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Ptesculentoside, a novel norsesquiterpene glucoside from the Australian bracken fern *Pteridium esculentum*

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ABSTRACT

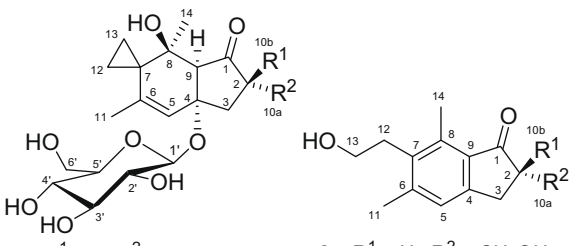
A novel norsesquiterpene glucoside ptesculentoside has been isolated from the Australian bracken *Pteridium esculentum*, together with the known bracken carcinogen ptaquiloside and lesser amounts of caudatoside. The structure of ptesculentoside is determined by analysis of 1D and 2D NMR spectra, and via its conversion into previously known pterosin G.

Bracken ferns (*Pteridium* species) are some of the most ubiquitous plants on earth, with an extensive history of poisoning grazing livestock.[1] The norsesquiterpenoid glucoside ptaquiloside (**1b**) has previously been isolated from bracken and shown to be responsible for a number of the associated syndromes, particularly acute haemorrhagic disease of cattle (bracken poisoning), bright blindness of sheep, bovine enzootic haematuria and upper alimentary carcinoma.[2] The biological activity of this reactive glycoside has been attributed to the facile elimination of glucose to form an unstable conjugated dieneone intermediate which acts as a powerful alkylating agent of amino acids and DNA.[2]

Taxonomic classification within the genus *Pteridium* remains controversial.[3,4] *Pteridium aquilinum* is the most widespread species with 11 subspecies occurring predominantly in the northern hemisphere,[4] and initial isolations of ptaquiloside (**1b**) relate to *P. aquilinum* subspecies.[5–8] Ptaquiloside and a series of similarly reactive analogues including caudatoside (**1c**) have also been isolated from what is now recognised as a separate species, *Pteridium caudatum*.[9–11] Previous chemical examination of the other three *Pteridium* species (*Pteridium arachnoideum*, *Pteridium esculentum* and *Pteridium semihastatum*) have been limited to surveys of their ptaquiloside content by HPLC–UV analysis of the more stable elimination product pterosin B (**2b**).[12–17]

We report here the first phytochemical study of *P. esculentum*, and the isolation of ptaquiloside (**1b**), caudatoside (**1c**) and the previously unknown hydroxy ptaquiloside analogue, ptesculentoside (**1a**) (Fig. 1). Treatment of the individual glucosides (**1a–c**) with dilute base produces the corresponding elimination products, pterosin G (**2a**), pterosin B (**2b**) and pterosin A (**2c**).

P. esculentum fern was collected from a site near Conondale, Queensland and the species identification confirmed by Queensland Herbarium (voucher AQ744801). An extract of milled freeze-dried immature croziers was partially purified by the method of Rasmus-



1a: R^1 = H; R^2 = CH_2OH
1b: R^1 = H; R^2 = CH_3
1c: R^1 = CH_3; R^2 = CH_2OH

2a: R^1 = H; R^2 = CH_2OH
2b: R^1 = H; R^2 = CH_3
2c: R^1 = CH_3; R^2 = CH_2OH

Figure 1. Glucosides isolated from *Pteridium esculentum* and their elimination products.

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sen et al.[18] to provide a glassy solid. A portion of this material (25.8 mg) was then subjected to repeated rounds of reverse phase HPLC to afford pure ptaquiloside (**1b**) (5.7 mg), caudatoside (**1c**) (3.2 mg) and ptesculentoside (**1a**) (6.7 mg). Ptaquiloside (**1b**) ($[\alpha]_D$ −170.8 (*c*, 0.42, MeOH)) and caudatoside (**1c**) ($[\alpha]_D$ −94.8 (*c*, 0.18, MeOH)) were identified by comparison of their ^{13}C and ^{1}H NMR spectral data with literature data for these compounds.[8,9] The measured optical rotation for ptaquiloside (**1b**) was consistent with literature,[7,10] but the optical rotation for caudatoside (**1c**) has not previously been reported.

Ptesculentoside (**1a**) ($[\alpha]_D$ −172.6 (*c*, 0.6, MeOH)) provided an ion at 437.1792 ($[M+Na]^+$) corresponding to a molecular mass formula of $C_{20}H_{30}O_9$ (calculated M+Na: 437.1782). Both proton and carbon spectra of ptesculentoside (**1a**) (Table 1) presented many signals characteristic of ptaquiloside (**1b**),[8] with a noted absence of the H-10 methyl singlet in the ^{1}H NMR and C-10 resonance in the ^{13}C NMR spectra. The presence of a CH_2OH group at this position was suggested by the existence of a characteristic δ_C 60.4 carbon resonance and two non-equivalent protons at δ_H 3.62 and δ_H 3.85 both with couplings to H-2 (δ_H 2.34), and is in agreement with the molecular formula. The only other significant differences in the NMR shifts of ptesculentoside (**1a**) relative to ptaquiloside (**1b**) are a downfield shift of 7.7 ppm for C-2 and an upfield shift of 5.7 ppm for C-3, which are consistent with β- and γ-hydroxy substituent effects.[19]

2D NMR techniques confirmed the proposed structure for ptesculentoside (**1a**) and enabled assignment of all resonances (Table 1), in agreement with previous assignments for ptaquiloside (**1b**).[8] Assignment of the protonated carbons of **1a** was performed by analysis of its HSQC spectrum, with the gross structure determined from HMBCs. The one-proton doublet at δ_H 4.61 (δ_C 99.4 via HSQC) is characteristic of H-1′ of a glycoside and the H-1′/H-2′ coupling of 7.9 Hz is indicative of the β-anomer. Measured coupling constants for the other protons of the sugar ring established the stereochemistry at each of the ring carbons and confirmed the depicted β-glucosyl moiety.

NOESY correlations (Fig. 2) between H-9/H-1′ and H-9/H-14 established that the A/B rings were *cis* fused with an α orientation of the β-glucosyl group, H-9 and C-14 methyl. H-1′ also showed a NOESY correlation with one of the H-3 resonances (δ_H 2.33) which is thus H-3α.

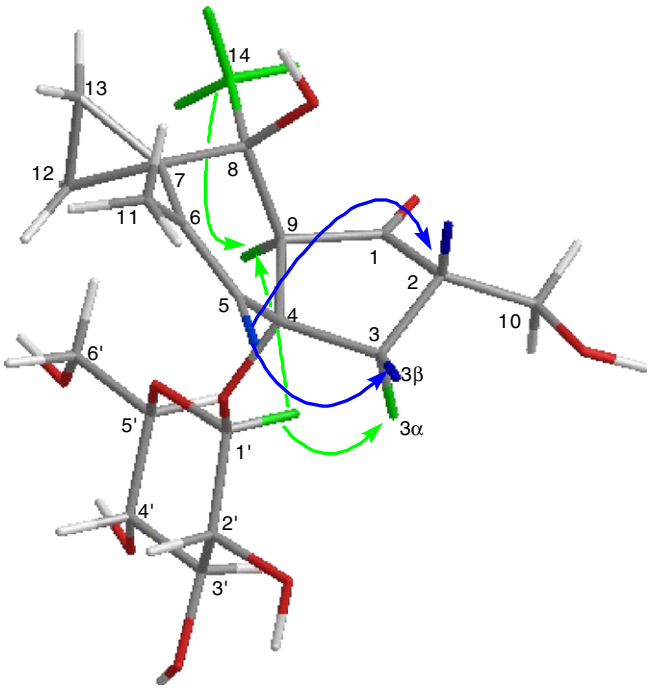

Figure 2. Selected NOE correlations for ptesculentoside (**1a**).

Therefore, the other H-3 resonance (δ_H 2.45) must be H-3β. NOESY correlations between H-5/H-3β and H-5 and the single H-2 resonance (δ_H 2.34) indicated that these hydrogens were on the same β face of the molecule, and hence the CH_2OH group at C-2 lies in the α position. Ptesculentoside (**1a**) thus has the 2*S* configuration.[20]

The structure and C-2 stereochemistry of ptesculentoside (**1a**) was further confirmed by chemical conversion. Treatment of ptaquiloside (**1b**) in aqueous base followed by weak acid readily

Table 1
^{13}C and ^{1}H NMR chemical shifts (δ, ppm) and coupling constants (Hz) determined for ptesculentoside (**1a**) compared with literature data for ptaquiloside (**1b**)

No.	Ptesculentoside (**1a**)[a,b]		Ptaquiloside (**1b**) [8,a,c]	
	^{13}C	^{1}H	^{13}C	^{1}H
1	222.0		224.0	
2	52.9	2.34, *m*, overlapping	45.2	2.20, *ddq* (6.5, 7.5, 12)
3	39.7	H-3α: 2.33, overlapping; H-3β: 2.45, *dd* (5.3, 9.2)	45.2	H-3α: 1.92, *dd* (12, 12); H-3β: 2.47, *dd* (8, 12)
4	82.3		82.0	
5	123.1	5.78, *br s*	123.1	5.75, *dq* (1, 1.5)
6	144.7		144.5	
7	30.1		30.1	
8	72.1		71.89	
9	63.4	2.64, *br s*	62.5	2.63, *d* (1.5)
10	60.4	3.62, *dd* (3.4, 12.0); 3.85, *dd* (4.4, 12.0)	13.6	1.05, *d* (6.5)
11	19.5	1.54, *s*	19.5	1.52, *d* (1)
12	6.1	H-12α: 0.89, *m*; H-12β: 0.69, *m*	5.9	H-12α: 0.85, *m* (overlap H-13β); H-12β: 0.68, *m*
13	10.7	H-13α: 0.53, *m*; H-13β: 0.88, *m*	10.6	H-13α: 0.48, *m*; H-13β: 0.85, *m* (overlap H-12α)
14	26.9	1.28, *s*	27.0	1.27, *s*
1′	99.4	4.61, *d* (7.9)	99.3	4.59, *d* (8)
2′	75.2	3.20, *dd* (7.8, 8.9)	75.2	3.19, *dd* (8, 8.5)
3′	78.1	3.35, *dd* (8.9, 8.9)	77.7	3.32
4′	71.8	3.26, *dd* (8.9, 8.9)	71.92	3.30
5′	77.8	3.30, *m*	78.2	3.32
6′	62.9	H-6′α: 3.91, *dd* (3.2, 12.4); H-6′β: 3.61, *dd* (1.8, 12.4)	62.9	H-6′α: 3.88, *dd* (2, 12); H-6′β: 3.66, *dd* (5.5, 12)

[a] Measured in CD_3OD. Coupling constants are in parentheses.
[b] ^{1}H NMR spectra were recorded at 278 K at 500 MHz and ^{13}C NMR at 125 MHz with the residual CD_3OD protonated signal (δ_H 3.31) and the central peak of the CD_3OD septet (δ_C 49.00) as internal standards.
[c] NMR data obtained for ptaquiloside (**1b**) from *P. esculentum* is in excellent agreement with the reported data (see Supplementary data)

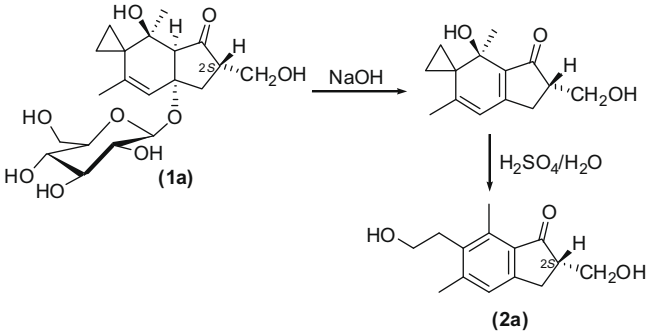

Figure 3. Elimination of ptesculentoside (**1a**) to form pterosin G (**2a**).

generates pterosin B (**2b**),[12] and caudatoside (**1c**) likewise provides pterosin A (**2c**).[9] Similar treatment of ptesculentoside (**1a**) (2 mg) afforded, after solvent partitioning, pure pterosin G (**2a**) (0.78 mg) ($[\alpha]_D$ −13.6 (*c*, 0.5, MeOH)) (Fig. 3).

Pterosin G has previously been characterised as a component from *P. aquilinum* var. *latiusculum* ($[\alpha]_D$ −14.6)[21,22] and from *Pteris podophylla* ($[\alpha]_D$ −14),[23] and ^{13}C and ^{1}H NMR data obtained here is consistent with that previously reported for this compound.[23] Fukuoka et al.[21] demonstrated that (−)-pterosin G has the 2*S* configuration by reduction with lithium aluminium hydride and oxidation with chromic anhydride to afford the same indanone as derived from (−)-2*R*-pterosin B.[20] The optical rotation obtained here for **2a** is in agreement with literature[21–23] indicating the same 2*S* stereochemistry in this compound, and hence also in ptesculentoside (**1a**).

Determination of the absolute configuration of the glucose unit was achieved by hydrolysis of ptesculentoside (**1a**) with 10% HCl in methanol followed by treatment with trifluoroacetic anhydride.[24] In enantioselective GC-coinjection studies, identical retention times were observed between the hydrolysate of ptesculentoside (**1a**) and authentic D-glucose.[25]

This study has revealed the presence of the previously unknown norsesquiterpene glucoside ptesculentoside (**1a**) in *P. esculentum*, together with comparable proportions of ptaquiloside (**1b**) and lesser amounts of caudatoside (**1c**). These three compounds demonstrate similar chemical reactivity and presumably have similar biological activity.

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Supplementary data

Supplementary data (Detailed description of experimental procedures and 1D and 2D NMR spectra of ptesculentoside (**1a**), ptaquiloside (**1b**), caudatoside (**1c**) and pterosin G (**2a**)) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.02.032.

References and notes

1. Vetter, J. *Acta Vet. Hung.* **2009**, *57*, 183–196.
2. Yamada, K.; Ojika, M.; Kigoshi, H. *Nat. Prod. Rep.* **2007**, *24*, 798–813.
3. Thomson, J. A.; Mickel, J. T.; Mehltreter, K. *Bot. J. Linn. Soc.* **2008**, *157*, 1–17.
4. Der, J. P.; Thomson, J. A.; Stratford, J. K.; Wolf, P. G. *Am. J. Bot.* **2009**, *96*, 1041–1049.
5. Niwa, H.; Ojika, M.; Wakamatsu, K.; Yamada, K.; Hirono, I.; Matsushita, K. *Tetrahedron Lett.* **1983**, *24*, 4117–4120.
6. Van der Hoeven, J. C. M.; Lagerweij, W. J.; Posthumus, M. A.; Van Veldhuizen, A.; Holterman, H. A. J. *Carcinogenesis* **1983**, *4*, 1587–1590.
7. Ojika, M.; Wakamatsu, K.; Niwa, H.; Yamada, K. *Tetrahedron* **1987**, *43*, 5261–5274.
8. Oelrichs, P. B.; Ng, J. C.; Bartley, J. *Phytochemistry* **1995**, *40*, 53–56.
9. Castillo, U. F.; Wilkins, A. L.; Lauren, D. R.; Smith, B. L.; Towers, N. R.; Alonso-Amelot, E.; Jaimes-Espinoza, R. *Phytochemistry* **1997**, *44*, 901–906.
10. Castillo, U. F.; Ojika, M.; Alonso-Amelot, M.; Sakagami, Y. *Bioorg. Med. Chem.* **1998**, *6*, 2229–2233.
11. Castillo, U. F.; Wilkins, A. L.; Lauren, D. R.; Smith, B. L.; Alonso-Amelot, M. *J. Agric. Food Chem.* **2003**, *51*, 2559–2564.
12. Alonso-Amelot, M. E.; Rodulfo-Baechler, S.; Jaimes-Espinoza, R. *Biochem. Syst. Ecol.* **1995**, *23*, 709–716.
13. Agnew, M. P.; Lauren, D. R. *J. Chromatogr.* **1991**, *538*, 462–468.
14. Smith, B. L.; Seawright, A. A.; Ng, J.; Hertle, A. T.; Thomson, J. A.; Bostock, P. D.. In Colegate, S. M., Dorling, P. R., Eds.; Plant-associated Toxins: Agricultural, Phytochemical and Ecological Aspects; CAB International: Wallingford UK, 1994; pp 45–50.
15. Smith, B. L.; Seawright, A. A.; Ng, J. C.; Hertle, A. T.; Thomson, J. A.; Bostock, P. D. *Nat. Toxins* **1994**, *2*, 347–353.
16. Smith, B. L.; Embling, P. P.; Agnew, M. P.; Lauren, D. R.; Holland, P. T. *N. Z. Vet. J.* **1988**, *36*, 56–58.
17. Rasmussen, L. H.; Lauren, D. R.; Smith, B. L.; Hansen, H. C. B. *N. Z. Vet. J.* **2008**, *56*, 304–309.
18. Rasmussen, L. H.; Bruun Hansen, H. C.; Lauren, D. *Chemosphere* **2005**, *58*, 823–835.
19. Williams, D. H.; Fleming, I. *Spectroscopic Methods in Organic Chemistry*, 5th ed.; McGraw-Hill: Glasgow, 1995.
20. The methyl substituent at C-2 in ptaquiloside (**2a**) [and pterosin (**2b**)] also lies in the α position,[7,8] but the C-2 stereochemistry in these compounds is 2*R* as the methyl group is of lower priority order.
21. Fukuoka, M.; Kuroyanagi, M.; Yoshihira, K.; Natori, S. *Chem. Pharm. Bull.* **1978**, *26*, 2365–2385.
22. Yoshihira, K.; Fukuoka, M.; Kuroyanagi, M.; Natori, S.; Umeda, M.; Morohoshi, T.; Enomoto, M.; Saito, M. *Chem. Pharm. Bull.* **1978**, *26*, 2346–2354.
23. Tanaka, N.; Murakami, T.; Saiki, Y.; Chen, C.-M.; Gomez, P. L. D. *Chem. Pharm. Bull.* **1981**, *29*, 3455–3463.
24. König, W. A.; Benecke, I.; Bretting, H. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 693–694.
25. Enantioselective GC analyses were performed on a Chirasil-L-Val capillary column (25 m × 0.32 mm × 0.20 μm). The retention times for the standards were: L-Glc (26.10 and 29.79 min), D-Glc (26.17 and 29.96 min). For the hydrolysate of ptesculentoside (**1a**), peaks were observed at 26.10 and 29.90 min. During coinjection studies, identical retention times were observed between the hydrolysate of (**1a**) and authentic D-Glc. The multiple peaks observed are a result of the formation of α and/or β anomers of pyranose forms and coincide with the number previously reported for this sugar.[24]