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ISOPTAQUILOSIDE AND CAUDATOSIDE, ILLUDANE-TYPE SESQUITERPENE GLUCOSIDES FROM *PTERIDIUM AQUILINUM* VAR. *CAUDATUM*

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Key Word Index—*Pteridium aquilinum* var. *caudatum*; Pteridaceae; illudane-type sesquiterpene glucosides; ptaquiloside; isoptaquiloside; caudatoside; pteroside A; pterosin A; pterosin B; pterosin Z; pterosin K.

Abstract—Ptaquiloside and two new illudane-type sesquiterpene glycosides were isolated from *Pteridium aquilinum* var. *caudatum*. One- and two-dimensional NMR analyses revealed the new glucosides to be isoptaquiloside and caudatoside. Four pterosins, A, B, K and Z, were also isolated from a base–acid treated extract.

INTRODUCTION

Ptaquiloside (aquilide A) (**1a**) [1, 2], which readily undergoes acid catalysed hydrolysis to give (2*R*)-pterosin B (**2a**), is a known mutagenic [2] and carcinogenic [3, 4] constituent of the *Pteridium* taxa. This plant has been implicated as a causative agent in many diseases of mammalian farm animals, including thiamine deficiency of monogastric animals, bright blindness of sheep, acute haemorrhagic disease of ruminants and the urinary bladder neoplasia, known as enzootic haematuria, of sheep and cattle [5, 6]. Several investigations have been made of the chemical constituents of the *Pteridium* taxa [7–10] using a variety of bioassay and chemical methods. High performance liquid chromatography (HPLC) has been used [11, 12] to isolate, identify and quantify compounds from the *P. esculentum*. HPLC has also been used [13, 14] to investigate the extractives of *P. aquilinum* var. *caudatum*, one of two taxa of bracken fern most commonly found in the Andes of Venezuela [15], and to isolate from it, (2*S*)-pterosin A (**2b**) and (2*R*)-pterosin B (**2a**), and ptaquiloside (**1a**). In this paper we report the isolation from *Pteridium aquilinum* var. *caudatum* of pterosins A (**2b**), B (**2a**), K (**2c**), Z (**2d**), ptaquiloside (**1a**), and two new illudane-type β-glucosides which we identified as isoptaquiloside (**3a**) and caudatoside (**1b**).

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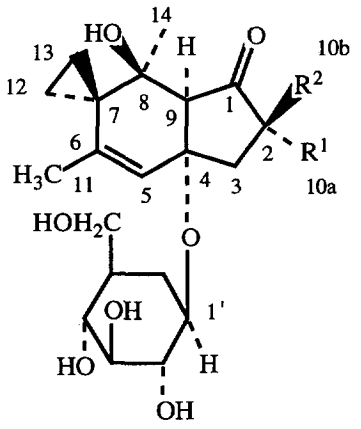
RESULTS AND DISCUSSION

Extracts of *P. aquilinum* var. *caudatum* were examined using methods based on those reported by Agnew and Lauren [11]. Cleaned-up extracts were analysed by analytical HPLC both before and after treatment with base then acid. The expected ptaquiloside and (2*R*)-pterosin B were identified by HPLC retention times compared with authentic specimens of these compounds, and other components potentially related to them were observed. Preparative HPLC of the cleaned-up extracts both before, and after treatment with base then acid, afforded pterosins A, B, K and Z, ptaquiloside, and two new glucosides which were identified as isoptaquiloside (**3a**) and caudatoside (**1b**), on the basis of one- and two-dimensional ^{1}H and ^{13}C NMR and mass spectral analyses, and chemical conversion to pterosins B and A, respectively. In keeping with the known instability of illudane-type glucosides, we observed **3a** and **1b** to be comparatively unstable. Evaporation of the HPLC solvent and sample handling can result in partial conversion of these compounds to pterosins B and A, respectively.

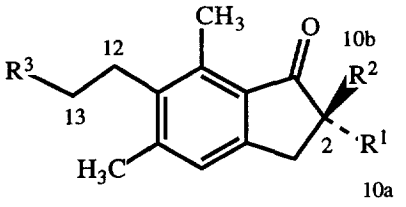
Pterosins A (**2b**), B (**2a**), K (**2c**) and Z (**2d**) were readily identified by comparisons of their published NMR spectral data. One- and two-dimensional NMR spectral data (see Tables 1 and 2) identified the third of three major glucosides as ptaquiloside (**1a**) [1, 2]. The ^{13}C NMR chemical shifts determined in pyridine-d_5 for ptaquiloside (**1a**) corresponded closely with those reported for this compound in methanol-d_4 [1]



1a $R^1 = CH_3$, $R^2 = H$

1b $R^1 = CH_2OH$, $R^2 = CH_3$

1c $R^1 = R^2 = CH_3$

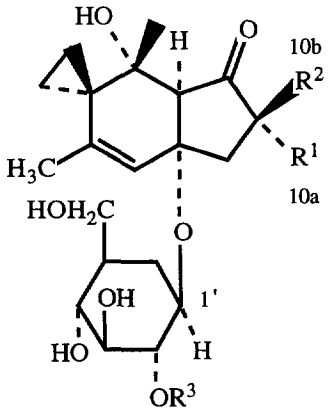


2a $R^1 = CH_3$, $R^2 = H$, $R^3 = OH$

2b $R^1 = CH_2OH$, $R^2 = CH_3$, $R^3 = OH$

2c $R^1 = CH_2OH$, $R^2 = CH_3$, $R^3 = Cl$

2d $R^1 = R^2 = CH_3$, $R^3 = OH$



3a $R^1 = CH_3$, $R^2 = R^3 = H$

3b $R^1 = R^2 = CH_3$, $R^3 = Ac$

3c $R^1 = CH_2OH$, $R^2 = CH_3$, $R^3 = Ac$

and in DMSO-d_6 [2]. Some of the ^1H NMR assignments established for (**1a**) in pyridine-d_5, as elucidated from a combination of conventional and NOE-difference spectra, and two-dimensional ^1H–^1H correlated COSY and ^{13}C–^1H correlated HMQC spectral data, differed significantly for those determined in methanol-d_4 [1] and DMSO-d_6 [2]. Irradiation of the H-9 resonance (δ2.98) of **1a** in an NOE-difference experiment enhanced the anomeric β-glucosyl resonance (5.27 ppm), thereby verifying the rings A and B to be *cis*-fused.

The ^1H and ^{13}C NMR data spectral presented in Tables 1 and 2 identified **3a** as the C-8 epimer of **1a**. Hitherto Saito *et al.* [8, 9] have reported the isolation from *Hypolepis punctata* and *Dennstaedtia hirsta* of some illudane-type sesquiterpene glucosides, including hypoloside A (**3b**), in which the C-8 configuration is epimeric to that established for **1a**. In methanol-d_4 the C-14 resonances of ptaquiloside and the hypolosides, occur in the vicinity of δ27 and 23–24, respectively. In pyridine-d_5 the equivalent resonance of **1a** occurs at δ27.4, while that of **3a** occurs at δ23.0 (see Tables 2 and 3). The marked differences in the ^1H NMR resonances of the 8-Me groups of **1a** (δ1.70)

Table 1. ^{1}H NMR chemical shifts (δ ppm in pyridine-d_5) and coupling constants (Hz) determined for some illudane type β-glucosides

	1a	**3a**	**1b**
H-2β	2.26 (*m*)*	2.75 (*m*)†	
H-3α	2.17 (*m*)*	2.75 (*m*)†	2.51 (*d*, J = 13.4 Hz)
H-3β	2.68 (*m*)	2.41 (*m*)	3.46 (*d*, J = 13.4 Hz)
H-5	6.06 (*s*)	6.23 (*s*)	6.18 (*s*)
H-9α	2.97 (*d*, J = 1.2 Hz)	3.14 (*br s*)	3.27 (*br s*)
H-10/10a	0.96 (*d*, J = 6.5 Hz)	1.14 (*d*, J = 6.6 Hz)	1.06 (*s*)
H′-10b			3.53 (*d*, J = 10.1 Hz)
H″-10b			4.09 (*d*, J = 10.1 Hz)
H-11	1.44 (*s*)	1.47 (*s*)	1.45 (*s*)
H′-12	0.47 (*m*)	0.58 (*m*)	0.62 (*m*)
H″-12	0.84 (*m*)	0.76 (*m*)	0.80 (*m*)
H′-13	0.88 (*m*)	0.76 (*m*)	0.86 (*m*)
H″-13	1.08 (*m*)	1.00 (*m*)	1.16 (*m*)
H-14	1.70 (*s*)	1.48 (*s*)	1.68 (*s*)
H-1′	5.27 (*d*, J = 7.7 Hz)	4.99 (*d*, J = 7.9 Hz)	5.27 (*d*, J = 7.7 Hz)
H-2′	4.05 (*m*)	4.01 (*m*)	4.04 (*m*)
H-3′	4.25 (*m*)	4.22 (*m*)	4.23 (*m*)
H-4′	4.25 (*m*)	4.22 (*m*)	4.23 (*m*)
H-5′	3.98 (*m*)	3.98 (*m*)	4.00 (*m*)
H-6′	4.35 (*m*)	4.35 (*m*)	4.35 (*m*)
H-6″	4.58 (*m*)	4.53 (*m*)	4.59 (*m*)

* Partial overlap of multiplets.
† Overlapping multiplets.

Table 2. ^{13}C NMR chemical shifts (δ ppm) determined for some illudane-type β-glucosides

	1a Methanol-d_4*	**3c** Methanol-d_4*	**1a** Pyridine-d_5	**1b** Pyridine-d_5	**3a** Pyridine-d_5
C-1	224.9	221.7	222.6	225.6	221.9
C-2	45.1	53.6	44.3	53.2	42.5
C-3	45.1	44.8	44.7	46.1	43.9
C-4	82.0	84.1	81.2	81.6	83.5
C-5	123.1	124.9	123.0	125.0	125.2
C-6	144.4	142.3	142.8	141.7	138.5
C-7	30.1	31.2	29.9	29.9	30.4
C-8	72.0	73.2	70.9	71.3	74.2
C-9	62.4	65.5	62.3	64.2	61.7
C-10/10a	13.6	67.8	13.5	67.8	14.9
C-10b	—	20.7	—	21.5	—
C-11	19.4	19.8	19.4	19.2	19.5
C-12	5.9†	8.0†	5.9†	6.2†	7.2†
C-13	10.6†	8.4†	10.2†	9.9†	9.7†
C-14	27.0	23.3	27.4	27.4	23.0
C-1′	99.3	97.4	99.6	99.6	99.9
C-2′	75.2	75.1	75.4	75.4	75.0
C-3′	77.7	76.6	78.4	78.4	78.5
C-4′	71.9	71.8	72.0	72.1	71.9
C-5′	78.2	78.6	78.9	78.9	79.1
C-6′	63.0	62.8	63.0	63.1	62.9
OAc		21.2			
		171.9			

* Assignments taken from ref. [8].
† Assignments are interchangeable.

Table 3. ^{13}C–^{1}H correlations (δ ppm) observed for the methyl group protons of some illudane type β-glucosides in two-dimensional HMQC and HMBC NMR spectra

	Correlated ^{13}C signals	
^{1}H signal	HMQC (^{1}J)	HMBC (^{2}J and/or ^{3}J)
Ptaquiloside (**1a**)		
0.96 (2-Me)	13.5 (C-10)	*
1.44 (6-Me)	19.4 (C-11)	*
1.70 (8-Me)	27.4 (C-14)	*
Isoptaquiloside (**3a**)		
1.14 (2-Me)	14.9 (C-10)	42.5 (C-2), 221.9 (C-1)
1.47 (6-Me)	19.5 (C-11)	30.4 (C-7), 125.2 (C-5), 138.5 (C-6)
1.48 (8-Me)	23.0 (C-12)	30.4 (C-7), 61.7 (C-9), 74.2 (C-8)
Caudatoside (**1b**)		
1.16 (2-Me)	21.5 (C-10b)	46.1 (C-3), 53.1 (C-2), 67.8 (C-10a), 225.6 (C-1)
1.45 (6-Me)	19.2 (C-11)	29.9 (C-7), 125.0 (C-5), 141.7 (C-6)
1.68 (8-Me)	27.4 (C-12)	29.9 (C-7), 64.2 (C-9), 71.3 (C-8)

* HMBC spectrum of **1a** not determined.

and **3a** (δ1.48), and some of the ring A protons (see Table 1), can be largely attributed to solvent shift (solvation) effects arising from the differing orientations of the 8-OH group in **1a** and **3a**, and the use of pyridine-d_5 as solvent.

The ^{1}H and ^{13}C NMR assignments presented for **3a** in Tables 1 and 2 were substantiated in a series of one- and two-dimensional NMR experiments, including NOE-difference, COSY and inverse mode HMQC and HMBC experiments (see Table 3). In accord with a *cis*-relationship between H-9 and the 4-glucosyl group of **3a**, irradiation of H-9 (δ3.14) enhanced the anomeric H-1′ glucosyl resonance (δ4.99), and to a lesser extent also the H-3α resonance (δ2.75).

The co-occurrence of **1a** and **3a** in the *P. aquilinum* var. *caudatum* extracts, and the optical rotation of the specimen of pterosin B (see Experimental section) which we isolated during the separation of the extracts (presumably derived from partial decomposition of a mixture of **1a** and **3a**), together with the absence of an NOE response between the H-9 and 2-Me protons of each of **1a** and **3a**, leads to the conclusion that in each of these compounds the C-2 configuration is (*R*). This conclusion is supported by our observation that for each of **1a** and **3a**, irradiation of the respective 2-Me resonances, enhanced the H-2β and H-3α resonances, while irradiation of the H-9 signals of **1a** and **3a** resulted in modest NOEs being experienced by the respective H-3α signals. The absence of an NOE response between H-9 and the 2α-CH_3 group of **1a** and **3a** is indicative of the preferred solution conformation of the A-ring of these compounds being the same as that established for **1a** in the solid state by X-ray crystallographic analysis [16]; namely that in which the 2α-CH_3 group is oriented pseudo-equatorially with respect to ring A, and in consequence it is too remote for H-9 for an NOE to be observed.

The one- and two-dimensional ^{1}H and ^{13}C NMR spectral data determined for **1b** (see Tables 1 and 2) identified this compound as an illudane-type sesquiterpene β-glucoside in which CH_2OH and CH_3 groups were attached to C-2. Irradiation of the anomeric H-1′ glucosyl resonance enhanced the glucosyl H-3′ and H-5′ resonances, and also the H-9 and H-5 resonances thereby revealing the ring A/B junction to be *cis*-fused. The ^{13}C resonance of C-14 (i.e. the 8-Me group) (δ27.4) revealed the C-8 configuration of **1b** to be the same as that determined for **1a**. The C-2 configuration of **1b** was established in a manner analogous to that described by Koyama *et al.* [10] for dennstoside A (**3c**). Irradiation of the H-3β resonance (δ2.52) of **1b** enhanced the C-2 methyl group (δ1.07) signal, while irradiation of H-3α (δ3.47) enhanced H-9 (δ3.27), and one of the protons of the CH_2OH group (δ4.09). It follows from these observations that the 2-CH_2OH group of **1b** is *cis*-oriented with respect to H-9, and that the configuration at C-2 is (*S*), as is also the case for **3c** [10].

EXPERIMENTAL

Plant extraction. Fresh fronds (2800 g) of *Pteridium aquilinum* var. *caudatum* were collected at 'El Vallecito' near Mérida, Venezuela during February 1994. A voucher specimen (UVI 95-002), identified using the key of Otega [15], has been deposited in the Herbarium of the Faculty of Pharmacy, Universidad de Los Andes, Mérida, Venezuela. The fresh fonds were blended with H_2O at room temp. for 20 min. This mixture was then stirred at 5° for 24 hr using a paddle stirrer to keep it in suspension. After filtration through a series of strainers, the aq. soln was reduced to 10% volume with the aid of a rotary evaporator ($\leqslant$35°), and the concd. plant extract centrifuged at 13 000 rpm for 30 min. The supernatant was decanted off, then

extracted with two portions of CH_2Cl_2 (300 ml). The organic layer was discarded and the aq. layer was freeze dried to a fine powder (23.4 g). The dry powder was kept at $-5°$ until required for analysis.

Nuclear magnetic resonance spectroscopy. One- and two-dimensional ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra were obtained for $CDCl_3$ or pyridine-d_5 solutions using a Bruker AC-300 instrument fitted with a 5 mm probe head. Chemical shifts are reported relative to TMS. ^{13}C NMR signal multiplicities (*s*, *d*, *t* or *q*) were determined using the DEPT135 sequence. NOE-difference spectra were obtained by subtracting an off-resonance control FID from an irradiated FID, and Fourier transforming the resulting difference FID. Two-dimensional COSY and inverse mode HMBC spectra were acquired in absolute value mode, while HMQC spectra were acquired in phase sensitive mode.

Isolation of pterosin A, B, Z and K. A portion (2 g) of freeze dried plant extract powder was dissolved in 50 ml of purified H_2O (Millipore Milli-Q) and cleaned-up by passage through a column consisting of 40 g of polyamide resin. Purified H_2O was used to elute the column, and 6 fractions of eluate (50 ml each) collected off the column. The 6 fractions were analysed by analytical HPLC using a Zorbax ODS column (4.6 mm ID × 15 cm) with a UV (220 nm) detector and MeCN–H_2O gradient programme commencing at 17:83. Aliquots (1 ml) of each fraction were also reacted with base then acid as described by Agnew and Lauren [11] and analysed using the same conditions. Fractions 1, 2 and 3 were shown by HPLC analysis to contain ptaquiloside (retention time 19.7 min) and other related compounds. These fractions were combined, freeze dried, then redissolved in H_2O (15 ml). The final soln was treated with 75 μl ml^{-1} of 2 M NaOH and allowed to react for 1 hr at 40°, then 35 μl $ml^{-1}$of 5 M HCl was added. The components of the reacted soln were then separated by prep. HPLC using a Zorbax ODS column (9.4 mm ID × 25 cm) with a UV (220 nm) detector and a mobile phase of MeCN–H_2O (A:B) to give in elution order, (2*S*)-pterosin A (3.4 mg), (2*R*)-pterosin B (7.3 mg), pterosin Z (2.3 mg) and (2*S*)-pterosin K (4.5 mg).

(2*S*)-pterosin A had mp 126–128° (lit. 125–127° [7]). ^1H NMR (300 MHz, $CDCl_3$): δ 1.22 (CH_3, *s*), 2.44 (CH_3, *s*), 2.67 (CH_3, *s*), 2.75 (1H, *d*, J = 17.0 Hz), 3.01 (2H, *t*, J = 7.5 Hz), 3.04 (1H, *d*, J = 17.0 Hz), 3.60 (1H, *d*, J = 10.6 Hz), 3.76 (2H, *t*, J = 7.4 Hz), 3.77 (1H, *d*, J = 10.6 Hz), 7.11 (1H, *s*). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.9 (*q*), 21.1 (*q*), 21.5 (*q*), 31.8 (*t*), 36.9 (*t*), 50.7 (*s*), 61.7 (*t*), 68.2 (*t*), 126.0 (*d*), 131.6 (*s*), 135.1 (*s*), 138.3 (*s*), 145.1 (*s*), 152.4 (*s*), 212.0 (*s*). (2*R*)-Pterosin B had mp 108–110° (lit. 109–110° [7]). $[\alpha]_D^{20}$ −3.6° (MeOH, *c* 0.12) (lit. $[\alpha]_D$ −6° (EtOH *c* 0.2 [17]). ^1H NMR (300 MHz, $CDCl_3$); δ 1.27 (CH_3, *d*, J = 6.3 Hz), 2.43 (CH_3, *s*), 2.55–2.65 (2H, *m*), 2.68 (CH_3, *s*), 3.02 (2H, *t*, J = 7.4 Hz), 3.23 (1H, *dd*, J = 16.6, 7.6 Hz), 3.76 (2H, *t*, J = 7.4 Hz), 7.09 (1H, *s*). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.7 (*q*), 16.6 (*q*), 21.4 (*q*), 31.9 (*t*), 33.9 (*t*), 42.6 (*d*), 61.7 (*t*), 125.8 (*d*), 132.5 (*s*), 134.8 (*s*), 138.0 (*s*), 144.4 (*s*), 152.6 (*s*), 210.3 (*s*). Pterosin Z had mp 86–89° (lit. 86–88° [7]). ^1H NMR (300 MHz), $CDCl_3$): δ 1.19 (CH_3 × 2, *s*) 2.44 (CH_3, *s*), 2.69 (CH_3, *s*), 2.85 (2H, *s*), 3.02 (2H, *t*, J = 7.4 Hz), 3.76 (2H, *t*, J = 7.4 Hz), 7.07 (1H, *s*). (2*S*)-pterosin K had mp 84–86° (lit. 85–87° [7]), ^1H NMR (300 MHz, $CDCl_3$): δ 1.22 (CH_3, *s*), 2.43 (CH_3, *s*), 2.67 (CH_3, *s*), 2.75 (1H, *d*, J = 17.0 Hz), 3.06 (1H, *d*, J = 17.0 Hz), 3.18 (2H, *t*, J = 8.3 Hz), 3.55 (2H, *t*, J = 8.3 Hz), 3.61 (1H, *d*, J = 10.7 Hz), 3.78 (1H, *d*, J = 10.7 Hz), 7.11 (1H, *s*). ^{13}C NMR ($CDCl_3$, 75.47 MHz) DEPT135 spectrum (protonated carbons only) δ 13.8 (*q*), 21.1 (*q*), 21.2 (*q*), 32.3 (*t*), 36.9 (*t*), 42.1 (*t*), 68.2 (*t*), 126.1 (*d*).

Isolation of β-glucosides. A portion (2 g) of the freeze dried plant extract was dissolved in 50 ml of purified H_2O (Millipore Milli-Q), and cleaned-up to give fractions 1, 2 and 3 as described above. These fractions were immediately frozen and then freeze dried separately, and stored at −20°. Separation of fraction 1 by preparative HPLC using the Zorbax ODS column (9.4 mm ID × 25 cm) with a UV (220 nm) detector and a mobile phase of MeCN–H_2O (17:83) at 2 ml min^{-1} afforded three major components identified in elution order as isoptaquiloside (**3a**) (8 mg) at 12.8 min, FAB-MS (NBA/ACNmatrix) *m/z* 399.2065 $[M+H]^+$, $C_{20}H_{31}O_8$ requires 399.2019: caudatoside (**1b**) (12 mg) at 19.2 min, FABMS (Na/glycerol matrix) *m/z* 451.1944 $[M+Na]^+$, $C_{21}H_{32}O_9Na$ requires 451.1935; and ptaquiloside (**1a**) (19 mg) at 30.2 min, identical (^1H and ^{13}C NMR, and analytical HPLC with an authentic specimen). Mps and optical rotations of **3a** and **1b** were not determined due to partial decomposition to pterosins B (**2a**) and A (**2b**), respectively, during isolation and storage (see Results). ^1H (300.13 MHz) and ^{13}C (75.47 MHz) NMR data for **3a**, **1b** and **1a** appear in Tables 1 and 2. Subsamples of each of **3a**, **1b** and **1a** were reacted with base then acid, as described by Agnew and Lauren [11] and analysed by analytical HPLC. These gave pterosins B (**2a**), A (**2b**) and B (**2a**), respectively.

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