Journal of Natural Products
Vol. 58, No. 12, pp. 1889-1891, December 1995

STRUCTURE ELUCIDATION OF 1,4-DIHYDROXY-2-*ISO*-PROPYL-5-METHYLPHENYL-1-*O*-β-GLUCOPYRANOSIDE, A CONSTITUENT OF *PTERIDIUM AQUILINUM* VAR. *CAUDATUM*

UVIDELIO F. CASTILLO,

Universidad de Los Andes, Facultad de Ciencias, Departamento de Química, Laboratorio de Química Ecológica, Mérida 5101, Venezuela

ALISTAIR L. WILKINS,*

Chemistry Department, University of Waikato, Hamilton, New Zealand

DENIS R. LAUREN, DONALD E. MCNAUGHTON,

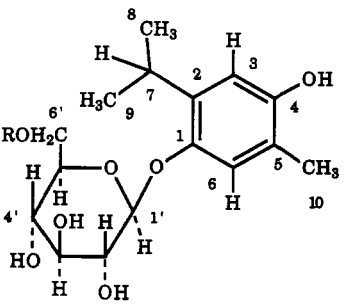
The Horticulture and Food Research Institute of New Zealand Ltd., Ruakura Research Centre, Hamilton, New Zealand

and BARRY L. SMITH

New Zealand Pastoral Agricultural Research Institute Ltd., Ruakura Research Centre, Hamilton, New Zealand

ABSTRACT.—A phenylglucoside isolated from the fresh fronds of *Pteridium aquilinum* var. *caudatum* was identified as 1,4-dihydroxy-2-*iso*-propyl-5-methylphenyl-1-*O*-β-D-glucopyranoside [**1**]. The natural occurrence of this compound is reported here for the first time.

Pteridium aquilinum (L. Kuhn) (Pteridaceae), considered to be one of the five most widely distributed organisms of the plant kingdom (1,2), has been associated with a number of diseases in farm animals (3,4). Chemical investigations of more than 30 species of the Pteridaceae have been reported (5). As a part of studies following from the work done by Alonso-Amelot *et al.* (6,7), we have been carrying out a detailed hplc analyses of the occurrence and distribution of illudane-type sesquiterpene glycosides in *Pteridium aquilinum* var. *caudatum*, one of two taxa of bracken fern most commonly found in the Venezuelan Andes (8). In the course of these studies a new phenylglucoside was isolated and identified as 1,4-dihydroxy-2-*iso*-propyl-5-methylphenyl-1-*O*-β-D-glucopyranoside [**1a**].



1a R=H
1b R=Galloyl

The ^{13}C-nmr spectrum of the phenylglucoside comprised sixteen resonances (see Table 1), six of which were reminiscent of a β-glucopyranosyl unit, and ten of which identified the aglycone portion as an aryl-terpenoid possessing isopropyl, methyl, and hydroxyl substituent groups. ^{13}C- and ^{1}H-nmr chemical shifts were correlated in inverse mode two-dimensional HMQC and HMBC experiments optimized for the detection of ^{1}J and longer range ^{2}J and ^{3}J couplings, respectively, while proton chemical shifts, including the aryl and glucosyl hydroxyl proton resonances (all of which were detected in the ^{1}H-nmr spectrum), were correlated in a COSY nmr experiment. Irradiation of the anomeric glucosyl resonance (H-1′, 5.45 ppm, d, J=7.4 Hz) in a nOe difference experiment enhanced the glucosyl H-3′ and H-5′ signals (4.32 and 4.02 ppm) and also the aryl proton

TABLE 1. ^{1}H- (300.13 MHz) and ^{13}C- (75.47 MHz) Nmr Data Determined in C_5D_5N for **1a**.

Position	δ_C	δ_H	NOe correlations	HMBC correlations $^{2,3}J_{(^1H-^{13}C)}$
1	148.9			
2	137.3			
3	113.0	7.10, s	OH-4, H-7, H-8, H-9	
4	152.2			
5	123.2			
6	120.3	7.54, s	H-1′, H-10	
7	26.6	3.86, sept., J=6.8 Hz	H-3, H-9, H-8	
8	23.4[a]	1.216, d, J=6.8 Hz	H-3, H-7	C-2, C-7, C-9
9	23.7[a]	1.238, d, J=6.8 Hz	H-3, H-7	C-2, C-7, C-8
10	16.5	2.38, s	H-6, OH-4	C-4, C-5, C-6
1′.....	104.8	5.45, d J=7.4 Hz	H-3′, H-5′, H-6	
2′.....	75.3	4.32, br m[b]		
3′.....	78.8	4.32 br m[b]		
4′.....	71.5	4.32, br m[b]		
5′.....	78.6	4.01, br m		
6′.....	62.6	4.42, dd, J=11.7, 6.4 Hz 4.52, ddd, J=11.7, 6.4, 2.4 Hz		
OH-4.....		10.64, s	H-3, H-10[c]	
OH-2′		7.20[a], br s		
OH-3′		7.29[a], br s		
OH-4′		7.48[a], br s		
OH-6′		6.44, br t, J=6.4 Hz		

[a]Assignments of similar chemical shift are interchangeable.
[b]Overlapping multiplet.
[c]Also displayed negative 'enhancement' peaks at δ 6.44, 7.20, 7.29, and 7.48 ppm due to hydroxyl proton exchange.

resonance which occurred at 7.54 ppm (H-6), while irradiation of the latter signal enhanced H-1′ and the aryl methyl group signal which occurred at 2.38 ppm (Me-5). Other nOe difference experiments (summarized in Table 1, and depicted in Figure 1) established the location of the remaining aryl substituent groups. The substitution pattern established for the aromatic portion of pteridioside corresponds to that of thymol [**2**] except for the incorporation of an additional hydroxyl at C-4, as in **3**. The strong ion at *m/z* 166 observed in the mass spectrum of **1a** can be assigned to a radical ion possessing structure **3**, arising from cleavage of the glucoside linkage with proton transfer.

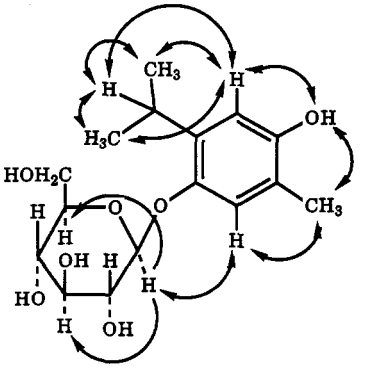
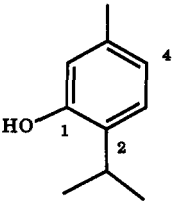
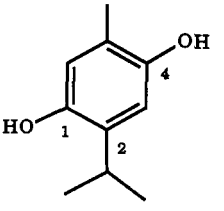


FIGURE 1. Enhancements observed for **1a** in nOe difference experiments.

The foregoing spectral data identified **1a** as 1,4-dihydroxy-2-*iso*-propyl-5-methylphenyl-1-*O*-β-D-glucopyranoside. Hitherto Sheu *et al.* (9) have reported the natural occurrence of the corresponding gallate querlanin [**1b**], which on tannase-treatment afforded **1a**. The ^{1}H- and ^{13}C-nmr spectral data reported for **1a** in Table 1 (C_5D_5N as solvent) correspond



2



3

closely with those determined by Sheu *et al.* (9) for this compound in Me_2CO-d_6. Our isolation of **1a** constitutes the first isolation this compound as a natural product.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Hplc was performed on Zorbax ODS columns (4.6 mm×15 cm i.d. for analytical and 9.4 mm×25 cm i.d. for prep.) at 35° and using a CH_3CN/H_2O gradient. Combined gc-ms analysis was performed using a 15 m×0.22 mm HP-1 (methylsilicone) capillary column (Hewlett-Packard) installed in a HP5890 gc instrument interfaced to a HP5970B mass selective detector. The gc column was temperature programmed from 160° to 220° at 30°/min, and then to 300° at 10°/min (15 min hold). One- and two-dimensional ^{1}H- (300.13 MHz) and ^{13}C-nmr (75.47 MHz) spectra, including nOe difference, COSY, and inverse-mode HMBC and HMQC spectra, were obtained in C_5D_5N on a Bruker AC-300 spectrometer using a standard 5-mm probe-head. Chemical shifts are reported relative to TMS. The hreims (direct-probe) of **1a** was determined on a Kratos MS80RFA instrument.

PLANT MATERIAL.—Young fronds (8–10 days after emergence) of *Pteridium aquilinum* var. *caudatum*, identified using the key of Ortega (8), were collected at "El Vallecito" near Mérida, Venezuela, during February 1994. Voucher specimens (UVI 93-001 and UVI 95-002) have been deposited in the herbarium of the Faculty of Pharmacy, Universidad de Los Andes, Mérida, Venezuela.

EXTRACTION AND ISOLATION.—Preliminary treatment to yield a dry powder of aqueous extracted material involved blending the fresh fronds in H_2O at room temperature, filtration, concentration and washing with CH_2Cl_2, and freeze-drying. A portion of the dried extract (2 g) was reconstituted in H_2O, then cleaned up and fractionated into six fractions by passage through polyamide 6S resin (Riedel-de Haen) with H_2O. These fractions were further subjected to analytical and prep. hplc. During the isolation of base-sensitive illudane-type sesquiterpene glucosides from the second fraction off the polyamide resin, a prominent unknown compound that was stable to base treatment was observed. Subjecting this fraction (213 mg) to prep. hplc with a mobile phase of CH_3CN-H_2O (29:171) at 2 ml min^{-1} gave 1,4-dihydroxy-2-*iso*-propyl-5-methylphenyl-1-*O*-β-D-glucopyranoside [**1a**] (at 15.3 min). Evaporation of the solvent afforded **1a** (26 mg) as a gum, and refrigeration afforded a glassy solid mp 162–164°; $[\alpha]^{20}_D$ −21.4° (*c*=0.03, MeOH); ^{1}H- (300.13 MHz) and ^{13}C- (75.47 MHz) nmr data (in C_5D_5N), see Table 1; eims *m/z* $[M]^+$ 328 (7), 208 (3), 181 (10), 166 (100), 151 (92). Hreims for $C_{16}H_{24}O_7$: found *m/z* 328.1524, calcd 328.1522. Purity was demonstrated by analytical hplc analysis, and by gc-ms analysis of the corresponding pentaacetate, prepared by *in situ* acetylation of a sub-sample of **1a** using pyridine-Ac_2O (1:1) (200 μl). The pentaacetate had *m/z* $[M]^+$ 538 (<0.1), 331 (9), 169 (33), 127 (8), 109 (32), 43 (100).

ACKNOWLEDGMENTS

The authors thank Universidad de los Andes, Fundacion Polar, Fundacion Gran Miriscal de Ayacucho, Fundacite Mérida of Venezuela, and AgResearch, New Zealand, for financial support given to U.F.C.

LITERATURE CITED

1. J.A. Taylor, in: "Bracken Biology and Management." Ed. by J.A. Thomson and R.T. Smith, Australian Institute of Agricultural Science, Sydney, Occasional Publication No. 40, July 1990, pp. 3–19.
2. C.M. Page, *Bot. J. Linn. Soc.*, **73**, 1 (1976).
3. G.R. Fenwick, *Sci. Food Agric.*, **46**, 147 (1988).
4. B.L. Smith, in: "Bracken Biology and Management." Ed. by J.A. Thomson and R.T. Smith, Australian Institute of Agricultural Science, Sydney, Occasional Publication No. 40, July 1990, pp. 227–232.
5. T. Murakami and N. Tanaka, *Prog. Chem. Org. Nat. Prod.*, **53**, 1 (1988).
6. M.E. Alonso-Amelot, M. Perez-Mena, M.P. Calcagno, and R. Jaimes-Espinoza, *Phytochem. Anal.*, **3**, 160 (1992).
7. M.E. Alonso-Amelot, M. Perez-Mena, M.P. Calcagno, R. Jaimes-Espinoza, and U.F. Castillo, *J. Chem. Ecol.*, **18**, 1405 (1992).
8. F. Ortega, *Biollania*, **7**, 47 (1990).
9. S.-Y. Sheu, F.-L. Hsu, and Y.-C. Lin, *Phytochemistry*, **31**, 2465 (1992).

Received 23 February 1995