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PASSICORIACIN AND EPIPASSICORIACIN: C-4 EPIMERS OF TETRAPHYLLIN B AND EPITETRAPHYLLIN B FROM *PASSIFLORA CORIACEA*

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Key Word Index—*Passiflora coriacea*; Passifloraceae; cynogenic glycosides; passicoriacin; epipassicoriacin.

Abstract—An epimeric pair of cyclopentenoid cyanogenic glycosides, for which we propose the trivial names passicoriacin and epipassicoriacin, has been isolated from *Passiflora coriacea*. These compounds are similar to tetraphyllin B and epitetraphyllin B, but differ in stereochemistry at the C-4 centre. Small amounts of compounds that appear to be the corresponding gentiobiosides were also isolated. These compounds, presumably derived from 2-cyclopentenylglycine, are unusual in section *Cieca*, subgenus *Plectostemma*, and suggest that the placement of this species should be re-examined.

INTRODUCTION

Passiflora coriacea L. is a member of section *Cieca* of subgenus *Plectostemma*, members of which typically produce isoleucine/valine and leucine-derived aliphatic cyanogenic glycosides [1, 2]. However, when treated with linamarase which hydrolyses aliphatic cyanogenic glycosides [3, 4], extracts of *P. coriacea* did not produce cyanide. Other enzyme preparations known to hydrolyse aromatic [5] or cyclopentenoid cyanogenic glycosides [6–12] hydrolysed the unknown compounds but not as efficiently as did an enzyme preparation from *P. coriacea* (see Experimental). The ^{1}H NMR spectrum (90 MHz, D_2O) suggested the presence of compounds similar to tetraphyllin B (**1**), in contradiction to these data. As previous work has shown that low-field NMR is not adequate to resolve epimers of underivatized cyclopentenoid cyanogenic glycosides [11], we anticipated the presence of previously undescribed epimers of tetraphyllin B.

RESULTS AND DISCUSSION

HPLC analysis of the original cyanogen mixture from *P. coriacea* showed the presence of four peaks in a 6:5:2:2 ratio. The largest peak was identical in retention time (7.1 min) to tetraphyllin B (**1**) [6]. This peak was closely preceded by a peak at 6.9 min. Two minor peaks corresponded in retention times (8.7 and 9.6 min) to cyanogenic diglycosides [6]. Sugar determinations for all cyanogenic fractions revealed only glucose to be present. Quantitative sugar and cyanide determination of the two major compounds showed each to have an equimolar ratio of cyanide to glucose.

These retention times were reproducible upon analysis of the separated monoglycoside and diglycoside fractions obtained by ascending paper chromatography. Separate collection of the peaks at 6.9 and 7.1 min yielded two fractions, the first upon derivatization giving an ^{1}H NMR spectrum identical to that of **1** and a small amount of epitetraphyllin B (**3**), and the second a novel compound (**2**) and a similar minor component (**4**) (Table 1). The ^{1}H NMR spectra of the latter two compounds were quite similar to that of **1**. Signals were assigned to the two separated compounds (**2** and **4**) based on a 4:1 difference in signal intensity.

The ^{1}H NMR data for the mixture of glycosides are presented in Table 1. The presence of four anomeric protons and four sets each of vinyl and geminal protons suggested the presence of four cyclopentenoid cyanogenic monoglucosides.

The ^{13}C NMR data for an underivatized sample of the second fraction are given in Table 2 and are compared with data for **1** and **3** obtained separately. The spectra of **3** and **2** are similar. The only significant difference is in the position of the C-4 signal, which is shifted δ 1.3 upfield (this was assigned to **2** rather than **4** on the basis of a significant difference in peak height). The absolute configuration of tetraphyllin B has recently been determined [13].

The ^{13}C NMR spectrum of **4** is observably different from those of **1** and **2** in that there is a greater difference in chemical shift of the vinyl carbon signals (2.6 and 0.8 ppm).

Although insufficient material was present to characterize fully the cyclopentenoid diglycosidic compounds present, evidence for their presence was obtained from the ^{1}H NMR spectrum of a sample containing **1** in which two doublets at δ 4.00 and 4.02 are also observed. Two sets of vinyl double doublets and four extra anomeric protons were also observed. The ^{13}C NMR spectrum of the sample showed two extra peaks at δ 103.6 and 103.7 corresponding to two first anomeric (C-1″) carbons of

Table 1. ^{1}H NMR spectral data for a mixture of tetraphyllin B (1), passicoriacin (2), epitetraphyllin B (3) and epipassicoriacin (4) as TMSi ethers in $CDCl_3$

H	1	2	3	4
2	6.19 (1, *dd*, 6, 1)	6.09 (1, *dd*, 5, 1)	6.17 (1, *dd*, 6, 1)	6.16 (1, *dd*, 5, 1)
3	6.04 (1, *dd*, 6, 1)	5.96 (1, *dd*, 5, 1)	6.02 (1, *dd*, 6, 1)	6.01 (1, *dd*, 5, 1)
4	5.00 (1, *m*)	4.98 (1, *m*)	5.00 (1, *m*)	4.82 (1, *m*)
5a	2.93 (1, *dd*, 15, 7)	2.99 (1, *dd*, 14, 5)	2.68 (1, *dd*, 14, 4)	3.02 (1, *dd*, 14, 5)
5b	2.22 (1, *dd*, 15, 5)	2.20 (1, *dd*, 14, 5)	2.20 (1, *m*)	2.20 (1, *m*)
1′	4.47 (1, *d*, 7)	4.48 (1, *d*, 7)	4.62 (1, *d*, 7)	4.58 (1, *d*, 7)
2′	3.28 (1, *t*, 8)	3.26 (1, *t*, 8)		
3′	3.40 (1, *t*, 8)	3.38 (1, *t*, 8)		
4′	3.43 (1, *t*, 8)	3.41 (1, *t*, 8)		
5′	3.20 (1, *m*)	3.20 (1, *m*)	Same as 1	Same as 2
6′a	3.76 (1, *d*, 11)	3.74 (1, *d*, 11)		
6′b	3.60 (1, *dd*, 11, 6)	3.60 (1, *dd*, 11, 6)		

*In ppm. Numbers in parentheses refer to integral value, multiplicity, J and J' (Hz).

Table 2. ^{13}C NMR data for a mixture of tetraphyllin B (1), passicoriacin (2), epitetraphyllin B (3) and epipassicoriacin (4) (D_2O, ref. DSS)

C	1	2	3	4
1	82.13	81.90	81.64	82.27
2	142.44	143.31	143.30	144.61
3	131.81	131.09	130.85	131.40
4	73.67*	74.25*	75.54	75.53
5	46.56	47.02	47.10	47.36
6	120.22	120.48	119.83	120.73
1′	100.36	100.12	99.89	100.30
2′	73.80*	73.81*	74.09	73.81
3′	77.02†	77.14†	76.50†	77.14†
4′	70.34	70.39	70.04	70.39
5′	76.46†	76.51†	76.18†	76.51†
6′	61.60	61.35	61.27	61.55

*,†Assignments within a spectrum may be interchangeable.

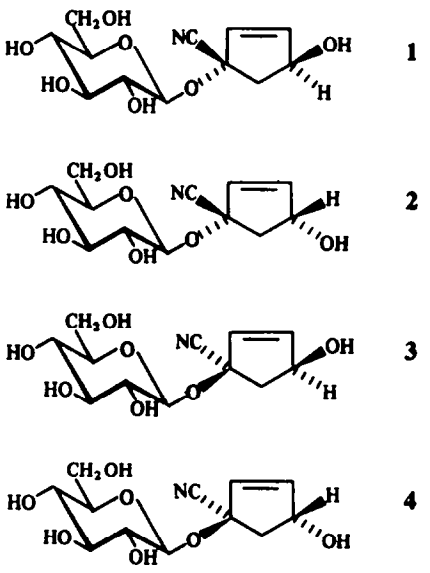

Fig. 1. The probable structures of tetraphyllin B (1) (see ref. [21]), passicoriacin (2), epitetraphyllin B (3) and epipassicoriacin (4).

glucose in gentiobiose [1, 14], and peaks corresponding to other sugar carbons. Additionally, extra vinyl carbon signal pairs were observed at δ144.28/133.38 and 142.98/132.46. A corresponding series of vinyl carbon signals in the ^{13}C NMR spectra of cyclopentenoid cyanogenic diglycosides in which intersignal shift distances are diminished relative to monoglycosides has been described previously.

We propose the trivial names passicoriacin and epipassicoriacin for compounds 2 and 4, respectively (Fig. 1).

EXPERIMENTAL

Plant material. P. coriacea was grown in a greenhouse at the University of Illinois, Urbana, IL. A voucher specimen has been deposited at the University of Illinois Herbarium (ILL).

Isolation of the glycosides. Fresh leaf material of *P. coriacea* (612 g) was ground in a blender with 80% MeOH. The suspension was filtered and the extract concentrated under vacuum. This material was placed on a large microcrystalline cellulose–Whatman CF1–Whatman CF11 (1:1:1) cellulose column and chromatographed in *iso*-PrOH–*n*-BuOH–H_2O (6:3:1). Fractions were collected (20 ml) and aliquots of the fractions were concentrated and tested for cyanide with an enzyme preparation (see below) and the Feigl–Anger method [15] as described previously [16].

The cyanogenic material (fractions 30–70) was pooled, concentrated and rechromatographed on a similar column with MeCOEt–Me_2CO–H_2O (15:5:3) as eluant. The eluate was tested as above and the fractions of interest (fractions 20–30) were rechromatographed on paper (Whatman 3MM) in Me_2CO–H_2O (5:1). The cyanogenic material was located by cutting a 1 cm strip from the centre of the chromatogram, taking 1 cm^2 sections from this strip and testing as above. The cyanogenes (R_f 0.7) were chromatographed again on paper in *iso*-PrOH–*n*-BuOH–H_2O (6:3:1) to yield a mixture of cyanogens (450 mg) (R_f 0.45).

Further resolution of the mixture was made by ascending PC (Whatman 3MM) in C_5H_5N–EtOAc–HOAc–H_2O

(36:36:21:7). One cyanogenic band corresponded to cyanogenic monoglycosides (440 mg, R_f 0.8) and another to cyanogenic diglycosides (R_f 0.3) [6]. Final resolution of the monoglycosides was made by HPLC.

HPLC analysis. A sample of the unknown mixture of cyanogenic glycosides was chromatographed by HPLC on an amine column (Alltech) with 85% MeCN at 1.0 ml/min. Compounds were detected with a refractive index detector.

Enzyme preparation. Fresh leaf material of *P. coriacea* (50 mg) was ground in a blender in cold Me_2CO. The suspension was filtered and rinsed with cold Me_2CO. The material retained was then placed under vacuum to remove traces of solvent and was suspended in cold Pi buffer (pH 6.8, 500 ml). After stirring for 1 hr in an ice bath, the suspension was filtered and the filtrate dialysed against Pi buffer, pH 6.8, for 12 hr. After three changes of buffer, the dialysate was filtered through cotton and brought to a final vol. of 1 l. The resulting enzyme preparation was assayed for activity towards the unknown compounds and found to be more active than commercial emulsin (Sigma) or linamarase prepared from seeds of *Linum usitatissimum* [6]. The preparation was also more active than preparations which efficiently hydrolyse tetraphyllin B and other cyclopentenoid cyanogens [6–12], although several of these hydrolysed the unknown mixture slowly.

Determination of sugars. A small sample of the major cyanogenic fraction from the final purification was hydrolysed in 1 M HCl [17, 18] and the hydrolysate chromatographed on paper (Whatman 3MM) in C_5H_5N–EtOAc–HOAc–H_2O (36:36:21:7) with standard sugars according to ref. [19]. The paper was developed in the ascending mode, dried, sprayed with *p*-anisidine hydrochloride [20] and heated at 100° for 10 min to visualize sugars. A sample of the minor cyanogenic fraction was also tested in this manner. Only glucose was detected in either sample.

Preparation of derivatives. A sample of the unknown mixture (50 mg) was dried under vacuum for 1 hr and then dissolved in C_5H_5N (0.5 ml). To this was added HMDS (0.5 ml). The sample was shaken gently with warming to effect homogeneity and TMCS (0.5 ml) was added. The reaction mixture was shaken in a water bath for 10 min (40°) and then dried with a stream of N_2. After removing traces of C_5H_5N by placing the sample under vacuum for several hours, the mixture was dissolved in $CHCl_3$ and filtered through a fine sintered glass filter. The filtrate was again dried and placed under vacuum to remove solvent. The sample was then dissolved in $CDCl_3$ for spectral analysis.

Spectral determination. ^{1}H NMR spectra of the trimethylsilyl (TMSi) ether derivative of the unknowns were measured on a Nicolet NT-360 (360 MHz) and on a Bruker WM-500 (500 MHz) FT spectrometer in $CDCl_3$. ^{13}C NMR spectra were determined on underivatized samples in D_2O on the Nicolet instrument (22.5 MHz) using dioxane as a reference.

Quantitative assay of cyanide and glucose was by a combination of the methods of Washko and Rice [21] and Lambert *et al.* [22] as previously described [6, 7].

Isolation and purification of tetraphyllin B and epitetraphyllin B. From a fresh tuber of *Adenia gracilis* Harms (350 g) according to ref. [10]. These samples were prepared to provide comparative ^{13}C NMR data.

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