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Pteroside A2—a New Illudane-Type Sesquiterpene Glucoside from *Pteridium caudatum* L. Maxon, and the Spectrometric Characterization of Caudatodienone

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Fractionation of an extract of *Pteridium caudatum* L. Maxon. (syn *P. aquilinum* L. Kuhn var. *caudatum*) which had earlier yielded the illudane-type sesquiterpene glucosides, ptaquiloside (**1a**), isoptaquiloside (**1b**), and caudatoside (**1c**) afforded a mixture containing **1a** and two minor components. Preparative HPLC afforded ptaquiloside Z (**1d**) and a new pteroside glucoside (pteroside A2) (**3e**), which was identified using a combination of mass spectral and one- and two-dimensional NMR analyses. The ^{1}H and ^{13}C NMR and mass spectrometric characterization of caudatodienone (**2b**), an unstable dienone derived from the degradation of caudatoside (**1c**) in pyridine solution, and the GC-MS characterization of some pterosin-type degradation products produced by reacting this solution with cosolvents is also reported.

KEYWORDS: ***Pteridium aquilinum*; *Pteridium caudatum*; bracken fern; illudane glucosides; caudatoside; ptaquiloside Z; caudatodienone; pteroside A2**

INTRODUCTION

Ptaquiloside (aquilide A) (**1a**), an illudane-type nor-sesquiterpene glucoside (*1*, *2*), is a known mutagen and carcinogenic constituent of *Pteridium aquilinum* L. Kuhn (bracken fern) (*2*−*7*). Bracken is the only vascular plant proven to cause cancer in animals naturally (*8*). Syndromes of bracken poisoning in livestock include thiamine deficiency of monogastric animals, bright blindness of sheep, acute haemorrhagic disease of ruminants, ileum cancer, and the urinary bladder neoplasia, known as enzootic haematuria, of sheep and cattle (*9*, *10*). In some parts of the world, young bracken fronds are used for human food. The direct and indirect consumption of bracken fern toxins by man, especially through the milk of lactating cows, is believed to constitute a risk to human health (*11*−*16*). Because the content of ptaquiloside in bracken can vary widely (*10*) and bracken is also known to contain many other components (*9*), it is important to understand the full content of putative carcinogens that occur in bracken species.

Previously, we have reported the isolation of ptaquiloside (**1a**), two new illudane-type sesquiterpene glucosides, isoptaquiloside (**1b**), caudatoside (**1c**), and after base/acid reaction of extracts, pterosins B, A, K, and Z (**3a**−**3d**) (*17*), from plant material which, at the time, was identified as *P. aquilinum* var. *caudatum* (*18*). The varietal status of *P. aquilinum* L. Kuhn has recently been clarified, and the standing of var. *caudatum* has now been raised to full species status, i.e., *P. caudatum* L. Maxon (*19*). High-performance liquid chromatography (HPLC) analysis of the extracts also revealed the probable presence of low levels of some other illudane-type sesquiterpene and/or pteroside glucosides. We now report the isolation and structure determination of a new pteroside glucoside (**3e**) and the mass spectrometric characterization of an unstable dienone (**2b**) derived from caudatoside (**1c**).

EXPERIMENTAL PROCEDURES

General Experimental Procedures. HPLC was performed using an LC-6A pump (Shimadzu, Rydalmere, Australia) and 15 cm × 4.6 mm i.d. analytical, or 25 cm × 9.4 mm i.d. preparative, Zorbax octadecylsilane (ODS) columns (Agilent Technologies) held at 35 °C, using acetonitrile−water gradients. Detection was by UV at 220 nm. Combined GC-MS analysis was performed using a 20 m × 0.22 mm i.d. HP-1 (methylsilicone) capillary column installed in an HP5890 GC instrument interfaced to an HP5970B mass selective detector (all from Hewlett-Packard, Palo Alto, California). The GC column was temperature-programmed from 100 to 150 °C at 25 °C/min and then to 275 °C at a rate of 6 °C/min (15 min hold). One- and two-dimensional

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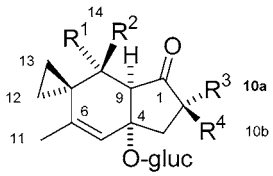


1a $R^1 = R^3 = CH_3$, $R^2 = OH$, $R^4 = H$

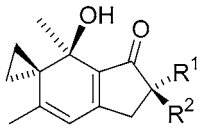
1b $R^1 = OH$, $R^2 = R^3 = CH_3$, $R^4 = H$

1c $R^1 = R^4 = CH_3$, $R^2 = OH$, $R^3 = CH_2OH$

1d $R^1 = R^3 = R^4 = CH_3$, $R^2 = OH$

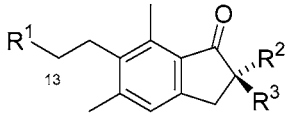
1e $R^1 = OH$, $R^2 = R^3 = R^4 = CH_3$

1f $R^1 = R^4 = CH_3$, $R^2 = OH$, $R^3 = CH_2O$-gluc



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

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



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

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

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

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

3e $R^1 = OH$, $R^2 = CH_2O$-gluc, $R^3 = CH_3$

3f $R^1 = OAc$, $R^2 = CH_3$, $R^3 = H$

3g $R^1 = OAc$, $R^2 = CH_2O$-gluc-$(Ac)_4$, $R^3 = CH_3$

3h $R^1 = OAc$, $R^2 = R^3 = CH_3$

3i $R^1 = OMe$, $R^2 = CH_2OH$, $R^3 = CH_3$

3j $R^1 = OEt$, $R^2 = CH_2OH$, $R^3 = CH_3$

3k $R^1 = OAc$, $R^2 = CH_2OH$, $R^3 = CH_3$

3l $R^1 = 12,13$-dehydro, $R^2 = CH_2OH$, $R^3 = CH_3$

3m $R^1 = O$-gluc, $R^2 = CH_2OH$, $R^3 = CH_3$

3n $R^1 = Cl$, $R^2 = CH_2O$-gluc, $R^3 = CH_3$

(HMBC, HMQC, COSY and ROESY) NMR spectra were determined for C_5D_5N solutions using a Bruker DRX-400 spectrometer and a 5 mm inverse probehead, or a Bruker AC-300 spectrometer and a standard 5 mm probehead. 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 determined at 300 MHz.

Plant Extraction and Fractionation of Extract. Young fronds (8−10 days after emergence) of *Pteridium caudatum (*syn *P. aquilinum* var. *caudatum),* identified using the key of Ortega (*18*), were collected at "El Vallecito" near Mérida, Venezuela, at 1900 m above sea level, during February 1994. A voucher specimen (UVI 95-002) has been deposited in the herbarium of the Faculty of Pharmacy, Universidad de Los Andes, Mérida, Venezuela. Aqueous extraction of the fresh fronds (2800 g) and fractionation of a 2 g (ca. 10%) subsample of the resulting freeze-dried product using polyamide 6 resin (Fluka, Buchs, Switzerland), as described previously (*17*), afforded three fractions which were shown to contain ptaquiloside (**1a**) and related compounds by analytical HPLC with 1 mL/min of an acetonitrile−water gradient program commencing at 17:83 and remaining isocratic for 22 min. Fraction 1 (158 mg) contained isoptaquiloside (**1b**) (8 mg) at 6.7 min, caudatoside (**1c**) (12 mg) at 11.6 min, and ptaquiloside (**1a**) (19 mg) at 19.6 min. Separation of fraction 2 (213 mg) by preparative HPLC using acetonitrile−water (29:171) at 2 mL/min afforded fourteen subfractions, three of which were identified as 1,4-dihydroxy-2-*iso*-propyl-5-methylphenyl-1-*O*-β-D-glucopyranoside (at 15.2 min; 26 mg) (*20*), isoptaquiloside (**1b**) (30.3 min; 10 mg), and caudatoside (**1c**) (35.6 min; 13 mg). Sub-fraction 14 (44−47 min; 36 mg), which included the major components from a gradient flush of the column, was evaporated and rechromatographed using acetonitrile−water (31:169) as the mobile phase. This afforded four fractions, three of which were found to be illudane-type, or pteroside-type, glucosides. All other fractions contained only minor amounts of material and were not investigated further.

Component 1 (37.5 min; 3.5 mg) was identified as ptaquiloside (**1a**), ^1H and ^{13}C NMR, identical with an authentic specimen (*17*). GC-MS analysis of an acetylated subsample of this component, prepared by treatment at room temperature of a portion (25 μL) of the C_5D_5N NMR solution with acetic anhydride−C_5H_5N (1:1) (150 μL), afforded peaks attributable to 13-acetoxypterosin B (**3f**), EIMS (70 eV) *m*/*z* 260 (31, M^+), 217 (19), 200 (100), 199 (54), 187 (33), 185 (54), 129 (21), 43 (58) (identical to the product produced by acetylation of **3a**); and β-D-glucose pentaacetate, EIMS (70 eV) *m*/*z* 331 (M^+ − OAc) (1), 242 (5), 200 (7), 169 (5), 157 (10), 140 (6), 115 (18), 98 (12), 43 (100).

Component 2 (46.9 min; 3.5 mg) was identified as pteroside A2 (**3e**), a gum; ^1H and ^{13}C NMR signal assignments appear in **Table 1**. HREIMS: found for $C_{21}H_{30}O_8$, *m*/*z* 410.1974; requires, *m*/*z* 410.1941. GC-MS analysis of an acetylated subsample of **3e**, prepared as described above, afforded a peak attributable to pteroside A2 pentaacetate (**3g**), EIMS (70 eV) *m*/*z*: 620 (3, M^+), 331 (46), 213 (48), 169 (100), 109 (38), 43 (68).

Component 3 (59.3 min; 0.3 mg) was identified as ptaquiloside Z (**1d**), ^1H NMR (300 MHz, C_5D_5N) δ: 1.03 (3H, s, Me), 1.11 (3H, s, Me), 1.47 (3H, s, Me), and 1.61 (3H, d, J = 1.2 Hz, Me), 3.2−4.6 (complex m, glucosyl and aglycone signals), 5.36 (1H, d, J = 7.8 Hz, β-glucosyl H-1′), and 5.96 (1H, br s, H-5). GC-MS analysis of an acetylated subsample of **1d** prepared as described above afforded peaks attributable to the following: 13-acetoxypterosin Z (**3h**), EIMS (70 eV) *m*/*z* 274 (31, M^+), 259 (11), 231 (14), 214 (85), 201 (27), 199 (100), 128 (14), 43 (62); and β-D-glucose pentaacetate, EIMS (70 eV) *m*/*z* 331 (M^+-OAc) (1), 242 (4), 200 (7), 169 (5), 157 (10), 140 (7), 115 (18), 98 (12), 43 (100).

Formation and Reaction of Caudatodienone (2b). A solution of caudatoside (**1c**) in C_5D_5N (in a 5-mm NMR tube) was allowed to stand for 24 d at room temperature, during which time the progressive conversion of **1c** to caudatodienone (**2b**) occurred. A complete assignment of the ^1H and ^{13}C NMR spectra of **2b** is given in **Tables 1** and **2**. HMBC correlations are reported in the Supporting Information. The aged (24 d) C_5D_5N solution was shown by ^1H NMR and GC-MS analysis to be composed of the following: caudatodienone (**2b**) (78%), EIMS (70 eV) *m*/*z* 248 (6, M^+), 233 (100), 216 (18), 199 (15), 187 (64), 159 (12), 128 (13); and pterosin A (**3b**) (22%), EIMS (70 eV) *m*/*z* 248 (99, M^+), 233 (81), 218 (49), 217 (97), 215 (61), 199 (84), 187 (100). A 200-μL portion of this solution was diluted to 2 mL with C_5H_5N and 200 μL aliquots, which were transferred to GC-MS vials.

Table 1. ^{1}H NMR Chemical Shifts (δ in C_5D_5N) of Pterosin A (**3b**), Pteroside A2 (**3e**), Caudatoside (**1c**), and Caudatodienone (**2b**)

signal	3b	3e	1c	2b
H-3α	3.60 (d, J = 16.9 Hz)	3.79 (d, J = 17.0 Hz)	2.51 (d, J = 13.4 Hz)	2.31 (d, J = 18.0 Hz)
H-3β	2.76 (d, J = 16.9 Hz)	2.65 (d, J = 17.0 Hz)	3.46 (d, J = 13.4 Hz)	3.14 (d, J = 18.0 Hz)
H-5	6.90 (s)	6.87 (s)	6.18 (s)	6.03 (s)
H-9			3.27 (br s)	
H-10a′	3.81 (d, J = 10.2 Hz)	3.64 (d, J = 9.4 Hz)	3.53 (d, J = 10.1 Hz)	3.74 (d, J = 10.3 Hz)
H-10a″	4.22 (d, J = 10.2 Hz)	4.55 (d, J = 9.4 Hz)	4.09 (d, J = 10.1 Hz)	4.11 (d, J = 10.3 Hz)
H-10b	1.25 (s)	1.17 (s)	1.06 (s)	1.10 (s)
H-11	2.37 (s)	2.31 (s)	1.45 (s)	1.59 (s)
H-12	3.10 (t, J = 7.4 Hz)	3.07 (t, J = 7.4 Hz)	0.62 and 0.80 (2 x m)	0.59 and 0.96 (2 x m)
H-13	3.93 (t, J = 7.4 Hz)	3.93 (m)[a]	0.86 and 1.16 (2 x m)	1.10 and 1.78 (2 x m)
H-14	2.82 (s)	2.78 (s)	1.68 (s)	1.48 (s)
H-1′		4.80 (d, J = 7.8 Hz)	5.27 (d, J = 7.7 Hz)	
H-2′		3.93[a] (m)	4.04 (m)	
H-3′		4.17 (m)	4.23 (m)	
H-4′		4.17 (m)	4.23 (m)	
H-5′		3.83 (m)	4.00 (m)	
H-6′		4.35 (m)	4.35 (m)	
H-6″		4.50 (m)	4.59 (m)	

[a] Co-incident –*CH*$_2$OH and –*CH*(OH)- signals.

Table 2. ^{13}C NMR Chemical Shifts (δ in C_5D_5N) of Pterosin A (**3b**), Caudatoside (**1c**), and Caudatodienone (**2b**)

signal	3b	1c	2b
C-1	211.2 (s)[a]	225.6 (s)	210.4 (s)
C-2	52.1 (s)	53.2 (s)	51.1 (s)
C-3	37.0 (t)	46.1 (t)	38.9 (t)
C-4	152.8 (s)	81.6 (s)	164.2 (s)
C-5	126.2 (d)	125.0 (d)	120.4 (d)
C-6	144.6 (s)	141.7 (s)	154.7 (s)
C-7	136.4 (s)	29.9 (s)	32.4 (s)
C-8	137.6 (s)	71.3 (s)	69.5 (s)
C-9	132.6 (s)	64.2 (d)	134.1 (s)
C-10a	67.9 (t)	67.8 (t)	67.4 (t)
C-10b	21.4 (q)	21.5 (q)	20.7 (q)
C-11	21.2 (q)	19.2 (q)	19.7 (q)
C-12	33.0 (t)	6.2 (t)	8.2 (t)
C-13	61.1 (t)	9.9 (t)	10.1 (t)
C-14	13.8 (q)	27.4 (q)	25.3 (q)
C-1′		99.6 (d)	
C-2′		75.4 (d)	
C-3′		78.4 (d)	
C-4′		72.1 (d)	
C-5′		78.9 (d)	
C-6′		63.1 (t)	

[a] Signal type: C (s), CH (d), CH_2 (t), or CH_3 (q) given in brackets.

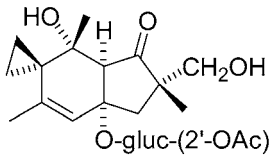
200 μL of methanol, ethanol, chloroform, or acetic acid were added to the GC-MS vials, which were capped, shaken, and allowed to stand at room temperature. GC-MS analyses, performed at 15−30 min and 3 and 6 h, showed that conversion of caudatodienone (**2b**) to pterosins possessing 13-methoxy, 13-ethoxy, 13-hydroxy, 13-chloro, 13-acetoxy, or 12(13)-dehydro groups (compounds **3i**, **3j**, **3b**, **3c**, **3k**, and **3l**, respectively) had occurred. EIMS (70 eV), **3i**, *m*/*z* 262 (59, M^+), 247 (73), 231 (23), 229 (37), 200 (28), 199 (100), 187 (44), 85 (27), 171 (23), 128 (39); **3j**, *m*/*z* 276 (84, M^+), 246 (28), 245 (39), 200 (80), 199 (100), 197 (28), 185 (45), 128 (22); **3b**, MS data given above; **3c**, *m*/*z* 266 (57, M^+), 268 (18), 237 (26), 236 (29), 235 (100), 213 (51), 199 (20), 87 (33), 185 (35), 173 (35), 128 (17); **3k**, *m*/*z* 290 (44, M^+), 230 (33), 200 (85), 199 (100), 197 (80), 185 (45), 128 (34); **3l**, *m*/*z* 230 (52, M^+), 215 (14), 212 (15), 200 (43), 199 (100), 97 (52), 185 (37), 128 (33).

RESULTS AND DISCUSSION

Preparative HPLC of a column fraction isolated during our earlier investigation afforded material which was rechromatographed by preparative HPLC to yield four fractions, three of which contained illudane-type compounds (components 1, 2, and 3). Component 1 was identified by ^{1}H NMR analysis as ptaquiloside (**1a**) (*1, 2, 17*). Although isolated as a single HPLC peak, the ^{1}H NMR specimen of ptaquiloside was contaminated by ca. 22% of pterosin B, the presence of which is most likely due to the slight acidity of the nonbuffered mobile phase used for preparative HPLC and purification. Ptaquiloside (**1a**) is stable over several months in standing bracken, but on extraction, it is unstable under both acidic and basic conditions. In weak acid, ptaquiloside is converted to a mixture of compounds such as pterosin B (**3a**), while in aqueous base ptaquiloside affords an aglycone dienone (**2a**), which readily undergoes acid-catalyzed hydrolysis to give (2*R*)-pterosin B (**3a**) (*1, 21*−*24*). This dienone (**2a**) is believed to be the active mutagen and has been shown to be a reactive alkylating agent toward a variety of nucleophiles, among them DNA (*22, 24*−*26*). GC-MS analyses of an acetylated subsample of a ptaquiloside (**1a**) afforded predominantly β-D-glucose pentaacetate and 13-acetoxypterosin B (**3f**) (Supporting Information). It can therefore be reasoned that treatment of an illudane-type glucoside (eg **1a**) with the basic mixture of pyridine−acetic anhydride initially affords partially acetylated β-D-glucose and dienone (**2a**). Thereafter, further reaction affords β-D-glucose pentaacetate and **3f**.

Component 2 was as identified as pteroside A2 (**3e**). The ^{1}H NMR spectrum of **3e** included signals attributable to a quaternary methyl group (1.17 ppm), two aryl methyl groups (2.31 and 2.78 ppm), a $-CH_AH_BO-$ group (3.64 and 4.55 ppm, J_{AB} = 9.4 Hz), an aryl proton (6.87 ppm), and a glucosyl group (see **Table 1**). Irradiation of the two-proton signal that resonated at 3.07 ppm (t, J = 7.4 Hz) sharpened the two-proton aryl−CH_2−CH_2O- signal centered at 3.93 ppm. These data indicated that the compound was either pteroside A (**3m**) (*27*) or positional isomer **3e**. The latter compound is the 13-hydroxy analogue of pteroside K (**3n**) (*27*).

The ^2J coupling constant (J_{AB} = 9.4 Hz) of the 10a−CH_AH_B-O-glucosyl protons of component 2 was comparable to that reported for pteroside K (**3n**) (J = 9.0 Hz) (*27*), and significantly different from those of the equivalent protons of pterosin A (**3b**) (J_{AB} = 10.2 Hz, **Table 1**), caudatoside (**1c**) (J_{AB} = 10.1 Hz, **Table 1**), dennstoside (**4**) (J_{AB} = 10.8 Hz) (*28*) and pteroside A (**3m**) (J_{AB} = 10.5 Hz) (*27*). Irradiation of the glucosyl H-1′ signal (4.80 ppm, d, J = 7.8 Hz) of **3e** in an NOE-difference experiment enhanced the 10a−CH_AH_B-O- signal, which occurred at 3.64 ppm. Because **3e** is a positional isomer of pteroside A, we propose the designation pteroside



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A2 for this compound. GC-MS analysis of an acetylated subsample of **3e** afforded a single peak attributable to pentaacetyl pteroside A2 (**3g**).

The ^{1}H and ^{13}C NMR signal assignments of **3e** were supported by a comparison of NMR spectrometric data determined for pterosin A (**3b**), which are reported for the first time in C_5D_5N (**Tables 1** and **2**). These assignments for **3b** were derived from a consideration of one- and two-dimensional NMR data, including ROESY, HMQC, and HMBC spectra. In particular, the H-3α and H-3β resonances of pterosin A (**3b**), and by analogy the equivalent resonances of pteroside A2 (**3e**), were differentiated on the basis of the correlation observed in the ROESY spectrum between the 2β-methyl group and the methylene resonance, which occurred at 2.76 ppm (H-3β). No correlations were observed for H-3α.

Only a very small amount of component 3 was obtained. The presence in the ^{1}H NMR spectrum of four methyl group signals (1.03, 1.11, 1.47, and 1.61 ppm, d, $J = 1.2$ Hz), olefinic- (5.96 ppm) and anomeric-glucosyl (5.36 ppm, d, $J = 7.8$ Hz) signals, and a complex cluster of signals in the 3.2−4.6 ppm region were consistent with the identification of the component as ptaquiloside Z (*29*). GC-MS analysis of an acetylated subsample afforded peaks attributable to β-D-glucose pentaacetate and 13-acetoxypterosin Z (**3h**). This is consistent with the detection of pterosin Z (**3d**) in reacted samples of the *P. caudatum* extracts (*29*). The occurrence of the H-1′, H-5, and H-14 resonances of **3d** at 5.36, 5.96, and 1.61 ppm, respectively, when compared with data for compounds **1a**, **1b**, and **1c** determined in C_5D_5N, indicates that the 8-hydroxyl group of **1d** is β-oriented as in ptaquiloside (**1a**), rather than α-oriented as in isoptaquiloside (**1b**), dennstoside (**4**) (*28*), and the hypolosides (*30*).

Caudatodienone (2b) Degradation Reactions. ^{1}H and ^{13}C NMR analyses demonstrated the progressive conversion of caudatoside (**1c**) to caudatodienone (**2b**) on standing in C_5D_5N. Negligible degradation of **1c** occurred when the NMR tube was stored between spectral data acquisition periods at 5 °C, however storage of the NMR tube for 24 d at room temperature resulted in the gradual conversion of **1c** to a compound that was identified, from the one- and two-dimensional NMR spectral data presented in **Tables 1** and **2**, as caudatodienone (**2b**). HMBC correlations observed for **2b** are given in the Supporting Information. Notable features of the ^{1}H NMR spectra of **2b** were the absence of an H-9 resonance and the comparatively large ^2J coupling constant of the 3α/3β-protons ($J_{AB} = 18.0$ Hz). Dienone **2b** is a 15-carbon analogue of the dienone (**2a**) derived from ptaquiloside (**1a**) (*20, 21, 23*). Dienones of illudane glycosides are considered to be the active alkylating agents responsible for mutagenicity.

GC-MS was employed to assess the stability of a pyridine solution of caudatodienone (**2b**) toward a selection of cosolvents. Addition of methanol, ethanol, chloroform, or acetic acid to subsamples of an aged pyridine solution of caudatoside (**1c**), which at the time of the GC-MS analyses was composed of 78% caudatodienone (**2b**), 22% pterosin A (**3b**), and a trace (<1%) of caudatoside (**1c**), resulted in the progressive conversion of (**2b**) to pterosins possessing 13-methoxy, 13-ethoxy, 13-hydroxy, 13-chloro, 13-acetoxy, or 12(13)-dehydro groups (compounds **3i**, **3j**, **3b**, **3c**, **3k**, and **3l**, respectively). The percentage contributions are given in **Table 3**. These were calculated assuming unit response factors for all compounds, and are corrected for the initial 22% contribution of **3b** arising from partial conversion of caudatoside after preparative HPLC separation (see earlier comment for ptaquiloside). Similar results were obtained in replicate experiments. Our observation that caudatoside (**1c**) slowly affords caudatodienone (**2b**) on standing in pyridine (a basic solvent), and that this dienone reacts more rapidly with acetic acid than is the case for neutral solvents such as methanol, ethanol, or chloroform (**Table 3**), is consistent with the previously reported formation of dienone (**2a**) from ptaquiloside (**1a**) in the presence of base and its reaction to give pterosin B (**3a**) in the presence of acid (*1, 23*).

Table 3. GC-MS Determined Product Outcomes (% Yields) for 1:1 Solutions of Dienone (**2b**) in Pyridine and Methanol, Ethanol, Chloroform, or Acetic Acid

		product						
cosolvent	time	**2b**	**3l**	**3i**	**3j**	**3b**	**3c**	**3k**
CH_3OH	3 h	28	3	68		1		
CH_3OH	6 h		2	98		tr[a]		
C_2H_5OH	3 h	tr[a]			48	52		
C_2H_5OH	6 h		2		51	47		
$CHCl_3$	3 h	68	13			1	18	
$CHCl_3$	6 h	44	14			2	40	
CH_3COOH	15 min					tr[a]		100

[a] tr = trace (<0.5%).

It can be inferred from our results that some of the pterosins previously reported in the literature (e.g., those possessing 13-methoxy or chloro groups) may be artifacts arising from the degradation of a dienone intermediate on exposure to extraction solvents such as methanol, dichloromethane, or chloroform. It is possible that pteroside A2 (**3e**) and its 13-chloro analogue (**3n**) (pteroside K) (*27*) are degradation products of an illudane-type diglucoside such as **1f**. Although only characterized as a degradation product in the present study, it is possible that caudatodienone occurs naturally in plant material and that the biosynthesis of pterosins occurs via the sequence: protoilludanes → illudanes → dienones → pterosins.

In previous studies directed toward an assessment of the carcinogenicity of members of the genus *Pteridium* taxa (*3, 4*), emphasis has been given to the occurrence of ptaquiloside (**1a**) in plant extracts. The various *Pteridium* species share a number of xenobiotic secondary metabolites, such as ptaquiloside, thiaminase, several phenolic acids, quercetin, condensed tannins, and prunasin. Other metabolites, such as coumarin, have a more restricted distribution with this taxa, occurring only from neotropical *P. caudatum* (*31*), which is also presently the only known source of caudatoside (**1c**) and a novel carvacrol glycoside (*20*). This may be the consequence of an intense compound search in crosiers of this species where the content of illudanes is highest. It is possible that other bracken species may also harbor the *P. caudatum* carcinogens identified in our investigations. Because of the limited quantity of caudatoside (**1c**) available to us, we have not as yet assessed its carcinogenicity, however we anticipate that the reactivity of the unstable dienone (**2b**), derived from caudotoside, toward amino acids, nucleosides, and nucleotides will be similar to that reported by Ojika et al. (*22*) for the unstable dienone (**2a**) derived from ptaquiloside.

SAFETY STATEMENT

Because of the presence of carcinogenic substances, plant material and extracts should be handled with care (wearing gloves) and disposed of with caution in sealed containers. Pyridine-containing samples should be prepared and reacted in a fumehood.

ABBREVIATIONS USED

GC, gas chromatograph; MS, mass spectrometer; COSY, correlated spectroscopy; DEPT135, distortionless enhancement by polarization transfer using a 135° pulse; HSQC, heteronuclear single quantum coherence; *m*/*z*, mass-to-charge ratio; NMR, nuclear magnetic resonance; NOE, nuclear Overhauser effect; ROESY, rotating frame Overhauser spectroscopy; SIM, selected ion mode; TMS, tetramethylsilane.

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Supporting Information Available: GC-MS profile of pyridine/acetic anhydride treated ptaquiloside (**1a**), GC-MS profile of pyridine/acetic anhydride treated 2β-methylptaquiloside (**1d**), mass spectrum of 13-acetoxypterosin B (**3f**), mass spectrum of 13-acetoxypterosin Z (**3h**), and a table of ^{13}C-^{1}H NMR correlations observed for caudatodienone (**2b**) and pterosin A (**3b**) in two-dimensional HMQC and HMBC spectra. This material is available free of charge via the Internet at http//pubs.acs.org.

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