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PASSIBIFLORIN, EPIPASSIBIFLORIN AND PASSITRIFASCIATIN: CYCLOPENTENOID CYANOGENIC GLYCOSIDES FROM *PASSIFLORA*

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Key Word Index—*Passiflora biflora*, *P talamancensis*, *P trifasciata*, Passifloraceae, cyanogenic glycosides, passibiflorin, epipassibiflorin, passitrifasciatin

Abstract—An epimeric mixture of two novel cyclopentenoid cyanogenic glycosides, passibiflorin [1-(6-*O*-*β*-D-rhamnopyranosyl-*β*-D-glucopyranosyloxy)-4-hydroxycyclopent-2-en-1-nitrile] and its C-1 epimer, epipassibiflorin, has been isolated from *Passiflora biflora* and *P talamancensis* The structures were determined by means of ^{1}H NMR and ^{13}C NMR Another novel cyclopentenoid cyanogenic glycoside, passitrifasciatin [1-(4-*O*-*β*-D-rhamnopyranosyl-*β*-D-glucopyranosyloxy)-4-hydroxycyclopent-2-en-1-nitrile] is described from *Passiflora trifasciata* The structure was determined by means of ^{1}H NMR The identification of the sugar moieties was made by HPLC and TLC The isolation of a *β*-1 → 4 and a *β*-1 → 6-rhamnoglucoside of cyclopentenoid cyanogens from three species of subgenus *Plectostemma* of *Passiflora* suggests that diglycosides of this type are taxonomically diagnostic for the section

INTRODUCTION

Cyclopentenoid cyanogens are restricted in distribution to the Passifloraceae and their close relatives To date, eight structural types have been isolated and identified Tetraphyllin A and deidaclin have been isolated from the Passifloraceae [1–4], Malesherbiaceae [5] and Turneraceae [6] Two monohydroxylated cyclopentenoid cyanogens, tetraphyllin B and epitetraphyllin B, have been identified from the same three families [2, 3, 7–11] Tetraphyllin B has also been isolated from the Flacourtiaceae [12], a family which more typically elaborates gynocardin [12–14], a dihydroxylated cyclopentenoid cyanogen The 4-sulphates of tetraphyllin B and epitetraphyllin B have been found to occur in the genus *Passiflora* [15], as has the unusual cyclopentenoid diglycoside, passicapsin [16]

As part of a continuing investigation into the distribution of cyclopentenoid cyanogens, we report here the discovery of two novel cyclopentene-ring containing cyanogenic glycosides from *Passiflora*

RESULTS AND DISCUSSION

The R_f values obtained by PC indicated that novel polar cyanogenic compounds were present in all samples This was confirmed when attempts to hydrolyse the compounds with enzyme preparations which hydrolyse the known cyanogenic glycosides [2, 6, 11, 18] failed Hydrolysis did occur when the reaction was carried out for an extended period (48 hr) using enzyme preparations specific for tetraphyllin B [9, 10] This suggested that the unknowns might be cyanogenic glycosides of the cyclopentenoid type [11, 15] similar to, but more polar than, tetraphyllin B Analysis of unknowns by HPLC (as for sugars, see Experimental) shows that the unknowns isolated from *P biflora* and *P talamancensis* had identical retention times of 11 7 min, while the unknown from *P trifasciata* had a retention time of 10 9 min

Analysis of the glycoside moieties by HPLC demonstrated the presence of rhamnose and glucose in a 1 1 ratio The ^{1}H NMR (Fig 1) of the TMSi derivatives of the cyanogenic glycoside obtained from both *P biflora* and *P talamancensis* were identical The presence of a cyclopentenoid ring structure was indicated by comparison with the ^{1}H NMR spectrum of the TMSi derivative of tetraphyllin B (Table 1) Relative to tetraphyllin B [9] the unknown H-2 peak (0 02 ppm) was shifted downfield by 0 14 ppm, the H-3 peak shifted slightly upfield, the H-4 signal shifted upfield (0 18 ppm) and signals for the geminal H-5 were shifted 0 06 ppm farther part Two anomeric proton signals (δ4 45 and δ4 62, 1H each) are observed in the spectrum, the coupling constant of these two peaks (J = 7 6 Hz) is indicative of *β*-linked glycosides The presence of a doublet at δ4 02 (J = 6 Hz) is due to one of the H-6′ protons of a *β*-1,6-linked glucose [25] The remaining signals in Table 1 are consistent with a rhamnoglucoside, including a diagnostic H-6″ rhamnose methyl signal at δ1 15

The ^{13}C NMR spectra in D_2O and as TMS ethers in $CDCl_3$ of samples of both origins were also identical (Table 2) and revealed the presence of small amounts of a second glycoside not clearly visible in the ^{1}H NMR spectrum The major compound shows signals consistent with the proposed structure of passibiflorin [1-(6-*O*-*β*-D-rhamnopyranosyl-*β*-D-glucopyranosyloxy)-4-hydroxycyclopent-2-en-1-nitrile] shown in Fig 2 The carbon shifts relative to tetraphyllin B are small [10]

The similar spectrum for the second compound obtained as its TMSi derivative corresponds to that of the epimer of passibiflorin These compounds were not separated As in the epimeric pair tetraphyllin B sulfate–epitetraphyllin B sulfate, all aglycone lines in the ^{13}C NMR spectrum are duplicated [15]

Assignment of sugar carbon peaks was facilitated

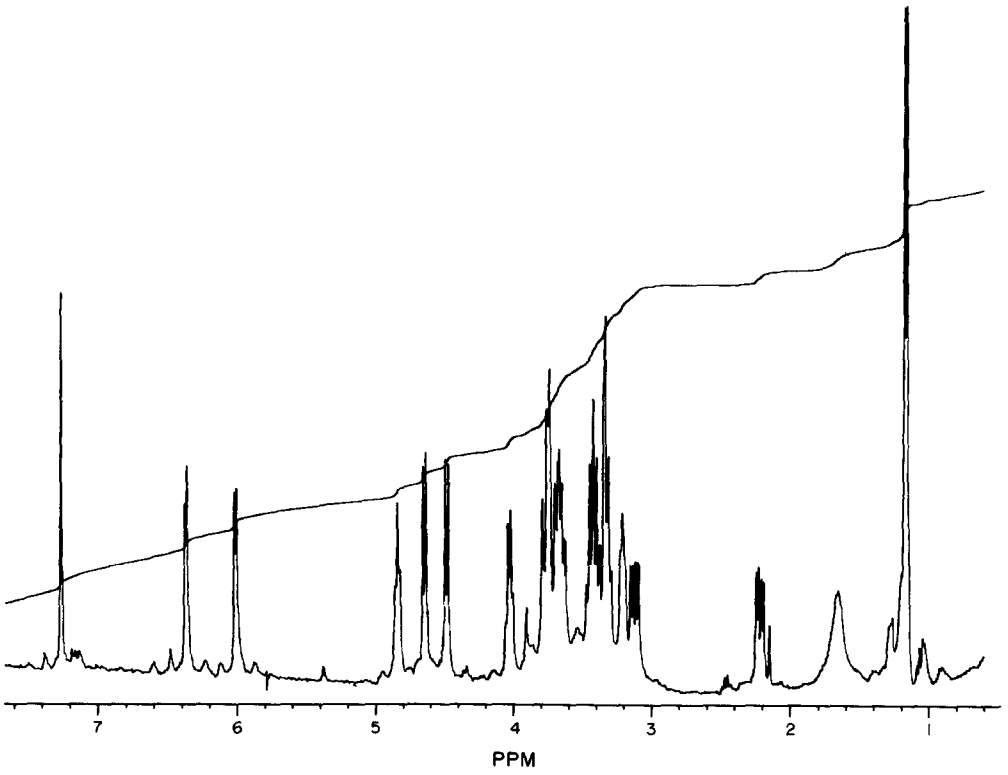

Fig 1 The ^{1}H NMR spectrum of the TMSi derivative of passibiflorin in $CDCl_3$

by comparison of ^{13}C NMR data for *O*-glucosides [26] and *O*-rhamnosides [27] in DMSO with those for an *O*-β-1 → 6-rhamnoglucoside in DMSO [26], allowing prediction of the magnitude expected for shifts in glucose peaks in a rhamnoglucoside in D_2O

The ^{1}H NMR spectrum of the TMSi derivative of the unknown from *P trifasciata* is shown in Fig 3 The identity of each proton of the aglycone portion of the molecule was established by decoupling experiments Irradiation of the double doublet at δ 6 35 collapsed the double doublet at δ 6 04 to a singlet and simplified the multiplet at δ 4 79 Irradiation of the peak at δ 6 04 caused a similar effect on the multiplet, and simplified the peak at δ 6 35 Irradiation of the multiplet at δ 4 79 collapsed the double doublets at δ 6 35 and δ 6 04 to singlets and simplified the 4-line patterns at δ 3 12 and δ 2 22 to doublets The spectrum is quite similar to that of the TMSi derivative of tetraphyllin B [9] Slight differences in chemical shifts relative to tetraphyllin B are noted in that the H-2 peak is shifted downfield by 0 14 ppm, the H-4 multiplet is shifted upfield 0 20 ppm, and the 4-line patterns of the H-5 protons are separated by an additional 0 20 ppm

Other decouplings gave expected results for rhamnose and glucose protons Irradiation of the anomeric proton doublet at δ 4 65 decoupled the doublet at δ 3 2 as did irradiation of the anomeric doublet at δ 4 46, indicating that the upfield doublets represent signals of the H-2′ and H-2″ protons Irradiation of the doublet at δ 1 18 (rhamnose Me protons) simplified the multiplet at δ 3 84 to a singlet This signal must therefore correspond to H-5″ Decoupling at this multiplet simplified the doublets at δ 1 18 and δ 3 22 (H-4″) to singlets Irradiation of the multiplet centred at δ 3 71 (H-6′) produced a change in the double doublet at δ 3 22 (H-5′) Decoupling of the multiplet centred at δ 3 32 (H-2′ and H-2″) simplified both the H-1″ and H-1′ doublets to singlets and simplified the multiplet at δ 3 43, although a complex pattern remained Irradiation of this system changed the apparent doublets at δ 3 22 and δ 3 32 to singlets Irradiation of the double doublet at δ 3 22 changed the 8-line H-6′ pattern at δ 3 71 to a simpler 4-line configuration, simplified the multiplet at δ 3 43 and changed the H-5″ multiplet to a singlet We thus assigned peaks according to Table 3 The presence of the 1 → 4 linkage is suggested as the glucose H-4′ peak is shifted downfield by 0 2 ppm as compared with the corresponding rhamnose proton The coupling constants of both anomeric protons are typical of those on β-linked carbons [15, 25]

The structure of the unknown is thus determined to be 1-(4-*O*-β-D-rhamnopyranosyl-β-D-glucopyranosyloxy)-4-hydroxycyclopent-2-en-1-nitrile (Fig 4) and the trivial name passitrifasciatin is proposed

The presence of passibiflorin and epipassibiflorin in both *P biflora* and *P talamancensis* is of taxonomic significance as it confirms their relationship as sister species [28] The presence of another rhamnoglucoside, passitrifasciatin, in *P trifasciata* suggests that diglycosides of this type are typical and diagnostic for Killip's [28] section *Decaloba* of subgenus *Plectostemma* [1, 18]

Table 1 ^{1}H NMR spectral data for passibiflorin as its TMSi derivative in $CDCl_3$

Compound		H-2	H-3	H-4	H-5	(2)	H-1′	H-2′–H-5′	H-6′	H-1″	H-2″-5″	H-6″
Tetraphyllin B*	Chemical shift (ppm)	6 21	6 02	4 99	2 93	2 23	4 47	3 2–3 7	3 8			
	Integral	1	1	1	1	1	1	4	2			
	Configuration	*dd*	*dd*	*m*	*dd*	*dd*	*d*	*m*	*d*			
	Coupling constant (J, Hz)	6	6	—	15	15	7 6	—	7 6			
	J''	—	—	—	7	7	—	—	—			
Passibiflorin	Chemical shift (ppm)	6 35	5 99	4 81	2 98	2 22	4 45	3 1–3 7	4 01†	4 62	3 1–3 7	1 15
	Integral	1	1	1	1	1	1	4	1	1	4	3
	Configuration	*dd*	*dd*	*m*	*dd*	*dd*	*d*	*m*	*d*	*d*	*m*	*d*
	Coupling constant (J, Hz)	5	5	—	14	14	7 6	—	6	7 6	—	6
	J''	—	—	—	7	6	—	—	—	—	—	—

*Data from [9, 15]

†The other H-6′ proton lies at δ 3 75

Table 2 ^{13}C NMR spectral data for the TMSi ethers of passibiflorin (**1a**) and epipassibiflorin (**1b**) from *Passiflora biflora* ($CDCl_3$, 90 MHz), for passibiflorin (**1c**) and tetraphyllin B (**1d**) (D_2O, 90 MHz, ref DSS) and for a flavonoid-7-*O*-β(1 → 6)-rhamnoglucoside* (**1e**) in DMSO

Carbon	1a	1b	1c	1d	1e
1	80 59	80 84	83 59	82 13	
2	141 32	141 76	143 55	142 44	
3	130 75	130 09	133 82	131 81	
4	75 33	73 58	75 47	73 67	
5	46 29	45 71	46 48	46 55	
6	118 74	119 14	121 96	120 22	
1′	100 01	100 29	102 26	100 36	101 30
2′	76 85†	76 49†	74 41†	73 80	73 70
3′	78 52§	79 94§	78 84§	77 02	77 00†
4′	71 82‡	71 55‡	72 23‡	70 34	70 40
5′	77 20§	78 06§	78 16§	76 46	76 40†
6′	62 24	62 00	63 22	61 60	66 70
1″	99 53	100 13	101 66		100 30
2″	74 87	74 80	73 88		71 50
3″	69 61‡	69 74‡	72 10‡		71 00
4″	75 18†	75 18†	74 41†		73 00
5″	68 89	68 89	70 40		69 00
6″	16 52	16 52	17 67		17 80

*Data for acacetin 7-*O*-glycoside from ref [26]
†‡§Assignments may be interchanged within a spectrum

EXPERIMENTAL

Plant material Living material of *P biflora* L was obtained from the Missouri Botanical Garden [voucher specimen University of Illinois Herbarium, (ILL)] Living *P talamancensis* Killip material was a gift of J M MacDougal, Department of Botany, Duke University, Raleigh, NC [voucher specimens (J M 410) at the University of Illinois (ILL) and Duke University (DUKE) herbaria] This species was originally collected by L E Gilbert near Rincon, Osa Peninsula, Costa Rica Living *Passiflora trifasciata* Lem was a gift of P Worley, Kartuz Greenhouses, Vista, CA 92083

Isolation of the glycosides Fresh leaf material (54 g) of *P trifasciata* was extracted with cold 80% MeOH in a Waring blender The suspension was then filtered and concd under vacuum to yield a thick syrup This material was partitioned between H_2O and $CHCl_3$ The aq phase was concd under vacuum and placed on a cellulose column (microcrystalline cellulose–Whatmann CF1–Whatman CF11, 1 1 1) and eluted with Me_2CO–H_2O (5 1) Small aliquots of the fractions collected were evapd and tested for cyanide using the Feigl–Anger method [17] and an enzyme preparation prepared as below Fractions 31–45 (10 ml) were found to contain the cyanogenic glycoside and were pooled and concd This fraction was then chromatographed on Whatman 3MM paper with MeCOEt–Me_2CO–H_2O (15 5 3) The cyanogenic material was located by cutting a 1-cm strip from the centre of the sheet, cutting 1-cm^2 sections from this strip, placing them in vials, adding enzyme preparation and testing above The band containing the compound of interest (R_f 0 3) was desorbed in H_2O and rechromatographed on paper with Me_2CO–H_2O (5 1), the cyanogenic glycoside as found at R_f 0 5 The final product was isolated as a viscous yellow solid (30 0 mg)

Fresh leaf material of *P biflora* (66 g) was extracted and partitioned as above, then chromatographed on Whatman 3MM paper in Me_2CO–H_2O (5 1) (R_f 0 5), MeCOEt–Me_2CO–H_2O (15 5 3) (R_f 0 2) and isopropOH–*n*-ButOH–H_2O (6 3 1) (R_f 0 2) The final product was desorbed on concd to yield a white solid (39 6 mg)

Fresh leaf material of *P talamancensis* (49 g) was extracted as above and chromatographed on Whatman 3MM paper in MeCOEt–Me_2CO–H_2O (15 5 3) The cyanogenic glycoside (R_f 0 2) was desorbed an rechromatographed in Me_2CO–H_2O (5 1) The glycoside (R_f 0 55) was desorbed and concd to yield a viscous white solid (24 5 mg)

Enzyme preparation Specific enzyme preparations were made in order to facilitate hydrolysis and location of cyanogens as has been previously described [6, 11, 18], from fresh leaves of *P biflora* (10 g), *P talamancensis* (10 g) and *P trifasciata* (10 g)

Determination of sugars A small (1 mg) sample of each unknown was checked for purity by ^1H NMR in D_2O Samples were then subjected to a quantitative determination of glucose [19] and were found to possess less than a 1 1 ratio of glucose to cyanide, suggesting that the glucose oxidase reaction did not proceed normally, and that another sugar might be present This was confirmed by hydrolysis of samples of the unknowns (1 mg) by heating with 1 M HCl for 10 min [20] The hydrolysates were then co-chromatographed with standard monosaccharides on microcrystalline cellulose plates in *n*-ButOH–EtOH–H_2O (4 1 2) [21, 22] The plates were dried and sprayed with aniline hydrogen phthalate or *p*-anisidine hydrochloride reagents [23], heating at 100° to visualize the sugars

Another sample of each hydrolysate was analysed by HPLC (Alltex 110A) The samples were dried under vacuum, resuspended in 85% ACN and chromatographed on an amine column (Alltech) (flow rate 1 2 ml/min) Compounds were detected with an RI detector Comparisons of retention and co-retention times were made using standard sugars

Spectral determination ^1H NMR spectra were determined on a Nicolet NT-360 (360 MHz) FT-NMR spectrometer in D_2O and as the TMSi derivates in $CDCl_3$ These were prepared as

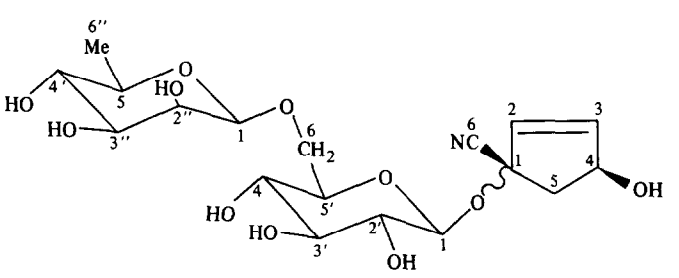

Fig 2 The proposed structures of passibiflorin and epipassibiflorin

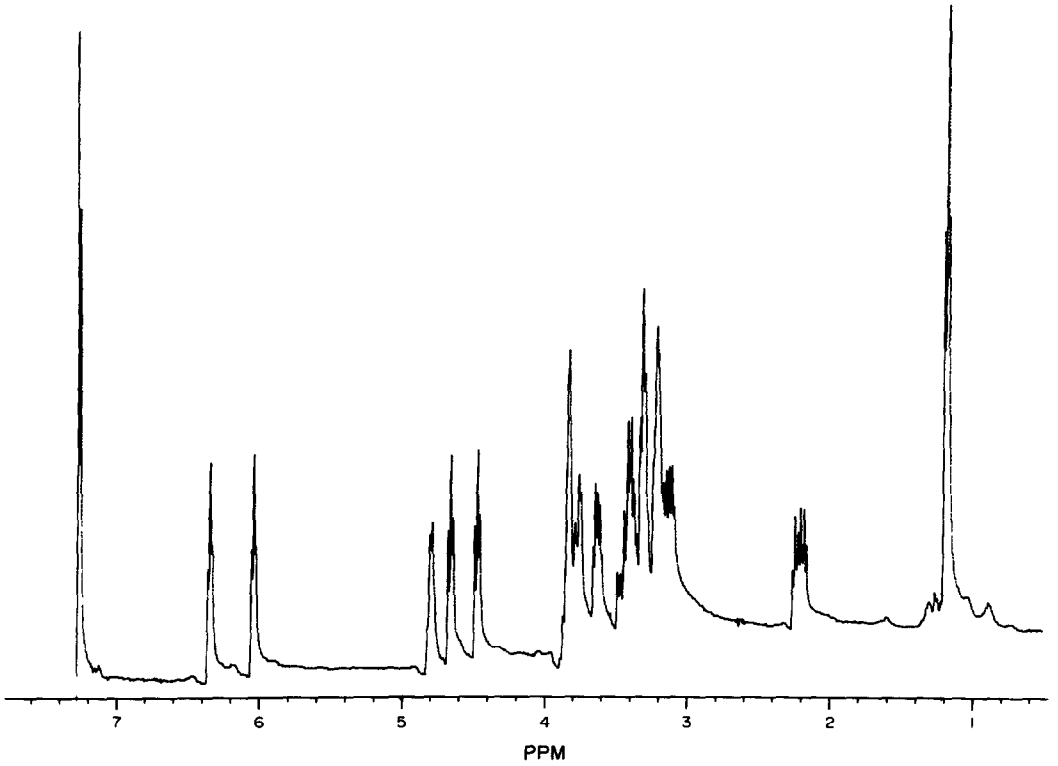

Fig 3 ^{1}H NMR of the TMSi derivative of passitrifasciatin in $CDCl_3$

Table 3 ^{1}H NMR spectral data for passitrifasciatin as its TMSi derivative in $CDCl_3$

	Passitrifasciatin
H-2	6 35 *dd* (1, 6, 1)
H-3	6 04 *dd* (1, 6, 1)
H-4	4 79 *t* (1, 13, 7)
H-5(2)	3 12 *dd* (1, 14, 7)
	2 22 *dd* (1, 14, 6)
H-1′	4 46 *d* (1, 7, —)
H-2′	3 22 *d* (1, 7, —)
H-3′	3 43 *t* (1, —, —)
H-4′	3 43 *t* (1, —, —)
H-5′	3 84 *m* (1, 0, —)
H-6′	3 80 *ddd* (2, 5, 3, 11)
H-1″	4 65 *d* (1, 7, —)
H-2″	3 32 *d* (1, 7, —)
H-3″	3 43 *t* (1, —, —)
H-4″	3 22 *t* (1, —, —)
H-5″	3 84 *m* (1, —, —)
H-6″	1 18 *d* (3, 6, —)

Figures in parentheses are integral value, coupling constant (Hz), *J*′

previously described [24] ^{13}C NMR spectra of the unknowns in D_2O (ref DSS) and their TMSi derivatives in $CDCl_3$ were measured on the same instrument (90 MHz) Decoupling experiments were performed on TMSi derivatives in $CDCl_3$

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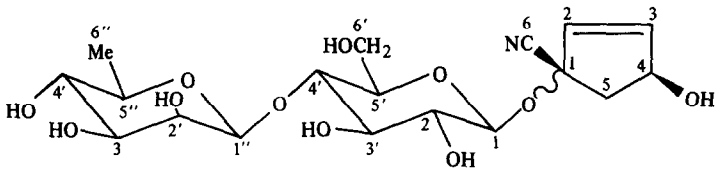

Fig 4 Proposed structure of passitrifasciatin

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