Identification of (*R*)-Vicianin in *Davallia trichomanoides* Blume

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Dedicated to Professor Hans Grisebach on the occasion of his 60th birthday

Davallia trichomanoides, Cyanogenic Glycoside (*R*)-Vicianin

The cyanogenic glycoside of young fronds and fiddleheads of the fern *Davallia trichomanoides* Blume was identified as (*R*)-vicianin (the β-vicianoside of (*R*)-mandelonitrile) by acid and enzymic hydrolysis, ^{1}H-NMR and ^{13}C-NMR spectroscopy, and by comparison with an authentic sample isolated from *Vicia angustifolia* seeds.

Introduction

(*R*)-Vicianin (the β-vicianoside of (*R*)-mandelonitrile; Fig. 1) displays a limited distribution within the Plant Kingdom [1]. Originally identified in *Vicia angustifolia* seeds [2, 3], this cyanogenic disaccharide also occurs in three species of the fern *Davallia,* namely *D. bullata* Wall., *D. denticulata* (Burm.) Mett., and *D. fijiensis* Diels [4]. In this paper, we describe the isolation and structure elucidation of (*R*)-vicianin from *Davallia trichomanoides* fronds and fiddleheads using authentic (*R*)-vicianin obtained from *V. angustifolia* seeds and (*R*)-amygdalin for comparative purposes.

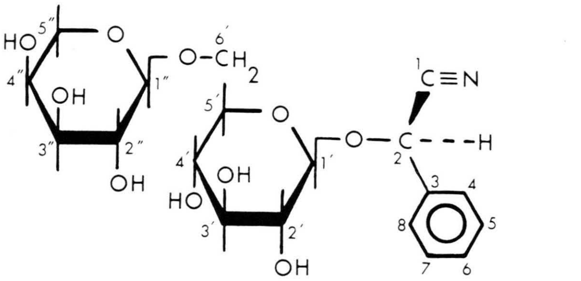

Fig. 1. Structure of (*R*)-vicianin with carbon numbering system used for ^{13}C-NMR chemical shift assignments.

Results and Discussion

Cyanogenic tissues of *D. trichomanoides* and *V. angustifolia* were extracted by similar methods to obtain the crude cyanogen. Subsequent recrystallization from benzene-methanol yielded white, needle-like crystals which were subjected to chemical and spectral analysis.

Partial acid hydrolysis of the *D. trichomanoides* cyanogen and authentic vicianin yielded identical products, namely D-glucose, L-arabinose, and (*R*)-prunasin (or (*S*)-sambunigrin). The presence of prunasin or sambunigrin amongst the hydrolysis products indicates that in both original cyanogens glucose was attached directly to the aglycone and that arabinose was the terminal sugar. The characteristic odor of benzaldehyde was also noted during hydrolysis, further indicating that the aromatic aglycone was derived from phenylalanine rather than tyrosine.

As described earlier [5], enzymic hydrolysis of authentic vicianin by the *D. trichomanoides* β-glycosidase released a disaccharide which yielded D-glucose and L-arabinose upon acid hydrolysis. The *D. trichomanoides* cyanogen behaved identically under these conditions.

^{1}H-NMR spectra of the *D. trichomanoides* cyanogen and authentic vicianin appeared virtually identical and correlated very closely with values obtained by Turczan *et al.* [6] for vicianin. The observed chemical shifts and their proton assignments are given in Table I. The presence of two methine peaks near 6.0 ppm indicated that both (*R*)- and (*S*)-epimers were probably present. Base-catalyzed racemization, as described by Turczan *et al.* [6], confirmed that the smaller, downfield peak was due to the (*S*)-epimer. Analysis of the peak areas showed that the *D. trichomanoides* cyanogen contained both (*R*)- and (*S*)-epimers in the ratio of 92:8, while the same ratio for vicianin was 83:17. Since, with one noted exception, both epimers of a particular cyanogenic glycoside generally do not co-occur in the same species [7], it appears that some epimerization of the *D. trichomanoides* and *V. angustifolia* cyanogens occurred during isolation. In subsequent studies (data not shown), vicianin was isolated from *V. angustifolia* seeds in 80% methanol at room temperature. The ^{1}H-NMR spectrum of the TMS-ether of vicianin in

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Table I. 360 MHz ^{1}H-NMR chemical shifts and assignments for *D. trichomanoides* cyanogen and *V. angustifolia* vicianin[a].

Proton	*D. trichomanoides* Cyanogen [ppm]	*V. angustifolia* Vicianin [ppm]	Vicianin [6] [ppm]
Phenyl	7.45−7.59	7.47−7.59	7.38−7.63
Methine	5.97	5.97	5.96
H1	4.23	4.23	4.22
H2	3.08	3.10	3.0−3.13
H3	3.08	3.10	3.0−3.13
H4	3.08	3.10	3.0−3.30
H5	3.28−3.44	3.28−3.41	3.3−3.44
H6	3.54−3.71	3.55−3.71	3.55
	3.99	3.99	3.95
H1′	4.32	4.32	4.31
H2′	3.28−3.44	3.28−3.41	3.0−3.13
H3′	3.28−3.44	3.28−3.41	3.3−3.44
H4′	3.54−3.71	3.55−3.71	3.63
H5′	3.28−3.44	3.28−3.41	3.3−3.46
	3.54−3.71	3.55−3.71	3.68
OH2	5.30	5.30	5.30
OH3	5.08	5.08	5.08
OH4	5.08	5.08	5.08
OH2′	4.51	4.50	4.50
OH3′	4.64	4.64	4.65
OH4′	4.90	4.90	4.91

[a] All data were obtained in DMSO-d$_6$ at 298 °K and compared to the internal standard TMS (δ = 0.00).

Table II. 90 MHz ^{13}C-NMR chemical shifts and assignments for *D. trichomanoides* cyanogen, *V. angustifolia* vicianin, and amygdalin[a].

Carbon	*D. trichomanoides* Cyanogen [ppm]	*V. angustifolia* Vicianin [ppm]	Amygdalin[b] [ppm]
C1	118.6	118.6	118.8
C2	69.9	69.9	66.7
C3	133.6	133.6	133.8
C4 C8	128.8	128.8	128.9
C5 C7	127.2	127.2	127.2
C6	129.4	129.4	129.5
C1′	101.4	101.4	101.5
C2′	73.0	73.0	73.1
C3′	76.4	76.4	76.5[c]
C4′	70.7	70.7	70.0
C5′	76.2	76.2	76.5[c]
C6′	68.1	68.1	68.4
C1″	103.8	103.8	103.7
C2″	72.4	72.4	73.7
C3″	67.5[c]	67.5[c]	76.5[c]
C4″	66.7[c]	66.7[c]	70.0
C5″	65.1	65.1	76.5[c]
C6″	–	–	61.0

[a] All data were obtained in DMSO-d$_6$ at 298 °K and compared to the internal standard TMS (δ = 0.00).

[b] Supplied by Sigma Chemical Co.

[c] Assignments interchangable.

CDCl$_3$ revealed only the (*R*)-epimer, supporting our assumption that higher extraction temperatures allowed vicianin epimerization.

In recent years, ^{13}C-NMR spectroscopy has become a powerful method for cyanogenic glycoside identification. Spectra are currently available for several mandelonitrile glycosides but these exclude (*R*)-vicianin [8−12]. In this study, the ^{13}C-NMR spectrum of vicianin was recorded in DMSO-d$_6$, and the chemical shifts and their assignments are summarized in Table II. The *D. trichomanoides* cyanogen displayed an identical spectrum which is shown in Fig. 2. The chemical shift assignments for C1–C8, C1′ and C1″ were based on the studies of Hübel *et al.* [9]. The presence of an inter-sugar β-linkage, characterized by shifts in the region 104−105 ppm, confirmed the assignment of C1″ [13]. The use of delayed decoupling allowed the assignment of C6′ and C5″ for vicianin and the *D. trichomanoides* cyanogen. The remaining carbons were assigned by analogy with data from Perlin *et al.* [14].

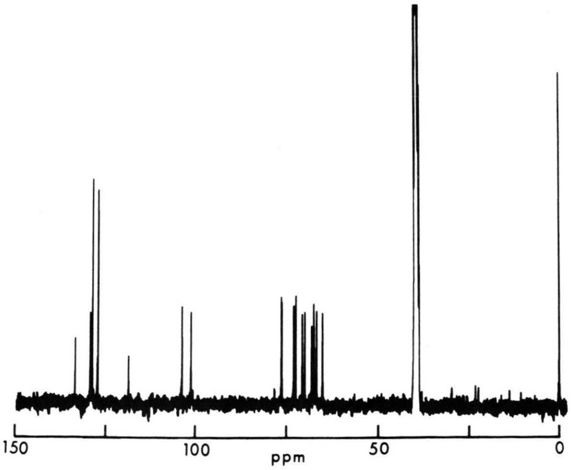

Fig. 2. 90 MHz ^{13}C-NMR spectrum of *D. trichomanoides* cyanogen in DMSO-d$_6$ at 23,810 Hz sweep width.

Although EI and CI mass spectrometry failed to give the expected molecular ion of 427, the *V. angustifolia* and *D. trichomanoides* cyanogens showed identical fragmentation patterns (data not shown). The major peak corresponding to MW 116 was attributed to the cyanobenzylic fragment, which is characteristic of aromatic cyanogenic glycosides [15].

Based on these findings, we conclude that the cyanogen in young fronds and fiddleheads of *D. trichomanoides* is (*R*)-vicianin.

Experimental

Plant materials

Davallia trichomanoides specimens were purchased from Fountain Square Nurseries (Sacramento, CA) and Alberts and Merkel Bros. Inc. (Boynton Beach, FL) and grown under natural conditions in the greenhouse. Voucher specimens have been deposited in the University of Iowa Herbarium. *Vicia angustifolia* seeds were a kind gift of Dr. E. E. Conn, University of California-Davis.

Isolation of cyanogens

Freshly collected young fronds and fiddleheads (30–60 g) of *D. trichomanoides* were extracted in boiling 80% methanol for 10 min. After removing the plant pulp by filtration, the filtrate was extracted twice with an equal volume of chloroform to remove unwanted lipid-soluble substances including chlorophyll. The methanolic fraction was reduced to dryness, redissolved in water, and applied to a column (45 × 2 cm diam) of cellulose (Avicel, Merck) which was pre-equilibrated and eluted with water-saturated *n*-butanol. Fractions giving a positive Feigl-Anger test [16] were pooled, evaporated to dryness, and redissolved in benzene-methanol (1:1, v/v). Crystals which formed initially at −20 °C were recrystallized at room temperature and dried under vacuum at 61 °C. Vicianin was obtained from *V. angustifolia* seeds as described above after first grinding this tissue in liquid N_2.

Acid hydrolysis

Cyanogens (50 nmol) were hydrolyzed with 8.3% trifluoroacetic acid for 3 h at 100 °C. Reaction products were analyzed by TLC on Whatman K5 Si gel plates (2 irrigations with acetonitrile:water, 85:15) with detection by methanolic H_2SO_4 [5].

Enzymic hydrolysis

Cyanogens (3 mg) were incubated at 30 °C for 4.5 h with vicianin hydrolase (isolated as previously described [5]) before terminating the reaction by exposure to 100 °C for 5 min. Hydrolysis products were analyzed by TLC on Whatman K5 Si gel plates (three irrigations with acetonitrile:water, 85:15). The disaccharide was eluted overnight with H_2O and hydrolyzed with 9.1% trifluoroacetic acid (3 h, 100 °C). Products were co-chromatographed with authentic sugars in the following systems: (i) on Bakerflex cellulose TLC plates (Pyr:EtOAc:HOAc:H_2O, 36:36:7:21) with detection by aniline phthalate [17], and (ii) on Whatman K5 Si gel plates (three irrigations with acetonitrile:H_2O, 85:15) with detection by methanolic H_2SO_4.

Nuclear magnetic resonance spectroscopy

^1H and ^{13}C-NMR spectra were recorded at 298 °K at 360 and 90 MHz respectively, on a Bruker WM-360 NMR spectrometer using tetramethylsilane (TMS) as internal standard. The TMS-ether of (*R*)-vicianin was prepared using Sigma Sil A reagent as directed by the manufacturer. After reaction, the solution was passed through a Millipore filter, dried under N_2, and then under vacuum to remove excess pyridine before dissolving in $CDCl_3$.

Mass spectroscopy

Electron impact (EI) and chemical ionization (CI) spectra of cyanogens were determined using a Hewlett-Packard 5985B mass spectrometer.

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