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PASSICOCCIN: A SULPHATED CYANOGENIC GLYCOSIDE FROM *PASSIFLORA COCCINEA*

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Key Word Index—*Passiflora coccinea*; Passifloraceae; passicoccin; cyanogenic glycoside; cyanogenic glycoside sulphate; tetraphyllin B sulphate.

Abstract—A novel cyclopentenoid cyanogenic glycoside (1-(6-*O*-β-D-rhamnopyranosyl-β-D-glucopyranosyloxy)-cyclopent-2-en-1-nitrile-4-sulphate) has been isolated from *Passiflora coccinea*. The structure was determined by means of the ^1H and ^{13}C NMR spectrum of the sulphate and its corresponding acetate derivative. Identification of the sugar constituents was made by HPLC and TLC. Passicoccin is so far unique to subgenus *Distephana* and its presence here is evidence for a phylogenetic relationship between *Distephana* and subgenera *Granadilla* and *Tacsonia*.

INTRODUCTION

Passiflora coccinea is a member of the small subgenus *Distephana* whose members are segregated based on having a dependent operculum, tubular flowers and a two-ranked corona, the inner of which is partly united into tubular membrane [1]. The large subgenus *Granadilla* differs in having open-campanulate flowers with a five-ranked corona and an erect to horizontally spreading operculum. Because of these morphological differences, the two subgenera have not been considered to be closely related. Subgenus *Distephana* has been considered to be more closely related to subgenus *Tacsonia* which also has a dependent operculum, a one- or two-ranked corona (though free), and a tubular flower (though the latter is considerably elongated). Subgenus *Distephana* has previously been included as a section of *Tacsonia* [1].

Isolation of tetraphyllin B sulphates from *Passiflora caerulea* and *P. alatocaerulea* has previously been reported [2] and these compounds have since been isolated from other members of subgenus *Granadilla* and from *P. mollissima* of subgenus *Tacsonia* [unpublished data].

RESULTS AND DISCUSSION

Sodium fusion analysis demonstrated the presence of sulphur in the unknown cyanogen. The compound had an R_f similar to that of tetraphyllin B sulfate [2] on PC and also had a similar R_t on HPLC.

The ^1H NMR data for the unknown (Table 1) confirm that it is a cyclopentenoid cyanogenic glycoside with a single oxygenated substituent [2, 3] at C-4. Failure of the compound to form a soluble TMSi derivative suggested that this substituent might be a sulphate [2]; this was confirmed by sulfatase hydrolysis. The presence of two anomeric protons and a doublet at δ4.0 ($J = 7$ Hz) suggests that the compound is a 1,6-diglycoside [4]. The doublet at δ1.2 corresponds to the C-6 Me group of rhamnose. The ^{13}C NMR data (Table 2) are consistent with the structure shown in Fig. 1. FD-MS of the unknown gave a parent ion at m/z 514 and a fragment at m/z 366 $[M - \text{rhamnose}]^+$. We propose the trivial name passicoccin for the compound herein described.

The presence of structurally related sulphated cyclopentenoid cyanogens in subgenera *Distephana*, *Granadilla* and *Tacsonia* supports their taxonomic relationship, but alteration of the basic floral structure in *Distephana* suggests that this subgenus has evolved under different selective influences.

EXPERIMENTAL

Plant material. Passiflora coccinea Aubl. was obtained from the Botanic Garden of Adelaide, Australia. A voucher specimen is on deposit in the University of Illinois Herbarium (ILL).

Isolation of glycoside. Leaf material of *P. coccinea* (266 g dry wt) was blended in cold 80% MeOH, filtered, concd under vacuum and extracted with $CHCl_3$. The aq phase was retained and chromatographed on a microcrystalline cellulose: Whatman CF 1 (1:1) cellulose column in Me_2CO–H_2O (1:1). Cyanogenic fractions (20 ml) were located by transferring small aliquots of fractions into vials, evaporating the solvent and adding enzyme prepn (see below). Cyanide as a hydrolysis product was detected with Feigl–Anger test strips [5]. The cyanogenic fractions were combined, concd under vacuum and rechromatographed on a microcrystalline cellulose: Whatman CF 11 (1:1) column in *iso*-PrOH–*n*-BuOH–H_2O (6:3:1). The cyanogenic material (fractions 50–70) was concd as before and chromatographed on paper (Whatman 3MM) in the same solvent for 7 days. The compound of interest was located on the paper by cutting a 1 cm wide strip from the centre of the paper, cutting 1 cm^2 sections from this strip, placing them in vials and testing as above. The cyanogenic compound (R_f 0.2) was desorbed in H_2O and concd under vacuum to yield an amorphous yellow solid (0.1% yield).

Enzyme preparation. From leaves of *P. alatocaerulea* as previously described [2].

Quantitative determination of cyanide and sugars. Quantitative estimation of glucose [6] established its presence in a 1:1 ratio with cyanide [7].

Hydrolysis of a sample (10 mg) in 1 M HCl [8] followed by cochromatography on Whatman 3MM paper in pyridine–

Table 1. ^{1}H NMR spectral data for passicoccin (**1a**) and tetraphyllin B sulphates epimeric mixture (**1b**) in D_2O and MeOD-d_4, and for their corresponding acetate derivatives of this mixture in $CDCl_3$ (**1c**, **1d**)

	1a	1b*	1c	1d
H-2	6.39 *dd* (1, 5, 2)†	6.41 *dd* (1, 6, 2) 6.53 *dd* (1, 6, 2)	6.34 *dd* (1, 6, 1)	6.34 *dd* (1, 5, 1)
H-3	6.26 *d* (1, 5, —)	6.16 *dd* (1, 6, 1) 6.39 *dd* (1, 6, 2)	6.08 *dd* (1, 6, 1)	6.08 *dd* (1, 6, 1)
H-4	5.46 *m* (1, —, —)	5.66 *m* (1, —, —) 5.59 *m* (1, —, —)	5.72 *m* (1, —, —)	5.74 *m* (1, —, —)
H-5 a	2.58 *dd* (1, 16, 6)	2.46 *dd* (1, 15, 4) 2.66 *dd* (1, 15, 7)	2.85 *dd* (1, 15, 8)	2.47 *dd* (1, 15, 7)
H-5 b	2.53 *dd* (1, 16, 3)	2.77 *dd* (1, 15, 3) 2.08 *dd* (1, 15, 7)	3.04 *dd* (1, 15, 8)	2.88 *dd* (1, 15, 7)
H-1′	4.35 (1, 6, —)	4.32 *d* (1, 8, —) 4.77 *d* (1, 8, —)		
H-2′–5′	3.1–3.7 *m* (4, —, —)	3.2–3.7 *m* (8, —, —)		
H-6′ a	4.02 *d* (1, 6, —)	3.88 *m* (2, —, —)		
6′ b	3.75 *dd* (1, 6, 2)			
H-1″	4.49 *d* (1, 6, —)			
H-2″–5″	3.1–3.7 *m* (4, —, —)			
H-6″	1.20 *d* (3, 7, —)			

*Data from ref. [2].
†Figures in parentheses are: integral value, J(Hz), J'.

Table 2. ^{13}C NMR data for passicoccin **2a** and passibiflorin **2b**, and revision of assignments of carbon signals for tetraphyllin B sulphate **2c** and epitetraphyllin B sulphate (**2d**) (D_2O, ref. dioxane)

Carbon	2a	2b*	2c†	2d†
1	82.39	83.59	83.50	82.37
2	140.38	143.55	139.51	140.30
3	134.14	133.82	135.62	134.07
4	76.34	75.47	73.71?[a]	76.41[a]
5	45.06	46.48	42.88	44.98
6	121.60	121.96	115.78	115.78
1′	102.34 (104.52?)	102.26	100.25	98.48
2′	73.65[a]	74.41[a]	73.71	73.71
3′	77.65[c]	78.84[c]	76.96[a]	76.58[a]
4′	70.53[b]	72.23[b]	70.44	70.21
5′	77.52[c]	78.16[c]	76.58[a]	76.88[a]
6′	63.22	63.22	61.39	61.39
1″	100.29	101.66		
2″	73.27	73.88		
3″	70.53[b]	72.10[b]		
4″	73.65[a]	74.41[a]		
5″	69.39	70.40		
6″	21.02	17.67		

*Data from ref. [13].
†Data from ref. [2]. Separation of signals from the two epimers was made possible through the reisolation of tetraphyllin B sulphate and epitetraphyllin B sulphate in a 3:1 ratio from another *P. caerulea* sample. Nomenclature is in reference to tetraphyllin B and epitetraphyllin B [13].
[a,b,c]Assignments within a single spectrum may be interchangeable.

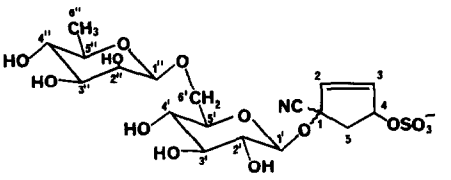

Fig. 1. The proposed structures for passicoccin and epipassicoccin.

EtOAc–HOAc–H_2O (32:32:21:7) [3, 9, 10] with standard sugars revealed the presence of both glucose and rhamnose. Compounds were visualized with *p*-anisidine HCl [11]. A sample of the above hydrolysate was also injected onto an HPLC column (Alltech NH_2) and eluted with 85% MeCN (flow rate 1.0 ml/min) [12] and visualized with a RI detector.

Qualitative determination of sulphur. A Na fusion analysis was carried out on a small sample of the cyanogen (10 mg) from both species [13]. The test proved positive for the presence of S.

Hydrolysis of cyanogen of P. coccinea *with sulphatase.* A sample of the cyanogen (1 mg) was dissolved in H_2O and sulphatase (0.5 mg, Sigma Chemical Co., aryl sulphatase H-1 from *Helix pomatia*) was added. After incubation at 25° for 5 min, three drops of $Ba(OH)_2$ (0.3 N) were added. A dense ppt of $BaSO_4$ resulted [2]. Controls with enzyme alone did not yield a ppt.

Preparation of acetate of cyanogen. A sample of the unknown (10 mg) was dried under vacuum and then dissolved in pyridine (0.5 ml). Ac_2O (1 mg) was added, the mixture warmed for 10 min and the sample then taken to dryness under vacuum. The solid material produced was extracted with $CHCl_3$ and the extract concd. This material was then purified by prep. TLC on silica gel

with $CHCl_3$–C_6H_6–MeOH (40:9:1). Duplicate plates were run. One plate was sprayed with H_2SO_4–chromic acid soln and charred for 15 min at 100°. This treatment revealed two major spots, one at R_f 0.5 and one at R_f 0.7. Each band was desorbed from the duplicate plate with $CHCl_3$. ^{1}H NMR showed that the material at R_f 0.5 contained the acetates of glucose and rhamnose, while the material at R_f 0.7 contained the acetylated cyanogen.

HPLC of unknown. A sample was subjected to HPLC on an Alltech NH_2 column in 80% MeCN (flow rate 1.2 ml/min). A broad major peak between R_t 24.7 and 27.5 min was observed. Tetraphyllin B sulphate has R_t 27 min [12] (RI detector).

Mass spectral measurement. The FD-MS was determined on a low resolution spectrometer.

NMR determination. ^{1}H NMR spectra were measured at 360 MHz. ^{13}C NMR spectra were measured on the same instrument at 22.5 MHz.

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