; Chemotaxonomy; Passiguatemalin; Dihydrogynocardin; α-Hydroxyamides; Passifloraceae; *Passiflora*; *Helix pomatia*

1. Introduction

The most profound chemical characteristic of the Passifloraceae is cyanogenesis resulting from the presence of cyanohydrin glycosides with cyclopentanoid aglycones (Olafsdottir et al., 1989a; Hegnauer, 1990; Clausen et al., 2001). Passifloraceae shares this characteristic with a narrowly defined cluster of higher plants also including Turneraceae (Olafsdottir et al., 1990; Wellendorph et al., 2001), Malesherbiaceae (Spencer and Seigler, 1985a), Achariaceae (Jensen and Nielsen, 1986), and the cyanogenic tribes of Flacourtiaceae (Spencer and Seigler, 1985c), which were recently separated as a new family Kiggelariaceae (Bernhard and Endress, 1999). Distribution of the cyclopentanoid cyanogenic glycosides within this group of taxa has a considerable chemotaxonomic interest.

For the purpose of the present discussion, cyanogenic glycosides found in the Passifloraceae can be divided

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* Corresponding author. Tel.: +45-3530-6499; fax: +45-3530-6040.
E-mail address: jch@dfh.dk (J. Christensen).

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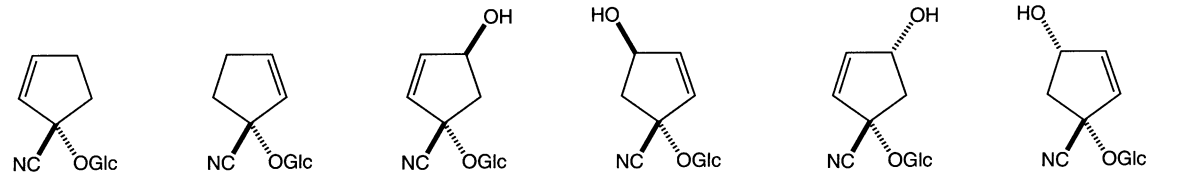


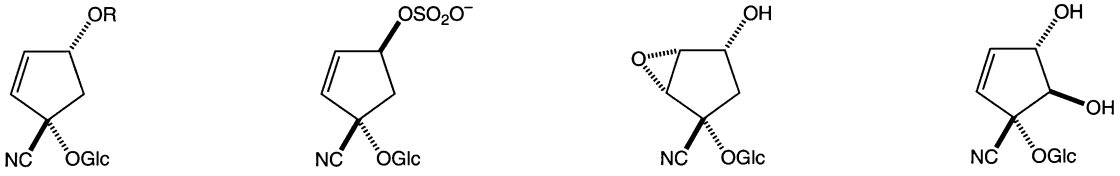
1 tetraphyllin A (*S*) **2** deidaclin (*R*) **3** tetraphyllin B (1*S*,4*S*) **4** volkenin (1*R*,4*R*) **5** epivolkenin (1*S*,4*R*) **6** taraktophyllin (1*R*,4*S*)

Fig. 1. Type I cyanogenic glycosides which occur in Passifloraceae as stereoisomeric pairs **1** and **2**, **3** and **4**, and **5** and **6**; Glc = β-D-glucopyranosyl.

into four distinct structural types. Type I (Fig. 1) includes β-D-glucopyranosides of the enantiomeric cyanohydrins of 2-cyclopentenone, tetraphyllin A (**1**) and deidaclin (**2**) (Jaroszewski and Jensen, 1985), together with their allylically hydroxylated derivatives, tetraphyllin B (**3**), volkenin (**4**), epivolkenin (**5**) and taraktophyllin (**6**) (Jaroszewski and Olafsdottir, 1986; Jaroszewski et al., 1987a,b). The characteristic structural property of these glycosides is that they usually (if not always) occur as pairs of β-D-glucopyranosides having enantiomeric aglycones, i.e. **1** co-occurs with **2**, **3** with **4**, and **5** with **6**. This indicates a certain lack of substrate specificity of the enzymes involved in their biosynthesis (Olafsdottir et al., 1992; Jaroszewski et al., 1996).

Type II includes compounds which may be regarded as a further structural elaboration of those belonging to Type I. Thus, these glycosides contain an additional, rare sugar residue, a sulfate group, or additional oxygenation of the cyclopentene ring (Fig. 2). Passibiflorin (**7**), passicapsin (**8**) (Olafsdottir et al., 1989b), passitrifasciatin (**9**) (Olafsdottir et al., 1991a), tetraphyllin B 4-*O*-sulfate (**10**) (Jaroszewski and Fog, 1989), the epoxide suberin A (**11**) (Olafsdottir et al., 1991b), and the classical cyclopentene gynocardin (**12**) reported from *P. incarnata* (Spencer and Seigler, 1984) belong to this group. None of the glycosides **7**–**12** has ever been found together with any stereoisomer, in contrast to the glycosides belonging to Type I where such co-occurrence appears to be a rule. All the glycosides that form Type II group have *identical stereochemistry* at C-1 (Fig. 2). The glycosides belonging to Type I and II are believed to be biosynthesized from 2-cyclopentenylglycine, although the possibility that they originate from oxygenated amino acids cannot be excluded (Tober and Conn, 1985; Olafsdottir et al., 1992).

Type III consists of linamarin (**13**), lotaustralin (**15**), the corresponding gentiobiosides **14** and **16**, and epilotaustralin (**17**) (Fig. 3) (Fischer et al., 1982; Spencer and Seigler, 1985b; Spencer et al., 1986). Although these glycosides do not contain a cyclopentene ring, their precursor amino acids (valine and isoleucine) occupy a similar conformational space as 2-cyclopentenylglycine (Jaroszewski et al., 1988). It appears that **13**, **15** and **17** normally co-occur in Passifloraceae and are biosynthesized by the



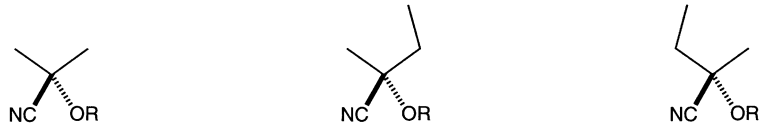
7 R = 6-deoxy-β-D-gulopyranosyl: passibiflorin (1*S*,4*R*)
8 R = 2,6-dideoxy-β-D-*xylo*-hexopyranosyl: passicapsin (1*S*,4*R*)
9 R = 6-deoxy-β-D-allopyranosyl: passitrifasciatin (1*S*,4*R*)

10 tetraphyllin B sulfate (1*S*,4*S*)

11 suberin A (1*R*,2*R*,3*R*,4*R*)

12 gynocardin (1*S*,4*S*,5*R*)

Fig. 2. Type II cyanogenic glycosides which occur in Passifloraceae as single stereoisomers; Glc = β-D-glucopyranosyl.



13 R = Glc: linamarin
14 R = GlcGlc: linustatin

15 R = Glc: lotaustralin (*R*)
16 R = GlcGlc: neolinustatin (*R*)

17 R = Glc: epilotaustralin (*S*)

Fig. 3. Type III cyanogenic glycosides found in Passifloraceae: glycosides derived from valine and isoleucine; Glc = β-D-glucopyranosyl, GlcGlc = 6-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl.

same enzyme complex (Jaroszewski et al., 1988; Olafsdottir et al., 1992), as in cassava (Andersen et al., 2000).

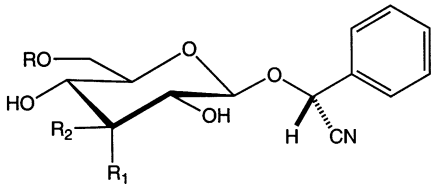
Finally, the last group (Type IV) consists of prunasin (**18**) and its derivatives **19** and **20** (Spencer and Seigler, 1983; Chassagne et al., 1996; Chassagne and Crouzet, 1998), as well as passiedulin (**21**) (Christensen and Jaroszewski, 2001), all isolated from *P. edulis* (Fig. 4). These glycosides must be assumed to originate from phenylalanine, an amino acid that lacks a β-branch in contrast to the precursor amino acids of the remaining cyanogenic glycosides found in Passifloraceae.

In this paper, we describe the cyanogenic constituents from a number of *Passiflora* species, report on a novel cyanogenic glycoside from *P. guatemalensis*, and discuss the distribution of cyanohydrin glycosides within the genus.

2. Results and discussion

2.1. Cyanogenesis of Passiflora guatemalensis

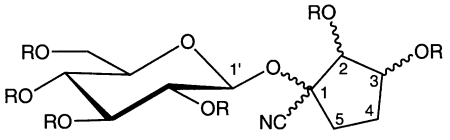
P. guatemalensis, not investigated prior to this work, was found to be only weakly cyanogenic. Purification of the cyanogenic constituent yielded a small amount (7 mg from 112 g of dry plant material, or about 0.006%) of a novel glycoside. ^{13}C NMR spectrum confirmed the presence of a single residue of β-D-glucopyranose. That glucose belongs to the D-series as expected was confirmed using the D-glucose oxidase test after acid-catalyzed hydrolysis of the glycoside. The ^{1}H and ^{13}C NMR spectra showed the presence of a cyclopentane ring without a double bond or an epoxide ring, contrary to all previously described cyclopentanoid cyanogenic glycosides (Figs. 1 and 2). Instead, two oxygenated methine carbons in addition to those belonging to the sugar moiety were present (δ 76.5 and 84.9). NOESY and COSY spectra demonstrated the presence of a –CH(OH)–CH(OH)–CH_2–CH_2– spin system; the two hydroxy groups in the cyclopentane ring are thus vicinal and placed next to the cyanohydrin center. The structure of the new glucoside can thus be described as shown in **22** (Fig. 5), as confirmed by high-resolution MS and by formation of a hexaacetate (**23**).

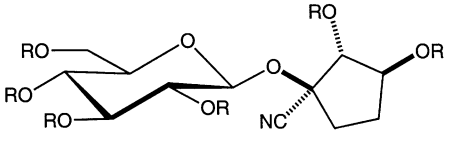


18 R = R_1 = H, R_2 = OH: prunasin (*R*)
19 R = Glc, R_1 = H, R_2 = OH: amygdalin (*R*)
20 R = α-L-rhamnopyranosyl, R_1 = H, R_2 = OH (*R*)
21 R = R_2 = H, R_1 = OH: passiedulin (*R*)

Fig. 4. Type IV of cyanogenic glycosides found in Passifloraceae: glycosides derived from phenylalanine.

In order to test the possibility that the new glucoside had the same stereochemistry as gynocardin (**12**), the latter was hydrogenated using palladium on carbon to afford dihydrogynocardin (**24**) quantitatively, also characterized as the hexaacetate **25**. Although it was previously reported that gynocardin (**12**) takes up one mole of hydrogen in the presence of palladium and three moles in the presence of platinum (Coburn and Long, 1966), dihydrogynocardin (**24**) has not been isolated and characterized prior to the present work. However, **24** was not identical with the new glycoside **22** isolated from *P. guatemalensis*. The absolute and relative configuration at C-1, C-2 and C-3 in **22** are thus left unspecified. Due to the flexibility of the saturated cyclopentane ring, relative configuration of the substituents cannot be determined reliably from vicinal interproton couplings or NOE effects, and use of chemical shift data to indicate stereochemistry of **22** would be speculative, even though the differences in the chemical shifts of C-1′ and H-1′ indicate that **22** and **24** have different stereochemistry at C-1. Because of a very limited amount of the material available, additional chemical studies could not be performed at this point. However, the new glycoside was fully characterized by ^{1}H and ^{13}C NMR spectroscopic and optical rotation data, which ascertain its identity and distinction from possible future isomers. We propose to call the new glycoside passiguatemalin; it belongs to the structural Type II (Fig. 2). Along with passiguatemalin (**22**), a





22 R = H: passiguatemalin
23 R = $COCH_3$: passiguatemalin hexaacetate

24 R = H: dihydrogynocardin
25 R = $COCH_3$: dihydrogynocardin hexaacetate

Fig. 5. New cyanogenic glycosides obtained in this work.

small amount of passibiflorin (**7**) was present in *P. guatemalensis* (see Experimental).

2.2. Distribution of cyclopentanoids in Passiflora

In addition to *P. guatemalensis*, 18 other *Passiflora* species were included in the study. Cyanogenesis was detected in concentrated extracts using the picrate sandwich TLC method (Brimer et al., 1983), as it was found to be more sensitive than detection of hydrogen cyanide in vials in fresh or dried plant material by tissue damage, and it does not depend on the function of endogenous enzymes. Since the exact identity of cyanogenic constituents was of major interest, the glycosides were purified by preparative, reversed-phase HPLC, followed by ^{1}H and ^{13}C NMR spectroscopy of the free glycosides as well as of their acetates. Reversed-phase HPLC on octadecylsilylsilica does not separate the glycosides having enantiomeric aglycones (i.e. **1** from **2**, **3** from **4**, and **4** from **5**), but the NMR spectra allow unambiguous identification of the glycosides and quantitative determination of the ratios between them.

The glycoside isolated from *P. apetala*, *P. cuneata*, *P. indecora*, *P. kalbreyeri*, *P. murucuja* and *P. perfoliata* was passibiflorin (**7**), which in the case of *P. murucuja* was accompanied by **5** and **6**. However, no isomers of **7** could be detected in any of the isolates. Re-investigation of *P. biflora*, the original source of passibiflorin (**7**) (Spencer and Seigler, 1985d; Olafsdottir et al., 1989b), with emphasis on detection of possible minor cyanogenic constituents, failed to detect any stereoisomer of **7**. *P. citrina* yielded passicapsin (**8**), whereas *P. discophora* and *P. herbertiana* contained glycosides belonging to the Type I. The hybrid *P.× violacea* (*P.× caeruleo-racemosa*) contained tetraphyllin B sulfate (**10**), in accord with the previous finding of this glycoside in *P. caerulea* and *P. racemosa* (Jaroszewski and Fog, 1989). Extracts of available specimens of *P. aurantia*, *P. gilbertii*, *P. ligularis*, *P. lindeniana*, *P. manicata*, *P. platyloba* and *P. tripartita* did not contain any detectable cyanogenic constituents, even though several collections made at different seasons were investigated.

2.3. α-Hydroxyamides as pseudocyanogenics and artefacts

During HPLC purification of the cyanogenic constituents of *P. citrina* a compound was detected which, similarly to passicapsin (**8**) also present in this plant, showed positive reaction in the cyanide-specific sandwich picrate TLC assay using *Helix pomatia* crude hydrolytic enzymes (Brimer et al., 1983). However, the ^{13}C NMR spectrum of this material failed to show the presence of a nitrile group, a resonance around 180 ppm characteristic of an amide group being observed instead. ^{1}H and ^{13}C NMR spectra, supported by high-resolution MS data, showed the material to have the structure **26** (Fig. 6). The relative stereochemistry of the cyclopentene ring was apparent from the NOESY spectra. Similarly, extracts of *P. cuneata* and *P. perfoliata*, which contain passibiflorin (**7**), gave the amide **27** (Fig. 6) identified as above. The amides **26** and **27** must be formed from the respective glycosides **8** and **7** by enzymatic hydrolysis of the glucosidic linkage and partial hydrolysis of the nitrile group during drying or extraction of the plant material. Similar amides (**28**–**32**), with or without the β-D-glucopyranosyl group, have previously been reported as artefacts during isolation of cyanohydrin glycosides from other *Passiflora* species (Jaroszewski et al., 1987b; Olafsdottir et al., 1991b; Adsersen et al., 1993). The fact that amides **26** and **27** retain the deoxy sugar at C-4 is a strong indication that hydrolysis of the glycosidic linkage involves enzymatic catalysis, since in an acid-catalyzed hydrolysis the deoxy sugar is expected to be

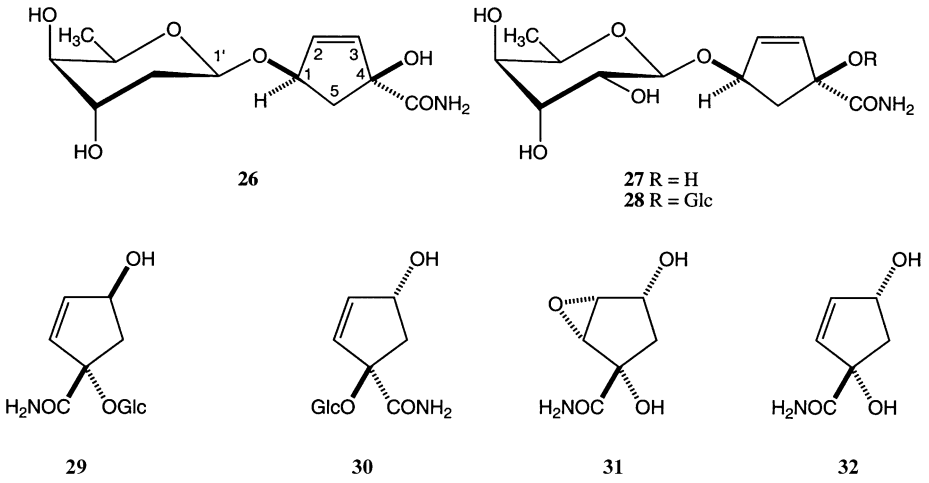


Fig. 6. α-Hydroxyamides isolated from *Passiflora* extracts; Glc = β-D-glucopyranosyl.

removed first, i.e. before the β-D-glucopyranose residue (Olafsdottir et al., 1989b). Also, the hydrolysis of the nitrile group to an amide seems to be the result of an enzymatic process because of the mildness of the conditions at which it occurs, unless acceleration of the chemical hydrolysis by some kind of intramolecular catalysis takes place.

Amides associated with cyclopentanoid glycosides appear to be formed, at least in a major part, during processing of the plant material. In the case of *P. cuneata* one extraction yielded a mixture of **7** and **27**, whereas another gave solely the amide **27**. An amide possibly corresponding to gynocardin (**12**) was isolated from *Lindackeria dentata* (Flacourtiaceae), apparently unaccompanied by a cyanogenic glycoside (Gibbons et al., 1998). Formation of amides was proposed to be an alternative way of catabolism of cyanogenic glycosides (Lechtenberg and Nahrstedt, 1999), but further work is necessary to clarify this point.

The formation of α-(β-D-glucopyranosyloxy)- and α-hydroxyamides is of interest for assessment of cyanide content of plant material. The above mentioned, positive reaction of **26** and **27** in the sandwich picrate assay specific for cyanide is also of interest. *Helix pomatia* crude enzyme preparation used in this assay apparently contains an amide dehydratase converting α-hydroxyamides into cyanohydrins, which liberate cyanide leading to the positive picrate test. To our knowledge, the presence of amide dehydratase activity in the *Helix pomatia* enzyme preparation (known as β-glucuronidase/sulfatase) has not been reported prior to this work.

2.4. Conclusions

The currently available information about cyanogenic constituents of *Passiflora* is compiled in Table 1. The sub-generic classification of *Passiflora* is based on Ulmer and Ulmer (1997). Although the picture is still far from being complete (33 species out of about 460 or about 7% have been studied so far), several distinct trends are apparent. Thus, all investigated species that belong to the series *Punctatae* contain passibiflorin (**7**) unaccompanied by Type 1 glycosides. All seven investigated species of the section *Decaloba*, which includes *Punctatae*, produce a bisglycoside, either **7** or **9**. However, passibiflorin (**7**) is not restricted to the section *Decaloba*, since it was found in two species belonging to *Murucuja* and *Pseudomurucuja*. Both investigated species belonging to the section *Xerogona* contain passicapsin (**8**), thus far not encountered outside this section. The sulfate **10** was only found in four closely related species belonging to the subgenera *Passiflora* and *Dysosmia*. Glycosides belonging to the Type III are apparently scattered throughout the genus, which is reminiscent of what is observed for dicotyledons at large, although all three examined species of the section *Pseudodysosmia* contain linamarin (Table 1). The glycosides belonging to the Type IV remain to be atypical cyanogenics of *Passiflora*. In conclusion, the distribution of various structural types of cyanogenic glycosides is highly structured at the subgenus level.

3. Experimental

3.1. General

NMR spectra were recorded on a Bruker AMX400 or AC250P spectrometer. High-resolution mass spectra were obtained on an IonSpec Ultima 4.7 Tesla FT mass spectrometer equipped with a matrix-assisted laser desorption ionization (MALDI) source based on a 337 nm nitrogen laser, using 2,5-dihydroxybenzoic acid for matrix preparation. All spectra were peak-matched using m/z 273.03936 ($[2M–2H_2O+H]+$) as a reference peak. Column chromatography was performed using open columns packed with Merck silica gel 60 (60–200 μm) or Matrex silica gel 60A (37–70 μm), or on a Büchi 681 MPLC system with a 5×30 cm column packed with Merck silica gel 60 (60–200 μm). EtOAc–Me_2CO–CH_2Cl_2–MeOH–H_2O (20:15:6:5:4) was used as the mobile phase. HPLC separations were performed either with a system consisting of a Gynkotek P 580A HPG pump, 2 ml Rheodyne 7725 injector, Shimadzu RID-10A refractive index detector, and a 2.1×25 cm Phenomenex Luna 5 C18(2) (5 μm) column, or on a system consisting of a HP 1050 pump, 0.5 ml Rheodyne 7725 injector, HP 1047 refractive index detector and a 1.6×25 cm Europrep 60 C-18 (10 μm) Knauer column. The HPLC columns were eluted with 10–30% aq. MeOH. Cyanogenic compounds were detected by TLC on Merck precoated aluminum plates (silica gel 60 F_{254}) run with EtOAc–Me_2CO–CH_2Cl_2–MeOH–H_2O (20:15:6:5:4), using the cyanide-specific sandwich picrate assay (Brimer et al., 1983). *Helix pomatia* enzyme preparation (β-glucuronidase, crude enzyme) was obtained from Sigma.

3.2. Plant material

All *Passiflora* species were grown in a greenhouse from seeds, and identified by the authors. Voucher specimens were deposited in Herbarium C (Botanical Museum, University of Copenhagen, Copenhagen) under the following accession numbers: *P. apetala* Killip: DFHJJ4; *P. biflora* Lam.: DFHJJ5; *P. citrina* MacDougal: DFHJJ6; *P. cuneata* Willd.: DFHJJ7; *P. discophora* Jørg. and Law.: DFHJJ8; *P. guatemalensis* Wats.: DFHJJ10; *P. herbertiana* Ker-Gawl.: DFHJJ11; *P. indecora* Kunth: DFHJJ12; *P. kalbreyeri* Mast.: DFHJJ13; *P. murucuja* L.: DFHJJ14; *P. perfoliata* L.: DFHJJ15; *P.×violacea* Loiseleur-Deslongchamps (*Passiflora×caeruleo-racemosa*): DFHJJ16.

Table 1
Occurrence of cyanogenic glycosides in subgenera of *Passiflora*

Subgenus	Section	Series	Number of species	Species studied	Compounds isolated				Source
					Type 1	Type 2	Type 3	Type 4	
1. *Apodogyne*			1						
2. *Astephia*			1						
3. *Decaloba (Plectostemma)*			177						
	Cieca		38	*P. coriacea* Juss.	**1-6**				Olafsdottir et al., 1989a
				P. suberosa L.	**1**, **5**, **6**	**11**			Olafsdottir et al., 1991b
	Deidamioides		1						
	Mayapathanthus		1						
	Decaloba		82						
		Auriculatae	2						
		Heterophyllae	2						
		Sexflorae	4						
		Apetalae	2	*P. apetala* Killip		**7**			This work
		Luteae	4	*P. lutea* L.		**7**[a]	**13**, **15**		Spencer and Seigler, 1985b
		Organenses	8						
		Miserae	6	*P. trifasciata* Lemaire		**9**			Olafsdottir et al., 1991a
		Punctatae	54	*P. biflora* Lam.		**7**			Olafsdottir et al., 1989b; this work
				P. colinvauxii Wiggins		**7**			Adsersen et al., 1993
				P. cuneata Willd.		**7**			This work
				P. talamancensis Killip		**7**[a]			Spencer and Seigler 1985d
	Xerogona		13	*P. capsularis* L.	**5**, **6**	**8**			Olafsdottir et al., 1989b
				P. citrina MacDougal		**8**			This work
	Pseudodysosmia		18	*P. adenopoda* DC.			**13**, **15**		Spencer et al., 1986
				P. pendens MacDougal			**13**, **15**		Spencer et al., 1986
				P. morifolia Mast. (= *warmingii*)			**13**, **15**		Olafsdottir et al., 1989a, 1992
	Pseudogranadilla		8	*P. indecora* Kunth		**7**			This work
				P. kalbreyeri Mast.		**7**			This work
	Hahniopathanthus		3	*P. guatemalensis* Wats.		**7**, **22**			This work
	Discophora		1	*P. discophora* Jørg. & Law.	**3**, **4**				This work
	Eueidipabulum		1						
	Distemma		4	*P. herbertiana* Ker-Gawl.	**1**, **2**, **5**, **6**				This work
	Octandranthus		2						
	Hollrungiella		1						
	Tryphostemmatoides		4						
4. *Chloropathanthus*			2						
5. *Murucuja*			4	*P. murucuja* L.	**5**, **6**	**7**			This work
6. *Pseudomurucuja*			7	*P. perfoliata* L.		**7**			This work
7. *Psilanthus*			3						
8. *Adenosepala*			1						
9. *Rathea*			2–3						
10. *Tacsonia*			49						
	Poggendorffia		5						

(*continued on next page*)

Table 1 (*continued*)

Subgenus	Section	Series	Number of species	Species studied	Compounds isolated				Source
					Type 1	Type 2	Type 3	Type 4	
	Colombiana		16						
		Leptomischae	6						
		Quindiensae	2						
		Colombianae	8						
	Parritana		2						
	Fimbriatistipula		2						
	Tacsoniopsis		2						
	Bracteogama		12						
	Tacsonia		4						
	Boliviana		2						
	Ampullacea		1						
	Trifoliata		3						
11. *Manicata* (*Grandillastrum*)			6						
12. *Distephana*			13						
	Glandulosa		4						
	Vitifolia		6	*P. coccinea* Aubl.		?[a]			Spencer and Seigler, 1985e
	Tholozaniella		3						
13. *Tacsonioides*			6						
14. *Passiflora* (*Granadilla*)			about 120						
	Quadrangularis		3	*P. quadrangularis* L.		**10**			Jaroszewski and Fog, 1989
	Digitata		1						
	Tiliifolia		11						
	Marginata		1						
	Laurifolia		20						
	Pachyantha		1						
	Serratifolia		5						
	Setacea		2						
	Pedata		1						
	Passiflora		8	*P. edulis* Sims				**18-21**	Spencer and Seigler, 1983; Chassagne et al., 1996; Chassagne and Crouzet, 1998; Christensen and Jaroszewski, 2001
				P. incarnata L.		**12**[a]			Spencer and Seigler, 1984
	Palmatisecta		1						
	Macdougaliana		1						
	Kermesiana		9						
	Imbricata		2						
	Simplicifolia		14						
	Calopathanthus		1	*P. racemosa* Brot.		**10**			Jaroszewski and Fog, 1989
	Lobatae		31	*P. caerulea* L.		**10**			Jaroszewski and Fog, 1989
				P. violacea Loiseleur-Deslongchamps		**10**			This work
				P. subpeltata Ortega			**13**		Olafsdottir et al., 1989a

(*continued on next page*)

Table 1 (*continued*)

Subgenus	Section	Series	Number of species	Species studied	Compounds isolated				Source
					Type 1	Type 2	Type 3	Type 4	
	Mensipermifolia		6						
15. *Dysosmia*			13	*P. foetida* L.	**1-4**	**10**	**13**		Andersen et al., 1998
16. *Dysosmioides*			5						
17. *Polyanthea*			1						
18. *Astrophea*			58						
	Dolichostemma		4						
	Euastrophea		17						
	Leptopoda		2						
	Pseudoastrophea		22						
	Botryastrophea		13						
19. *Tetraphathaea*			1	*P. tetrandra* Banks & Sol. ex DC.	**1-4**				Jaroszewski et al., 1987a
20. *Tetrastylis*			1						
21. *Porphyropathanthus*			1						

[a] Insufficient evidence for the structure reported was presented.

3.3. General extraction and identification procedure

Plant material (leaves and branches) was either freeze-dried or air-dried, and finely milled. The material was added in small portions into boiling, 80% aq. MeOH, and the mixture boiled for five min. The suspension was filtered and the plant material was re-extracted by boiling for 2 min with 80% aq. MeOH. The combined extracts were evaporated in vacuo and the residue freeze-dried, and the crude extract suspended in methanol and adsorbed on silica gel by evaporation in vacuo. The material was applied on top of a silica gel column packed with EtOAc–Me_2CO–CH_2Cl_2–MeOH–H_2O, 20:15:6:5:4, and the column eluted with the same solvent mixture. Fractions were monitored by TLC using the picrate sandwich assay to visualize cyanogenic compounds. Appropriate fractions were combined, evaporated, and the cyanogenic constituents purified by preparative HPLC. In some cases the column chromatography was repeated before final purification by HPLC.

3.4. Identification of known cyclopentanoid cyanogenics in Passiflora species

The isolated compounds were subjected to analysis by ^1H and ^{13}C NMR in CD_3OD. Subsequently, the compounds were acetylated by overnight treatment with pyridine–Ac_2O (1:1) and the identity of the isolates was confirmed by ^1H and ^{13}C NMR spectra of the acetates in $CDCl_3$. When the isolate consisted of a mixture of glycosides with enantiomeric aglycones (which were not resolved by the chromatographic procedures used), their ratio was determined by integration of appropriate ^1H NMR signals. *P. apetala*: 13 g of dried plant material yielded 140 mg (1.1%) of passibiflorin (**7**); *P. biflora*: 30 g yielded 65 mg (0.18%) of passibiflorin (**7**); *P. citrina*: 48 g yielded 6 mg (0.01%) of passicapsin (**8**) and 17 mg (0.035%) of amide **26**; *P. cuneata*: 61 g yielded 532 mg (0.87%) of passibiflorin (**7**) (in another experiment 28 g of the plant material from a different collection from the same specimen gave 53 mg or 0.19% of the amide **27** and no **7**); *P. discophora*: 135 g yielded 29 mg (0.02%) of a 5:1 mixture of tetraphyllin B (**3**) and volkenin (**4**); *P. herbertiana*: 2.5 g yielded 3 mg (0.12%) of a 2:3 mixture of tetraphyllin A (**1**) and deidaclin (**2**), and 3 mg (0.12%) of a 4:1 mixture of epivolkenin (**5**) and taraktophyllin (**6**); *P. indecora*: 13 g yielded 46 mg (0.35%) of passibiflorin (**7**); *P. kalbreyeri*: 64 g yielded 26 mg (0.04%) of passibiflorin (**7**), 48 mg (0.075%) of the amide **27**, and 4 mg (0.006%) of the amide **32**, identified by ^1H and ^{13}C NMR spectra in CD_3OD identical with those previously reported (Olafsdottir et al., 1991b); *P. murucuja*: 13 g yielded 2 mg (0.015%) of passibiflorin (**7**) and 2 mg (0.015%) of a 1:3.7 mixture of epivolkenin (**5**) and taraktophyllin (**6**); *P. perfoliata*: 18 g yielded 40 mg

(0.12%) of passibiflorin (**7**), 12 mg (0.07%) of a 1:1 mixture of epivolkenin (**5**) and taraktophyllin (**6**), and 9 mg (0.05%) of the amide **27**; *P.× violacea* (*P.×caeruleo-racemosa*): 36 g yielded 14 mg (0.04%) of tetraphyllin B sulfate (**10**).

3.5. Isolation of passibiflorin (7) and passiguatemalin [1ξ-(β-D-glucopyranosyloxy)-2ξ,3ξ-dihydroxycyclopentane-1ξ-carbonitrile] (22) from P. guatemalensis

Extraction of dry plant material (112 g) as described above yielded 18.4 g of a residue, which after chromatography on silica gel yielded 300 mg of a cyanogenic fraction. Repeated preparative HPLC (10% MeOH) yielded, besides 8 mg (0.007%) of passibiflorin (**7**), 7 mg (0.006%) of passiguatemalin (**22**). $[\alpha]_D^{25}$ −38.5° (*c* 0.1, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ 1.70–1.79 and 2.10–2.20 (each 1H, *m*, H-4 geminal pair), 2.22–2.29 and 2.35–2.42 (each 1H, *m*, H-5 geminal pair), 3.23 (1H, *dd*, *J* = 9.0 and 7.6 Hz, H-2′), 3.29–3.43 (3H, *m*, H-3′, H-4′, H-5′), 3.69 (1H, *dd*, *J* = 12.0 and 5.0 Hz, H-6′A), 3.84 (1H, *dd*, *J* = 12.0 and 2.0 Hz, H-6′B), 4.00–4.05 (2H, *m*, H-2 and H-3), 4.71 (1H, *d*, *J* = 7.6 Hz, H-1′) (assignments based on COSY and NOESY correlations). ^{13}C NMR (100 MHz, CD_3OD): δ 30.1 (C-4), 35.2 (C-5), 62.4 (C-6′), 71.2 (C-4′), 75.0 (C-2′), 76.5 (C-3), 77.9 and 78.2 (C-3′ and C-5′), 84.6 (C-1), 84.9 (C-2), 102.1 (C-1′), 119.1 (CN) (assignments based on an ^{1}H,^{13}C-correlation). HR MALDI FT MS *m*/*z* (rel. int.) 328.1007 (100%, $[MNa]^+$), $[C_{12}H_{19}NO_8 + Na]^+$ requires 328.1003.

Passiguatemalin hexaacetate (**23**) was prepared by overnight treatment of **22** (1.5 mg) with pyridine–Ac_2O. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.01, 2.04, 2.06, 2.08, 2.09 and 2.18 (each 3H, *s*, CH_3), 1.81–1.93 and 2.29–2.37 (each 1H, *m*, H-4 geminal pair), 2.20–2.26 and 2.44–2.50 (each 1H, *m*, H-5 geminal pair), 3.80 (1H, *ddd*, *J* = 10.1, 5.6 and 2.5 Hz, H-5′), 4.15 (1H, *dd*, *J* = 12.3 and 2.5 Hz, H-6′A), 4.25 (1H, *dd*, *J* = 12.3 and 5.6 Hz, H-6′B), 5.02–5.12 (4H, *m*, H-3, H-1′, H-2′, H-4′), 5.17 (1H, *dd*, *J* = 4.2 and 1.5 Hz, H-2), 5.26 (1H, *m*, H-3′). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.5, 20.6 (2C), 20.7, 20.8 and 20.9 (CH_3), 27.9 (C-4), 35.9 (C-5), 61.7 (C-6′), 68.2 (C-4′), 70.6 (C-2′), 72.4 (C-5′), 72.6 (C-3′), 76.8 (C-3), 81.6 (C-1), 82.1 (C-2), 98.4 (C-1′), 115.5 (CN), 169.3, 169.4, 169.5, 170.1 (2C) and 170.6 (CO) (assignments based on an ^{1}H,^{13}C shift correlation and for glucosyl carbons on comparison with model compounds). HR MALDI FT MS *m*/*z* (rel. int.) 580.1658 (65%, $[MNa]^+$), $[C_{24}H_{31}NO_{14} + Na]^+$ requires 580.1637; 520.1437 (100%, $[M–AcOH + Na]^+$), $[C_{22}H_{27}NO_{12} + Na]^+$ requires 520.1426.

A solution of passiguatemalin (**22**) (4.5 mg) in 1 M HCl (300 μl) was heated at 90–100 °C for 1 h. The solution was freeze-dried, the residue dissolved in 30 μl H_2O, and the presence of D-glucose was demonstrated using D-glucose oxidase test (Diabur-Test 5000, Roche).

3.6. Synthesis of (1R,2R,3R)-1-(β-D-glucopyranosyloxy)-2,3-dihydroxycyclopentane-1-carbonitrile (dihydrogynocardin) (24)

Gynocardin (150 mg) in 20 ml EtOH was hydrogenated at ambient temperature and pressure over 50 mg of 5% Pd/C for 2 h. Filtration and evaporation afforded an essentially pure crude product as a colorless gum; an analytical sample was obtained by prep. HPLC (10% MeOH). $[\alpha]_D^{25}$ −11° (*c* 1.1, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ 1.66–1.75 and 2.04–2.14 (each 1H, *m*, H-4 geminal pair), 2.25–2.40 (2H, *m*, H-5 geminal pair), 3.23 (1H, *dd*, *J* = 9.8 and 8.8 Hz, H-4′), 3.26 (1H, *dd*, *J* = 9.1 and 7.7 Hz, H-2′), 3.35–3.52 (2H, *m*, H-3′ and H-5′), 3.57 (1H, *dd*, *J* = 11.4 and 7.8 Hz, H-6′A), 3.94 (1H, *dd*, *J* = 11.4 and 2.5 Hz, H-6′B), 3.99 (1H, apparent *q*, *J* ≈ 7 Hz, H-3), 4.08 (1H, *d*, *J* = 6.6 Hz, H-2), 4.66 (1H, *d*, *J* = 7.7 Hz, H-1′) (assignments based on COSY and NOESY correlations). ^{13}C NMR (100 MHz, CD_3OD): δ 29.1 (C-4), 34.7 (C-5), 63.0 (C-6′), 72.1 (C-4′), 74.9 (C-2′), 76.9 (C-3), 78.1 (C-3′), 78.2 (C-5′), 84.6 (C-1), 85.4 (C-2), 101.7 (C-1′), 118.9 (CN) (assignments based on ^{1}H,^{13}C shift correlation). HR MALDI FT MS *m*/*z* (rel. int.) 328.1005 (100%, $[MNa]^+$), $[C_{12}H_{19}NO_8 + Na]^+$ requires 328.1003.

Dihydrogynocardin hexaacetate (**25**) was obtained as a colorless syrup by overnight treatment of **24** (15 mg) with pyridine-Ac_2O. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.01, 2.04, 2.05, 2.06, 2.08 and 2.18 (each 3H, *s*, CH_3), 1.96–2.02 (1H, *m*, H-4A), 2.29–2.36 (3H, *m*, H-5 geminal pair and H-4B), 3.89 (1H, *ddd*, *J* = 10.2, 5.3 and 2.5 Hz, H-5′), 4.17 (1H, *dd*, *J* = 12.4 and 2.5 Hz, H-6′A), 4.22 (1H, *dd*, *J* = 12.4 and 5.3 Hz, H-6′B), 4.99–5.08 (4H, *m*, H-3, H-1′, H-2′, H-4′), 5.27 (1H, *t*, *J* = 9.0 Hz, H-3′), 5.72 (1H, *d*, *J* = 3.0 Hz, H-2) (assignments based on COSY and NOESY correlations). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.6 (4C, CH_3), 20.8 (2C, CH_3), 28.4 (C-4), 36.9 (C-5), 61.9 (C-6′), 68.0 (C-4′), 70.8 (C-2′), 72.5 and 72.7 (C-3′ and C-5′), 77.8 (C-3), 79.5 (C-2), 83.4 (C-1), 99.0 (C-1′), 116.3 (CN), 169.3, 169.4, 169.6, 170.0, 170.3 and 170.6 (CO) (assignments based on an ^{1}H,^{13}C shift correlation and for glucosyl carbons on comparison with model compounds). HR MALDI FT MS *m*/*z* (rel. int.) 580.1646 (61%, $[MNa]^+$), $[C_{24}H_{31}NO_{14} + Na]^+$ requires 580.1637; 520.1426 (100%, $[M–AcOH + Na]^+$), $[C_{22}H_{27}NO_{12} + Na]^+$ requires 520.1426.

3.7. (1S,1R)-1-Hydroxy-4-(2,6-dideoxy-β-D-xylo-hexapyranosyloxy)-2-cyclopentene-1-carboxamide (26)

The amide **26** was obtained as a colorless syrup during HPLC purification of passicapsin-containing fraction of *P. citrina* (17 mg from 48 g of the dried plant material). $[\alpha]_D^{25}$ +40° (*c* 0.1, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ 1.24 (3H, *d*, *J* = 6.6 Hz, H-6′), 1.69 (1H, *dm*, *J* = 13.6 Hz, H-2′α), 1.71 (1H, *dd*, *J* = 13.4 and

5.1 Hz, H-5 *cis* to $CONH_2$), 1.84 (1H, *ddd*, $J = 13.6$, 9.9 and 3.2 Hz, H-2′β), 2.85 (1H, *dd*, $J = 13.4$ and 7.3 Hz, H-5 *trans* to $CONH_2$), 3.23 (1H, *dm*, $J = 3.5$ Hz, H-4′), 3.96 (1H, *q*, $J = 3.2$ Hz, H-3′), 3.98 (1H, *dq*, $J = 6.6$ and 1.3 Hz, H-5′), 4.88 (1H, *dd*, $J = 9.9$ and 2.2 Hz, H-1′), 4.91 (1H, *m*, H-4), 5.74 (1H, *dd*, $J = 5.5$ and 1.4 Hz, H-2), 6.12 (1H, *dd*, $J = 5.5$ and 1.9 Hz, H-3). ^{13}C NMR (100 MHz, CD_3OD): δ 17.2 (C-6′), 35.1 (C-2′), 46.6 (C-5), 70.6, 70.7 and 71.3 (C-3′, C-4′, C-5′), 83.7 (C-4), 85.9 (C-1), 99.5 (C-1′), 136.8 (C-2), 138.9 (C-3), 180.5 (CO). HR MALDI FT MS *m/z* (rel. int.) 296.1097 (100%, $[MNa]^+$), $[C_{12}H_{19}NO_6 + Na]^+$ requires 296.1105.

3.8. (1S,1R)-1-Hydroxy-4-(6-deoxy-β-D-gulopyranosyloxy)-2-cyclopentene-1-carboxamide (**27**)

The amide **27** was obtained as a colorless syrup during HPLC purification of passibiflorin-containing fraction of *P. cuneata* (40 mg from 28 g of the dried plant material) and from *P. perfoliata* (9 mg from 18 g of the plant material). $[\alpha]_D^{25}$ +68° (*c* 0.76, MeOH). ^1H NMR (400 MHz, CD_3OD): δ 1.23 (3H, *d*, $J = 6.6$ Hz, H-6′), 1.86 (1H, *dd*, $J = 13.7$ and 4.5 Hz, H-5 *cis* to $CONH_2$), 2.82 (1H, *dd*, $J = 13.7$ and 7.2 Hz, H-5 *trans* to $CONH_2$), 3.46 (1H, *dd*, $J = 3.5$ and 1.3 Hz, H-4′), 3.55 (1H, *dd*, $J = 8.2$ and 3.5 Hz, H-2′), 3.97 (1H, *t*, $J = 3.5$ Hz, H-3′), 4.03 (1H, *dq*, $J = 6.6$ and 1.3 Hz, H-5′), 4.87 (1H, *d*, $J = 8.2$ Hz, H-1′), 4.92 (1H, *m*, H-4), 5.77 (1H, *dd*, $J = 5.5$ and 1.4 Hz, H-2), 6.11 (1H, *dd*, $J = 5.5$ and 2.0 Hz, H-3) (assignments based on COSY and NOESY correlations). ^{13}C NMR (100 MHz, CD_3OD): δ 16.4 (C-6′), 46.2 (C-4), 69.5 (C-2′), 70.2 (C-5′), 73.4 (C-3′), 73.6 (C-4′), 83.7 (C-4), 85.7 (C-1), 101.4 (C-1′), 137.0 (C-2), 138.2 (C-3), 180.3 (CO) (the assignments are based on a ^1H,^{13}C-correlation). HR MALDI FT MS *m/z* (rel. int.) 312.1054 (100%, $[MNa]^+$), $[C_{12}H_{19}NO_7 + Na]^+$ requires 312.1053.

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