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PASSISUBEROSIN AND EPIPASSISUBEROSIN: TWO CYCLOPENTENOID CYANOGENIC GLYCOSIDES FROM *PASSIFLORA SUBEROSA*

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Key Word Index—*Passiflora suberosa*; Passifloraceae; cyanogenic glycoside; passisuberosin; epipassisuberosin.

Abstract—A novel cyclopentenoid cyanogenic glycoside, passisuberosin (1-(β-D-glucopyranosyloxy)-4-hydroxy-2,3-epoxycyclopentanenitrile), has been isolated from *Passiflora suberosa*. Its structure was determined by means of ^{1}H NMR and ^{13}C NMR and the identity of the glycosidic moieties by HPLC and TLC. A probable C-1 epimer, epipassisuberosin, was also present, as were smaller amounts of passicoriacin and epipassicoriacin, previously isolated from *Passiflora coriacea*. In addition, the presence of diglucosides of passisuberosin and epipassisuberosin was detected. These compounds differ in structure from those produced by other members of section *Cieca*, subgenus *Plectostemma* of *Passiflora*; the data suggest that the taxonomic placement of these two species should be re-evaluated.

INTRODUCTION

Passiflora suberosa L. is a member of subgenus *Plectostemma* section *Cieca* [1]; members of this subgenus typically elaborate valine/isoleucine-derived aliphatic cyanogenic glycosides [2–4]. Initial screening of cyanogenic glycosides in this species with different enzyme preparations [5] suggested that the major cyanogenic constituent of *P. suberosa* was a unique cyanogenic glycoside and probably a cyclopentenoid diglycoside. Similar compounds have recently been described from section *Decaloba* of subgenus *Plectostemma* [6].

As part of a larger chemosystematic study of cyanogenesis in *Passiflora*, we undertook to isolate the cyanogens of *P. suberosa* and to compare them with those elaborated by species considered to be closely related.

RESULTS AND DISCUSSION

The cyanogenic material isolated from *P. suberosa* gave very slow cyanide tests when hydrolysed with enzyme preparations made from *Turnera ulmifolia* or *Passiflora foetida* acetone powders [7], emulsin or linamarase, but hydrolysed readily when exposed to a *P. suberosa* enzyme fraction prepared as previously reported [8]. These results are consistent with those obtained for cyanogenic cyclopentenoid diglycosides reported previously [6]. However, treatment with other enzyme preparations known to hydrolyse the latter compounds (*P. biflora*, *P. trifasciata*) also gave poor results, suggesting the presence of a novel compound. Treatment with an enzyme preparation from *Passiflora coriacea* Juss. did cause some hydrolysis.

HPLC analysis of the unknown mixture showed that several compounds were present, including at least one with a retention time similar to that of cyanogenic diglycosides (9.0 min) and two with retention times similar to that of passicoriacin (7.0 min) [9].

R_f values from PC for the various cyanogens in the mixture were consistent with the presence of a diglycoside, passicoriacin, and a novel compound.

The ^{1}H NMR data for the unknowns are summarized in Table 1. Proton assignments were made by double resonance techniques. Irradiation of the doublets at δ4.60 in **1a** or δ4.85 in **1b** simplified a multiplet at δ3.33. Irradiation of the apparent triplet at δ4.27 in **1a** collapsed the two four-line patterns at δ2.38 and 1.93 to doublets; other experiments show that all three systems are coupled. Irradiation of the apparent triplet at δ4.48 in **1b** collapsed the two four-line patterns at δ2.06 and 1.57 in a similar fashion. Irradiation of either of the four-line patterns in a pair simplified the other. Double resonance experiments also demonstrated the presence of passicoriacin and its epimer, which have been described previously [9].

Table 1. ^{1}H NMR data for a mixture of passisuberosin (**1a**) and epipassisuberosin (**1b**) as their TMSi derivatives in $CDCl_3$ (chemical shifts, ppm)

H	1a	1b
2	3.83 (1, *d*, 3)	3.82 (1, *d*, 3)
3	3.48 (1, *dd*, 2, 1)	3.47 (1, *d*, 2)
4	4.27 (1, *dd*, 8)	4.48 (1, *m*)
5a	2.38 (1, *dd*, 12, 7)	2.06 (1, *dd*, 12, 7)
5b	1.93 (1, *dd*, 13, 8)	1.57 (1, *dd*, 12, 8)
1′	4.60 (1, *d*, 7)	4.85 (1, *d*, 7)
2′	3.33 (1, *m*)	3.33 (1, *m*)
3′	3.41 (1, *m*)	3.42 (1, *m*)
4′	3.44 (1, *m*)	3.43 (1, *m*)
5′	3.26 (1, *m*)	3.26 (1, *m*)
6′a	3.76 (1, *dd*, 11, 2)	3.76 (1, *dd*, 11, 2)
6′b	3.62 (1, *dd*, 11, 5)	3.62 (1, *dd*, 11, 5)

Numbers in parentheses following the chemical shifts are integral, type of signal, J (Hz) and J'.

The ^{13}C NMR data (Table 2) are consistent with the structures proposed in Fig. 1. The compounds differ from tetraphyllin B and passicoriacin as they possess signals from two epoxide carbons between δ 56 and 59, and the C-1, C-4 and C-5 carbons show an upfield shift. The epoxide carbon signals are virtually identical with those of cyclopentene oxide (δ 57.2 and 58.7) [10].

The presence of the corresponding gentiobiosides was demonstrated by the presence of a second pair of anomeric carbon (1') signals downfield from those of passisuberosin and epipassisuberosin at δ 100.70 and 100.29, by two additional anomeric carbon (1") peaks at δ 104.07 and 103.71, and by the presence of an extra carbon peak (C-6') typical of gentiobiose [11, 12] at δ 70.0.

This was confirmed in the ^1H NMR spectrum of the gentiobiosides where two sets of doublets (δ 4.05 and 4.00) are found. These are typical of one H-6 proton of the internal glucose of a β-(1 → 6)-linked diglucoside. A second apparent quartet at δ 3.68 (2, *dd*, 11, 5) accounted for the second H-6 proton of the same sugar.

Additional anomeric proton signals were seen in the region δ 4.2–4.8, but the assignments of each could not be made with certainty.

The presence of an epoxide group in passisuberosin was confirmed by the periodate and chlorohydrin tests [13].

Elaboration of passicoriacin and epipassicoriacin by *P. suberosa* is suggestive of a close relationship to *P. coriacea* (section *Cieca*, of subgenus *Plectostemma*), as has been proposed by Killip [1]. However, as other members of the section typically produce isoleucine/valine and leucine-derived cyanogenic glycosides, these two species may be considered to be less closely allied with the section as a whole, and should, perhaps, be placed in another cyclopentenoid-producing section.

Table 2. ^{13}C NMR data for a mixture of passisuberosin (**1a**) and epipassisuberosin (**1b**) in D_2O (chemical shifts, ppm)

C	1a	1b
1	76.74	76.20
2	58.43	58.22
3	57.23	56.59
4	68.94	66.99
5	36.88	38.64
6	118.20	119.17
1'	100.77	99.81
2'	73.62	73.94
3'	77.05	76.75
4'	70.07	70.50
5'	76.27	76.61
6'	61.24	61.60

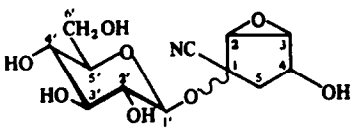

Fig. 1. Proposed structures of passisuberosin (**1a**) and epipassisuberosin (**1b**).

EXPERIMENTAL

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

Isolation of the glycosides. Fresh leaf material (388 g) was ground in a blender with 80% MeOH, filtered and concentrated under vacuum. The resulting extract was partitioned between H_2O and $CHCl_3$; the aq. phase was concentrated under vacuum, placed on a Sephadex G-10 column and eluted with H_2O. The cyanogenic material was located (fractions 4–6) as previously described [14]. This material was concentrated and chromatographed over a microcrystalline cellulose–Whatman CF 1–Whatman CF 11 (1:1:1) cellulose column with *iso*-PrOH–*n*-BuOH–H_2O (6:3:1). The cyanogenic material (fractions 20–80) was then rechromatographed with a gradient of Me_2CO–H_2O (5:1 to 1:1) over 8 hr. Cyanogens (fractions 80–160) were chromatographed on paper (Whatman 3MM) in *iso*-PrOH–*n*-BuOH–H_2O (6:3:1) and the cyanogen was located as previously reported [15]. The cyanogenic material (R_f 0.25) was rechromatographed on paper in MeCOEt–Me_2CO–H_2O (15:3:3) and found at R_f 0.33. Greater resolution of the cyanogens was achieved by PC with Me_2CO–H_2O (5:1) which yielded two cyanogenic bands, one at R_f 0.6 (corresponding to passicoriacin and its epimer) and another at R_f 0.7 (corresponding to an unknown mixture of cyanogenic compounds). The latter was a white solid (357 mg, 0.1%). Additional purification was accomplished by PC in *iso*-PrOH–*n*-BuOH–H_2O (6:3:1). In this solvent, two bands were observed, one at R_f 0.35 and the other at R_f 0.60.

Determination of sugars. A small sample of each of the unknowns (1 mg) was hydrolysed in hot 1 M HCl for 10 min [16, 17]. The hydrolysate was chromatographed with standard sugars on microcystalline cellulose TLC plates in *n*-BuOH–EtOH–H_2O (4:1:2) [18]. The plates were dried and the sugars visualized with *p*-anisidine hydrochloride and aniline hydrogen phthalate reagents [19] by heating at 100°. The hydrolysates were also analysed by HPLC (Altex 110A, Alltech amine column, flow rate 1.2 ml/min, 85% MeCN, refractive index detection). Comparison with standard sugars was made.

HPLC of cyanogens. Using the system described above, several cyanogenic components were separated. The major peak (7.0 min) overlapped a minor peak at 6.9 min. Another peak occurred at 9.0 min. These were subsequently assigned to passisuberosin, passicoriacin and passisuberosin gentiobioside, respectively.

Quantitative determination of sugar and cyanide. Using the method of Washko and Rice [20] in conjunction with a Lambert quantitative cyanide assay [21] as previously described [3, 6], it was determined that cyanide and glucose were present in equimolar amounts in passicoriacin and passisuberosin, and that the ratio of glucose to cyanide was 2:1 for the passisuberosin gentiobiosides.

Spectral determination. ^1H NMR spectra were measured on a Nicolet NT-360 (360 MHz) FT spectrometer and decoupling experiments were carried out on a Bruker WM-500 (500 MHz) spectrometer as the TMS; derivatives in $CDCl_3$. These were prepared as described previously [22]. ^{13}C NMR spectra of the unknowns in D_2O (reference TSP) were measured on the Nicolet instrument at 22.5 MHz (reference DSS).

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