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Full Papers

Diterpenoid Glycosides from the Bitter Fern *Gleichenia quadripartita*

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Fifteen new diterpenoid glycosides (**1a**–**n**, **2**) were isolated from an Argentine collection of the bitter fern *Gleichenia quadripartita* along with the known flavonoid glycoside afzelin. Structure elucidation was accomplished by 1D and 2D NMR spectroscopy and by high-resolution MS analyses. In addition, X-ray crystallographic analysis of a monocrystal of **1a** as well as chemical derivatization of **1h** and **1m** were performed to confirm their structures.

Gleichenia quadripartita (Poiret) T. Moore is a bitter-tasting fern that is endemic to southern Argentina and Chile. Ferns from the Gleicheniaceae family have been studied to some extent due to their interesting diterpenoid glycosides. From the root-stalks of ferns from this family, clerodane- and labdane-type diterpenoids and their glycosides have been reported.[1–4] Labdanes and clerodanes glycosilated at C-13 are rare in angiosperms, although they are rather common in ferns.[5] Within the Gleicheniaceae, *Dicranopteris pedata* contains (6*S*,13*S*)-cleroda-3,14-diene-6,13-diol and its 6,13-*O*-diglycosides, which are also present in the root-stalks of *D. linearis*, *G. microphylla*, and *G. japonica*. The latter also contains 13-*O*-glycosides and 3,13-*O*-diglycosides of (3*S*,13*R*)-labda-8(17),14-diene-3,13-diol, known as 3β-hydroxymanool.[6]

Continuing our search for bitter and pungent substances from ferns,[7,8] we report herein the isolation and identification of 15 new diterpenoid glycosides and a flavonoid from the bitter fern *G. quadripartita*.

Results and Discussion

The dried fronds of *G. quadripartita* were extracted successively with Et_2O and MeOH. The bitter-tasting MeOH extract was fractionated on a Si gel column, and the bitter fractions were further processed by RP-HPLC to afford 15 new diterpenoid glycosides along with the known flavonoid glycoside afzelin (kaempferol-3-*O*-α-L-rhamnopyranoside). The present report is the first on the chemistry of *G. quadripartita*.

Compounds **1a**–**h** are 13-*O*-monoglycoside *ent*-labdanes, **1i** and **1j** are 19-*O*-monoglycoside *ent*-labdanes, **1k**–**n** are 13,19-*O*-diglycoside *ent*-labdanes, and **2** is the only clerodane-type diterpenoid glycoside isolated from the present collection.

The HRFABMS of compound **1a** showed a quasimolecular ion peak at *m*/*z* 639.3746 $[M + Na]^+$, consistent with the molecular formula $C_{32}H_{56}O_{11}$, indicating five degrees of unsaturation. The structure and absolute configuration of **1a** was established as *ent*-14-labden-3β,8β-diol 13α-*O*-[β-D-quinovopyranosyl-(1→2)-α-L-rhamnopyranoside] as a result of X-ray crystallographic analysis and determination of the absolute configuration of the sugars. The colorless crystals, obtained from an MeOH solution, are monoclinic, belonging to the space group $P2_1$. In the ORTEP drawing of compound **1a** (Figure 1), the labdane-type skeleton of the aglycone, with the six-membered rings *trans*-fused in chair conformations, is clearly observed.[9] The results of the X-ray analysis agree with the structure derived from 1D and 2D NMR data of compound **1a**. Assignment of NMR spectra (Tables 1 and 2) was accomplished by extensive analysis of $^1H-^1H$ COSY, HMQC, NOESY, and HMBC (Supporting Information, Table A) NMR spectra.

The molecular formula of compound **1b** was $C_{26}H_{46}O_7$ and its molecular weight 470, as deduced by a quasimolecular ion peak $[M + Na]^+$ at *m*/*z* 493.3161 in the HRFABMS spectrum of **1b**. The NMR resonances for the aglycone moiety were almost identical to those of compound **1a**, but the resonance for the carbinol proton at C-3 of compound **1a** was absent in **1b**; therefore the aglycone of compound **1b** was established as *ent*-14-labden-8β,13α-diol. In

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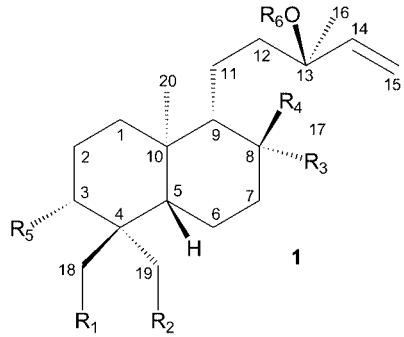

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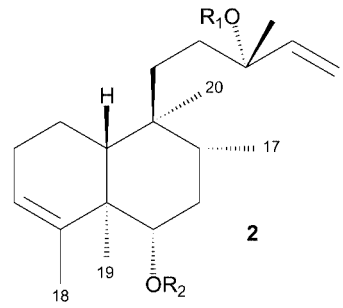

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1a- R_1=H, R_2=H, R_3=OH, R_4=CH_3, R_5=OH, R_6=β-D-quinovopyranosyl-(1→2)-α-L-rhamnopyranosyl
1b- R_1=H, R_2=H, R_3=OH, R_4=CH_3, R_5=H, R_6=β-D-glucopyranosyl
1c- R_1=H, R_2=OH, R_3=OH, R_4=CH_3, R_5=H, R_6=β-D-glucopyranosyl
1d- R_1=H, R_2=OH, R_3=CH_3, R_4=OH, R_5=H, R_6=β-D-glucopyranosyl
1e- R_1=OH, R_2=H, R_3=OH, R_4=CH_3, R_5=H, R_6=β-D-glucopyranosyl
1f- R_1=OH, R_2=H, R_3=CH_3, R_4=OH, R_5=H, R_6=β-D-glucopyranosyl
1g- R_1=H, R_2=OH, R_3=OH, R_4=CH_3, R_5=H, R_6=α-L-rhamnopyranosyl
1h- R_1=H, R_2=OH, R_3=OH, R_4=CH_3, R_5=H, R_6=β-D-quinovopyranosyl-(1→2)-α-L-rhamnopyranosyl
1i- R_1=H, R_2=*O*-β-D-glucopyranosyl, R_3=CH_3, R_4=OH, R_5=H, R_6=H
1j- R_1=H, R_2=*O*-β-D-glucopyranosyl, R_3=OH, R_4=CH_3, R_5=H, R_6=H
1k- R_1=H, R_2=*O*-α-L-rhamnopyranosyl, R_3=OH, R_4=CH_3, R_5=H, R_6=β-D-glucopyranosyl
1l- R_1=H, R_2=*O*-α-L-rhamnopyranosyl, R_3=OH, R_4=CH_3, R_5=H, R_6=α-L-rhamnopyranosyl
1m- R_1=H, R_2=*O*-α-L-rhamnopyranosyl, R_3=OH, R_4=CH_3, R_5=H, R_6=β-D-quinovopyranosyl-(1→2)-α-L-rhamnopyranosyl
1n- R_1=H, R_2=*O*-α-L-rhamnopyranosyl, R_3=OH, R_4=CH_3, R_5=H, R_6=β-D-quinovopyranosyl-(1→2)-3′-*O*-acetyl-α-L-rhamnopyranosyl
1o- R_1=H, R_2=OAc, R_3=OH, R_4=CH_3, R_5=H, R_6=2″,3″,4″-tri-*O*-acetyl-β-D-quinovopyranosyl-(1→2)-3′,4′-di-*O*-acetyl-α-L-rhamnopyranosyl
1p- R_1=H, R_2=*O*-2‴,3‴,4‴-tri-*O*-acetyl-α-L-rhamnopyranosyl, R_3=OH, R_4=CH_3, R_5=H, R_6=2″,3″,4″-tri-*O*-acetyl-β-D-quinovopyranosyl-(1→2)-3′,4′-di-*O*-acetyl-α-L-rhamnopyranosyl
2- R_1=β-D-glucopyranosyl, R_2=β-D-quinovopyranosyl

the ^{1}H NMR spectrum of **1b** (Table 1), a resonance for one anomeric proton at δ 4.34 (1H, d, $J_{1',2'}$ = 8.0 Hz) indicated the presence of a sugar moiety. The nature of the sugar could be inferred from the peak observed at *m/z* 290 in the EI mass spectrum assigned to the fragment $[M - H_2O - 162]^+$, consistent with the loss of a hexose moiety. The sugar was identified as β-D-glucopyranose by analysis of the chemical shifts, coupling constants, and multiplicity of its NMR resonances, together with chiral detection after acid hydrolysis of the glycoside. In addition, the HMBC spectrum of **1b** (Supporting Information, Table A) showed correlation between the anomeric proton of glucose and C-13 (δ 82.1) of the aglycone; thus, the structure of **1b** was established as *ent*-14-labden-8β-ol 13α-*O*-β-D-glucopyranoside.

The bitter-tasting compound **1c** was obtained as a white, amorphous solid. Its positive HRFABMS spectrum showed a quasimolecular ion peak at *m/z* 509.3100 $[M + Na]^+$, consistent with the molecular formula $C_{26}H_{46}O_8$, accounting for four degrees of unsaturation. The anomeric proton at δ 4.34 (1H, d, $J_{1',2'}$ = 7.9 Hz) in the ^{1}H NMR spectrum of **1c** (Table 1) indicated the presence of a sugar moiety identified as β-D-glucopyranose by comparison of its NMR data (Tables 1 and 2) with those of compound **1b**, as well as chiral detection of the sugar. The presence of a labdane-type diterpenoid aglycone in **1c** was confirmed by comparison of the NMR data with those of **1b**. The C-19 CH_2OH group could be assigned from the doublets at δ 3.75 and 3.30 (1H each, J = 11.0 Hz), assigned to a methylene in the proton spectrum, and from the cross-peaks in the HMQC and HMBC spectra that linked the doublets with C-19 (δ 64.9) and C-4 (δ 39.7), respectively. A long-range ^{1}H−^{13}C correlation between the proton at δ 4.34 and the ^{13}C NMR resonance at δ 82.1 revealed the location of the glucose attached to C-13 of the aglycone. The position of the sugar, as depicted, was confirmed by the NOESY spectrum (Supporting Information, Figure B). Therefore, compound **1c** was identified as *ent*-14-labden-8β,19-diol 13α-*O*-β-D-glucopyranoside.

The bitter-tasting compound **1d** was an isomer of **1c**, with the molecular formula $C_{26}H_{46}O_8$ and a molecular weight of 486, as deduced by its HRFABMS, which showed a quasimolecular peak at *m/z* 509.3089 $[M + Na]^+$. The specific rotation for **1d**, $[\alpha]_D^{20}$ −31.0, was slightly different from that for **1c**, $[\alpha]_D^{22}$ −27.1. The NMR spectroscopic features of **1d** (Tables 1 and 2) were very similar to those of **1c**, which indicated that the structures were almost identical. However, the ^{13}C NMR resonance assigned to C-17 was shifted 7.1 ppm upfield (δ 23.9), and H-9 was shifted 0.35 ppm downfield (δ 1.07, br t), in comparison with the corresponding resonances in the NMR spectra of **1c**, indicating that the hydroxy group at C-8 in compound **1d** was β-oriented. Further evidence was provided by NOESY correlations (Supporting Information, Figure B) observed between CH_3-17 and both H-19 and CH_3-20, as well as between H-1β and H-9 (both β-axially oriented). In addition, the absence of any NOESY correlation between CH_3-17 and H-9 or H-5 indicated that, in **1d**, CH_3-17, CH_3-20, and CH_2-19 were cofacial. Therefore, compound **1d** was identified as *ent*-14-labden-8α,19-diol 13α-*O*-β-D-glucopyranoside.

Compound **1e**, with the molecular formula $C_{26}H_{46}O_8$, isomeric with **1c** and **1d**, showed ^{1}H and ^{13}C NMR traces very similar to those of compound **1c**. A comparative glance at the ^{13}C NMR spectra of **1c** and **1e** (Table 2) indicated that the main differences were in the chemical shifts of C-5, C-18, and C-19. In addition, in the ^{1}H NMR spectra of both compounds (Table 1), the differences were observed in the resonances assigned to H-3α, H-3β, H-5, H-18, and H-19, indicating that **1e** could be a C-4 epimer of **1c**. In order to confirm this hypothesis, the NOESY spectrum of **1e** (Supporting Information, Figure C) was measured and clearly showed correlations between CH_3-19 and CH_3-20, indicating that in **1e** a methyl group at C-4 was α-oriented, while the CH_2OH moiety attached to C-4 was β-equatorially oriented. Thus, compound **1e** is *ent*-14-labden-8β,18-diol 13α-*O*-β-D-glucopyranoside.

Compound **1f** was an isomer of **1e**, with the molecular formula $C_{26}H_{46}O_8$ and a molecular weight of 486, as deduced by its HRFABMS, showing a quasimolecular ion peak at *m/z* 509.3111 $[M + Na]^+$. The specific rotation for **1f**, $[\alpha]_D^{20}$ −37.3, was slightly different from that for **1e**, $[\alpha]_D^{22}$ −35.0. The ^{1}H and ^{13}C NMR spectroscopic features of **1f** (Tables 1 and 2) were similar to those of **1e**, indicating that the structures were almost identical. However, the ^{13}C NMR resonance assigned to C-17 was shifted 7.1 ppm upfield (δ 24.0), and H-9 was shifted around 0.30 ppm downfield

Table 1. ^{1}H NMR Data of Compounds **1a–f** (600 MHz, MeOH-d_4)[a]

H	**1a**	**1b**	**1c**	**1d**	**1e**	**1f**
1α	1.73–1.67[b]	1.68–1.62[b]	1.72–1.68[b]	1.68 br d (13.2)	1.67–1.61[b]	1.68–1.61[b]
1β	1.00 td (13.2, 3.6)	0.89–0.82[b]	0.94–0.88[b]	1.00 td (13.2, 3.6)	0.85 td (13.2, 4.0)	0.96 td (11.7, 3.0)
2a	1.65–1.60[b]	1.66–1.56[b]	1.64–1.56[b]	1.60–1.52[b]	1.60–1.53[b]	1.68–1.61[b]
2b	1.59–1.52[b]	1.42–1.34[b]	1.40–1.33[b]	1.26 tt (13.5, 3.0)	1.45–1.39[b]	1.50–1.43[b]
3α		1.42–1.34[b]	1.84 br d (13.5)	1.82–1.76[b]	1.47–1.41[b]	1.50–1.44[b]
3β	3.13 dd (11.5, 4.7)	1.16 td (13.5, 3.8)	0.88 td (13.5, 4.1)	0.90 td (13.2, 4.0)	1.22–1.17[b]	1.20 br d (12.4)
5β	0.82 dd (12.1, 1.9)	0.88–0.82[b]	0.98 dd (11.8, 2.8)	1.07 dd (NC, 3.8)	1.23–1.18[b]	1.31–1.27[b]
6a	1.66–1.60[b]	1.57 td (13.2, 3.0)	1.62–1.52[b]	1.61 td (12.9, 3.8)	1.55 td (12.4, 2.7)	1.61–1.52[b]
6b	1.50–1.44[b]	1.52–1.42[b]	1.62–1.52[b]	1.43–1.37[b]	1.37 br d (14.0)	1.32–1.26[b]
7α	1.74 dt (13.5, 3.0)	1.74 dt (13.5, 3.0)	1.73 dt (13.5, 3.0)	1.82–1.77[b]	1.70 dt (13.5, 2.7)	1.77 dt (12.6, 2.8)
7β	1.41 td (13.5, 4.1)	1.46–1.34[b]	1.42–1.34[b]	1.40–1.34[b]	1.48 td (13.5, 3.8)	1.52–1.44[b]
9β	0.67 br t (3.0)	0.71 br t (3.5)	0.72 br t (4.5)	1.07 br t (3.5)	0.76–0.73[b]	1.10 br t (3.8)
11a	1.54–1.49[b]	1.50–1.40[b]	1.46 tt (14.6, 4.5)	1.75–1.70[b]	1.49–1.42[b]	1.62–1.53[b]
11b	1.36–1.29[b]	1.29 br t (16.0)	1.29 br t (14.6)	1.39–1.32[b]	1.30 br t (13.0)	1.30–1.23[b]
12a	1.64–1.56[b]	1.68 td (13.5, 5.5)	1.72–1.59[b]	1.84 td (12.9, 4.0)	1.71–1.58[b]	1.85 td (13.0, 4.0)
12b	1.54–1.50[b]	1.66–1.58[b]	1.72–1.59[b]	1.64–1.54[b]	1.71–1.58[b]	1.66–1.58[b]
14	5.83 dd (17.4, 11.0)	5.91 dd (17.7, 11.0)	5.91 dd (17.7, 11.0)	5.96 dd (17.7, 11.0)	5.92 dd (17.8, 11.0)	5.96 dd (17.7, 11.0)
15 *trans*	5.21 dd (17.4, 1.1)	5.23 dd (17.7, 1.4)	5.23 dd (17.7, 1.4)	5.19 dd (17.7, 1.2)	5.23 dd (17.8, 1.2)	5.19 dd (17.7, 1.4)
15 *cis*	5.22 dd (11.0, 1.1)	5.22 dd (11.0, 1.4)	5.22 dd (11.0, 1.4)	5.16 dd (11.0, 1.2)	5.22 dd (11.0, 1.2)	5.16 dd (11.0, 1.4)
16	1.35 s	1.40 s	1.40 s	1.36 s	1.40 s	1.36 s
17	1.11 s	1.12 s	1.12 s	1.10 s	1.13 s	1.12 s
18	0.78 s	0.87 s	0.93 s	0.94 s	3.35 d (11.0)	3.35 d (11.0)
					2.99 d (11.0)	2.99 d (11.0)
19	0.96 s	0.84 s	3.75 d (11.0)	3.64 d (11.3)	0.75 s	0.72 s
			3.30 d (11.0)	3.33 d (11.3)		
20	0.96 s	0.95 s	0.91 s	0.80 s	0.98 s	0.85 s
13-*O*-	Rha	Glc	Glc	Glc	Glc	Glc
1′	5.18 d (1.8)	4.34 d (8.0)	4.34 d (7.9)	4.34 d (7.7)	4.34 d (7.7)	4.35 d (7.7)
2′	3.68 dd (3.6, 1.8)	3.14 dd (8.0, 9.1)	3.14 dd (7.9, 9.0)	3.15 dd (7.7, 8.9)	3.14 dd (7.7, 8.8)	3.15 dd (7.7, 9.0)
3′	3.75 dd (3.6, 9.5)	3.30 t (9.1)	3.31 t (9.0)	3.30 t (8.9)	3.30 t (8.8)	3.30 t (9.0)
4′	3.32 t (9.5)	3.26 t (9.1)	3.26 t (9.0)	3.26 t (8.9)	3.26 t (9.4)	3.27 t (9.0)
5′	3.77 dq (9.5, 6.2)	3.16 ddd (9.1, 2.5, 5.6)	3.16 ddd (9.0, 2.5, 5.8)	3.15 ddd (8.9, 2.6, 5.6)	3.16 ddd (9.4, 2.4, 5.8)	3.15 ddd (9.0, 2.5, 5.8)
6′	1.22 d (6.2)	3.80 dd (11.8, 2.5)	3.80 dd (11.8, 2.5)	3.80 dd (12.1, 2.6)	3.80 dd (12.0, 2.4)	3.79 dd (11.8, 2.5)
		3.62 dd (11.8, 5.6)	3.62 dd (11.8, 5.8)	3.62 dd (12.1, 5.6)	3.62 dd (12.0, 5.8)	3.62 dd (11.8, 5.8)
2′-*O*-Qui (**1a** only)	H1″: 4.34 d (7.2), H-2″: 3.25 dd (7.2, 9.4), H-3″: 3.28 t (9.4), H-4″: 2.91 t (9.4), H-5″: 3.28 dq (9.4, 6.0), H-6″: 1.25 d (6.0)					

[a] NC: coupling constant not calculated because of overlapping resonances; Rha: α-L-rhamnopyranosyl, Glc: β-D-glucopyranosyl, Qui: β-D-quinovopyranosyl. [b] Overlapped resonances.

Table 2. ^{13}C NMR Data of Compounds **1a–g**, **1i**, and **1j** (150 MHz, MeOH-d_4)

C	1a	1b	1c	1d	1e	1f	1g	1i	1j
1	38.9	40.7	40.6	41.3	40.2	40.7	40.7	41.3	40.7
2	27.6	19.3	18.9	20.4	18.7	18.9	18.9	19.2	19.0
3	79.8	43.3	36.6	36.8	36.5	36.5	36.6	37.7	37.4
4	39.9	34.0	39.7	39.7	38.6	38.6	39.7	38.9	39.0
5	56.5	57.5	58.2	58.2	49.9	50.2	58.2	58.3	58.3
6	19.3	19.6	19.4	19.1	19.1	21.1	19.4	20.9	19.7
7	43.2	43.1	43.5	44.8	42.7	44.5	43.5	45.6	43.5
8	73.7	74.0	73.9	75.1	74.0	75.2	73.9	75.2	73.9
9	60.6	60.8	60.9	63.2	60.7	63.1	60.9	62.9	61.0
10	40.1	40.4	40.3	40.4	40.2	40.4	40.3	40.5	40.3
11	20.2	20.4	20.6	21.6	20.4	20.2	20.4	21.9	20.8
12	47.1	46.6	46.5	45.2	46.6	44.8	47.3	46.7	47.6
13	81.1	82.1	82.1	81.8	82.1	81.9	80.9	74.4	74.3
14	143.9	144.6	144.5	145.2	144.6	145.2	143.8	146.7	146.2
15	116.0	116.1	116.1	115.2	116.1	115.2	116.1	111.8	112.2
16	22.8	22.5	22.5	23.1	22.5	23.1	22.6	27.4	27.3
17	30.9	31.1	31.0	23.9	31.1	24.0	30.9	23.8	30.9
18	28.8	34.2	27.7	27.7	72.0	72.0	27.7	28.3	28.2
19	16.2	22.2	64.9	65.1	16.1	17.8	64.9	74.1	73.9
20	15.8	15.7	16.5	16.6	18.1	16.5	16.5	16.5	16.4
13-*O*- or 19-*O*-	Rha	Glc	Glc	Glc	Glc	Glc	Rha	Glc	Glc
1′	96.0	99.5	99.5	99.4	99.5	99.4	96.7	105.0	105.1
2′	83.6	75.3	75.2	75.2	75.3	75.2	73.4	75.2	75.3
3′	72.3	78.3	78.2	78.3	78.3	78.3	72.5	78.3	78.2
4′	74.6	71.7	71.7	71.7	71.7	71.7	74.4	71.7	71.7
5′	69.5	77.6	77.6	77.6	77.6	77.6	69.6	77.8	77.7
6′	18.0	62.8	62.8	62.8	62.8	62.8	18.0	62.8	62.7
2′-*O*-Qui (**1a** only)			106.7 (C-1″), 75.6 (C-2″), 77.7 (C-3″), 76.8 (C-4″), 73.3 (C-5″), 18.2 (C-6″)						

(δ 1.10, br t), in comparison with the corresponding resonances in the NMR spectra of **1e** (Tables 1 and 2). This indicated that the C-8 hydroxy group in compound **1f** was β-oriented. Further evidence was provided by the NOESY spectrum of **1f** (Supporting Information, Figure C). NOESY correlations between CH_3-20 and both CH_3-17 and CH_3-19, as well as between H-1β and H-9 (both β-axially oriented), were observed. Therefore, compound **1f** was identified as *ent*-14-labden-8α,18-diol 13α-*O*-β-D-glucopyranoside.

The bitter-tasting compound **1g** was isolated as an amorphous solid, whose molecular formula, $C_{26}H_{46}O_7$, was obtained from its HREIMS (*m/z* 470.3256, M^+). Extensive analysis of the NMR spectra of **1g** (Tables 2 and 3) showed that the aglycone was the same as that of **1c**. The resonance at δ 1.22 (3H, d, $J = 6.3$ Hz) assigned to a methyl group together with a group of oxygenated methine resonances between δ 3.1 and 4.9 in the ^1H NMR spectrum (Table 3) indicated the presence of a 6-deoxy sugar unit. This was further confirmed by the peak at *m/z* 306 in the EIMS of **1g** assigned to $[M - 146 - H_2O]^+$. Analysis of the chemical shifts, coupling constants, and multiplicity of the resonances for the sugar residue, as well as chiral detection after hydrolysis of the glycoside, allowed us to identify it as an α-L-rhamnopyranosyl moiety. The β-orientation of the anomeric proton was clear through the value of the coupling constant of the doublet at δ 4.83, $J_{1',2'} = 1.8$ Hz. A cross-peak between the anomeric proton of rhamnose and the resonance assigned to C-13 (δ 80.9) of the aglycone was observed in the HMBC spectrum of **1g** (Supporting Information, Table B). Thus, the structure of this compound was established as *ent*-14-labden-8β,19-diol 13α-*O*-α-L-rhamnopyranoside.

The bitter glycoside **1h** exhibited a quasimolecular ion peak $[M + Na]^+$ at *m/z* 639.3730, consistent with the molecular formula

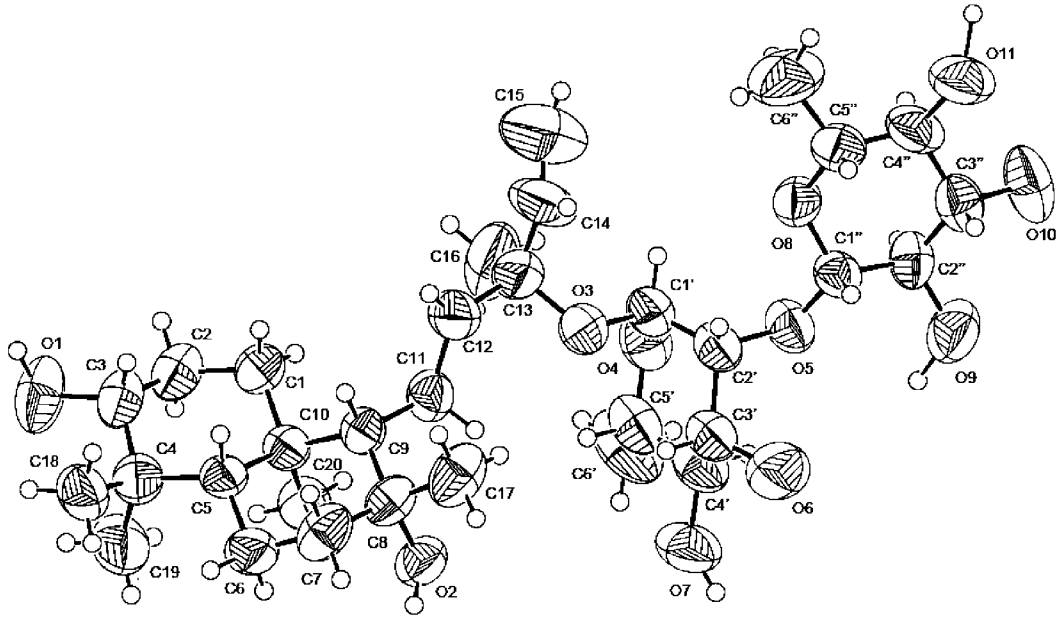

Figure 1. ORTEP diagram for compound **1a**.

Table 3. ^{1}H NMR Data of Compounds **1g**, **1i**, and **1j** (600 MHz, MeOH-d_4)[a]

H	1g	1i	1j
1α	1.71 br d (13.2)	1.74–1.68*	1.74–1.70*
1*β*	0.89 td (13.2, 3.6)	0.97 td (13.5, 4.1)	0.90 td (13.5, 3.6)
2a	1.64–1.50*	1.64 br dt (13.5, 3.5)	1.65–1.59*
2b	1.40–1.34*	1.42–1.34*	1.38–1.32*
3α	1.84 br d (13.5)	1.84 br d (13.5)	1.88 br d (14.0)
3*β*	0.89 td (13.5, 4.0)	0.94 td (13.5, 4.1)	0.91 td (14.0, 4.1)
5*β*	0.99 dd (11.8, 2.7)	1.04 dd (12.1, 2.2)	0.96 dd (12.1, 2.5)
6a	1.62–1.50*	1.50–1.43*	1.66–1.60*
6b	1.62–1.50*	1.31 dt (12.1, 4.1)	1.60–1.53*
7α	1.73 dt (13.5, 3.0)	1.80 dt (12.1, 2.8)	1.72 dt (13.5, 3.2)
7*β*	1.41–1.34*	1.41–1.34*	1.38 td (13.5, 4.6)
9*β*	0.70 br t (3.6)	1.05, t (4.0)	0.71 br t (4.4)
11a	1.51 tt (13.2, 3.6)	1.75–1.70*	1.45 tt (13.2, 4.4)
11b	1.34–1.28*	1.47–1.42*	1.34–1.28*
12a	1.65–1.59*	1.75–1.67*	1.61–1.56*
12b	1.54–1.49*	1.55 td (12.9, 4.1)	1.53 td (13.2, 4.4)
14	5.81 dd (17.7, 11.0)	5.91 dd (17.6, 10.8)	5.92 dd (17.4, 10.8)
15 *trans*	5.21 dd (17.7, 1.1)	5.18 dd (17.6, 1.6)	5.19 dd (17.4, 1.4)
15 *cis*	5.23 dd (11.0, 1.1)	5.00 dd (10.8, 1.6)	5.03 dd (10.8, 1.4)
16	1.36 s	1.23 s	1.25 s
17	1.10 s	1.10 s	1.10 s
18	0.93 s	1.01 s	1.00 s
19	3.75 d (11.0)	3.98 d (9.6)	4.08 d (9.3)
	3.30 d (11.0)	3.32 d (9.6)	3.29 d (9.3)
20	0.92 s	0.84 s	0.94 s
13-*O*-	Rha	Glc	Glc
1′	4.83 d (1.8)	4.17 d (7.8)	4.17 d (7.9)
2′	3.68 dd (1.8, 3.3)	3.16 dd (7.8, 9.0)	3.16 dd (7.9, 9.2)
3′	3.70 dd (3.3, 9.3)	3.33 t (9.0)	3.33 t (9.2)
4′	3.33 t (9.3)	3.29 t (9.0)	3.29 t (9.2)
5′	3.16 dq (9.3, 6.3)	3.23 ddd (9.0, 2.2, 5.5)	3.23 ddd (9.2, 2.2, 5.5)
6′	1.22 d (6.3)	3.85 dd (11.8, 2.2)	3.84 dd (12.1, 2.2)
		3.67 dd (11.8, 5.5)	3.68 dd (12.1, 5.5)

[a] * = overlapping signals.

$C_{32}H_{56}O_{11}$. Analysis of the NMR data for **1h** (Tables 4 and 5) indicated that the aglycone was *ent*-14-labden-8*β*,13α,19-triol as in **1g**. Resonances for two anomeric protons at *δ* 5.18 (1H, d, $J_{1',2'}$ = 1.7 Hz) and 4.34 (1H, d, $J_{1'',2''}$ = 7.4 Hz) were detected in the ^{1}H NMR spectrum of this compound (Table 4). Comparison of the NMR data for the sugar moieties of compound **1h** with those for **1a** revealed identical disaccharide moieties. The HMBC spectrum of **1h** (Supporting Information, Table C) revealed correlation between the anomeric proton of rhamnose and C-13 (*δ* 81.0), as well as a cross-peak between the anomeric proton of quinovose and C-2′ of rhamnose (*δ* 83.6), which indicated the position and nature of the sugar moiety. Additional evidence supporting the proposed structure was obtained by acetylation of **1h** to furnish the hexaacetate **1o**, whose NMR data are compiled in Tables 4 and 5. The foregoing evidence allowed us to establish the structure of **1h** as *ent*-14-labden-8*β*,19-diol 13α-*O*-[*β*-D-quinovopyranosyl-(1→2)-α-L-rhamnopyranoside].

From the HRFABMS of the nonbitter compound **1i**, the molecular formula $C_{26}H_{46}O_8$ was obtained, indicating that this compound was an isomer of compounds **1c**−**f**. The ^{13}C and ^{1}H NMR spectra of **1i** (Tables 2 and 3) were similar to those of **1d**, which indicated that the structures were almost identical except for the location of the sugar moiety. The HMBC spectrum (Supporting Information, Table B) showed a cross-peak between the anomeric proton of glucose (*δ* 4.17, d, $J_{1',2'}$ = 7.8 Hz) and the resonance assigned to C-19 of the aglycone (*δ* 74.1). Therefore, compound **1i** was identified as *ent*-14-labden-8α,13α-diol 19-*O*-*β*-D-glucopyranoside.

The HRFABMS of compound **1j** showed a quasimolecular ion peak at *m/z* 509.3102 $[M + Na]^+$ with a molecular formula of $C_{26}H_{46}O_8$, indicating that **1j** is an isomer of compounds **1c**−**f** and **1i**. The ^{13}C and ^{1}H NMR traces of **1j** (Tables 2 and 3) were almost identical to those of **1i** except for the resonance assigned to H-9 (*δ* 0.71, br t), which was shifted 0.37 ppm upfield, and C-17 (*δ* 30.9), located 7.1 ppm downfield in comparison with the corresponding resonances in the NMR spectra of **1i**. Analysis of the NOESY spectrum of **1j** showed correlation between H-9 and CH_3-17, indicating that the C-8 methyl group was *β*-oriented. Therefore, the hydroxy group attached to C-8 was α-oriented and the structure of **1j** is *ent*-14-labden-8*β*,13α-diol 19-*O*-*β*-D-glucopyranoside.

The molecular formula of the bitter compound **1k** was assigned as $C_{32}H_{56}O_{12}$ on the basis of the quasimolecular ion peak $[M + Na]^+$ at *m/z* 655.3687 in the HRFABMS. The ^{1}H and ^{13}C NMR spectra of **1k** (Tables 4 and 5) displayed similar features to those of **1c**, since the aglycones were the same. However, in the NMR spectra of **1k**, resonances for two anomeric protons at *δ* 4.57 (1H, d, $J_{1'',2''}$ = 1.6 Hz) and 4.34 (1H, d, $J_{1',2'}$ = 8.0 Hz) as well as for two anomeric carbons at *δ* 101.8 and 99.5 indicated the presence of two sugar units, identified as α-L-rhamnose and *β*-D-glucose. Long-range ^{1}H−^{13}C correlations (Supporting Information, Table C) were observed between the anomeric proton (*δ* 4.57) of rhamnose and C-19 (*δ* 71.5) and between the anomeric proton of glucose (δ4.34) and C-13 (*δ* 82.1) of the aglycone moiety, revealing the location of the sugars. The structure of **1k** was therefore established as *ent*-14-labden-8*β*-ol 13α-*O*-*β*-D-glucopyranosyl-19-*O*-α-L-rhamnopyranoside.

The bitter-tasting compound **1l** had the molecular formula $C_{32}H_{56}O_{11}$ on the basis of its HRFABMS. The ^{1}H and ^{13}C NMR features of this compound (Tables 4 and 5) closely resembled those of **1k**, but two rhamnose units were detected. The HMBC correlations observed for **1l** (Supporting Information, Table C) revealed that one rhamnose was linked to C-13 (*δ* 80.9) and the other to C-19 (*δ* 71.5). Therefore, the location of the sugar residues attached to the aglycone was the same as in **1k**. These data supported the identification of **1l** as *ent*-14-labden-8*β*-ol 13α-*O*-α-L-rhamnopyranosyl-19-*O*-α-L-rhamnopyranoside.

Compound **1m** was obtained as a bitter-tasting, amorphous solid. A quasimolecular ion peak at *m/z* 785.4316 $[M + Na]^+$ was

Table 4. ^{1}H NMR Data of Compounds **1h**, **1k**, **1l** (600 MHz, MeOH-d_4), and **1o** (600 MHz, $CDCl_3$)[a]

H	1h	1k	1l	1o
1α	1.72 br d (13.5)	1.73–1.68*	1.75–1.70*	1.74–1.68*
1β	0.90 td (13.5, 4.1)	0.92 td (13.2, 3.4)	0.91 td (12.9, 3.2)	0.89 td (12.9, 3.3)
2a	1.64–1.51*	1.60–1.52*	1.62–1.51*	1.62–1.53*
2b	1.42–1.36*	1.40–1.35*	1.42–1.35*	1.46–1.38*
3α	1.84 br d (13.7)	1.80 br d (13.5)	1.80 br d (13.5)	1.72–1.66*
3β	0.89 td (13.7, 4.1)	0.98–0.91*	0.99–0.93*	1.01–0.95*
5β	0.99 dd (12.1, 2.4)	0.98 dd (12.4, 2.2)	0.99 dd (NC, 2.5)	1.01–0.95*
6a	1.64–1.51*	1.67–1.60*	1.63 td (13.5, 2.5)	1.63–1.54*
6b	1.64–1.51*	1.60–1.52*	1.60–1.52*	1.63–1.54*
7α	1.74 dt (13.5, 3.0)	1.73 dt (13.2, 3.0)	1.73 dt (13.5, 3.0)	1.76 dt (14.0, 3.2)
7β	1.40 td (13.5, 4.4)	1.37 td (13.2, 4.4)	1.43–1.36*	1.46–1.38*
9β	0.71 br t (3.6)	0.73 br t (4.6)	0.72 br t (3.8)	0.75 br t (3.5)
11a	1.52 tt (12.9, 3.6)	1.48 tt (14.7, 4.6)	1.53 tt (13.5, 3.8)	1.46–1.38*
11b	1.36–1.29*	1.31 br t (14.7)	1.36–1.31*	1.35–1.28*
12a	1.64–1.56*	1.72–1.59*	1.66–1.58*	1.62 td (13.5, 4.9)
12b	1.56–1.50*	1.72–1.59*	1.58–1.50*	1.54 td (13.5, 4.7)
14	5.83 dd (17.4, 11.3)	5.92 dd (17.8, 11.0)	5.82 dd (17.6, 11.0)	5.70 dd (17.6, 11.0)
15 *trans*	5.21 dd (17.4, 1.1)	5.24 dd (17.8, 1.4)	5.22 dd (17.6, 1.2)	5.17 dd (17.6, 0.8)
15 *cis*	5.22 dd (11.3, 1.1)	5.23 dd (11.0, 1.4)	5.24 dd (11.0, 1.2)	5.20 dd (11.0, 0.8)
16	1.35 s	1.40 s	1.37 s	1.34 s
17	1.11 s	1.12 s	1.11 s	1.15 s
18	0.94 s	0.98 s	0.98 s	0.97 s
19	3.75 d (11.7)	3.83 d (9.3)	3.84 d (9.2)	4.23 d (11.0)
	3.28 d (11.7)	3.16 d (9.3)	3.16 d (9.2)	3.92 d (11.0)
20	0.93 s	0.94 s	0.96 s	0.96 s
13-*O*-	Rha	Glc	Rha	Rha
1′	5.18 d (1.7)	4.34 d (8.0)	4.84 d (1.6)	5.03 d (1.4)
2′	3.68 dd (1.7, 3.4)	3.14 dd (8.0, 9.2)	3.69 dd (1.6, 3.4)	3.83 dd (1.4, 3.0)
3′	3.76 dd (3.4, 9.3)	3.31 t (9.2)	3.70 dd (3.4, 9.2)	5.19 dd (3.0, 9.9)
4′	3.33 t (9.3)	3.26 t (9.2)	3.34 t (9.2)	4.88 t (9.9)
5′	3.77 dq (9.3, 6.3)	3.16 ddd (9.2, 2.5, 5.8)	3.78 dq (9.2, 6.3)	3.97 dq (9.9, 6.3)
6a′	1.26 d (6.3)	3.80 dd (11.8, 2.5)	1.22 d (6.3)	1.15 d (6.3)
6b′		3.62 dd (11.8, 5.8)		
	2′-*O*-Qui	19-*O*-Rha	19-*O*-Rha	2′-*O*-Qui
1″	4.34 d (7.4)	4.57 d (1.6)	4.58 d (1.5)	4.41 d (7.4)
2″	3.26 dd (7.4, 9.1)	3.78 dd (1.6, 3.5)	3.78 dd (1.5, 3.3)	5.03 dd (7.4, 9.6)
3″	3.29 t (9.1)	3.62 dd (3.5, 9.4)	3.62 dd (3.3, 9.4)	5.16 t (9.6)
4″	2.99 t (9.1)	3.36 t (9.4)	3.36 t (9.4)	4.78 t (9.6)
5″	3.28 dq (9.1, 6.3)	3.57 dq (9.4, 6.3)	3.57 dq (9.4, 6.3)	3.50 dq (9.6, 6.2)
6″	1.26 d (6.3)	1.25 d (6.3)	1.26 d (6.3)	1.18 d (6.2)

[a] NC: coupling constant not calculated because of overlapping resonances. Rha: α-L-rhamnopyranosyl, Glc: β-D-glucopyranosyl, Qui: β-D-quinovopyranosyl. * = overlapped resonances.

observed in the HRFABMS of this compound, indicating a molecular formula of $C_{38}H_{66}O_{15}$. The ^{1}H NMR spectrum of **1m** (Table 6) exhibited three anomeric protons at δ 5.18 (1H, d, $J_{1',2'}$ = 1.6 Hz), 4.34 (1H, d, $J_{1'',2''}$ = 7.2 Hz), and 4.58 (1H, d, $J_{1''',2'''}$ = 1.8 Hz). The NMR data of **1m** resembled those of **1h**, indicating the presence of the same aglycone and disaccharide as found in **1h**, but additionally one more α-rhamnose unit was detected. HMBC correlations (Supporting Information, Table D) between the anomeric proton of rhamnose (H-1′) and C-13 (δ 81.1) of the aglycone revealed the linkage of the disaccharide to C-13. Another cross-peak was observed between the anomeric proton of the other rhamnose unit (H-1‴) and C-19 (δ 71.5) of the aglycone. Additional evidence for the proposed structure was provided by acetylation of **1m** to furnish **1p**, whose NMR features are compiled in Tables 5 and 6. On the basis of the foregoing evidence, compound **1m** was identified as *ent*-14-labden-8β-ol 13α-*O*-[β-D-quinovopyranosyl-(1→2)-α-L-rhamnopyranosyl]-19-*O*-α-L-rhamnopyranoside.

Glycoside **1n** gave a quasimolecular ion peak at *m/z* 827.4423 $[M + Na]^+$ in its HRFABMS, accounting for a molecular formula of $C_{40}H_{68}O_{16}$. The ^{13}C NMR spectrum of this compound (Table 5) was identical to that of **1m**, but two additional resonances at δ 172.6 and 21.0 were observed. These resonances, along with a singlet at δ 2.09 (3H, s) in the ^{1}H NMR spectrum of **1n** (Table 6), revealed the presence of an *O*-acetyl group that was evident by the IR C═O band at 1723 cm^{-1}. The acetate location was clear from the 1.25 ppm downfield shift of the proton NMR resonance assigned to H-3′ (δ 5.01) of the rhamnose moiety of **1n** in comparison with the corresponding resonance in the spectrum of **1m**. The acetylated rhamnose was linked to C-13 (δ 81.2) of the aglycone. In addition, a 2.8 ppm downfield shift of the resonance assigned to C-3′ (δ 75.1) of the acetylated sugar unit was observed in the ^{13}C NMR spectrum of **1n**. Therefore, the structure of **1n** was *ent*-14-labden-8β-ol 13α-*O*-[β-D-quinovopyranosyl-(1→2)-3′-*O*-acetyl-α-L-rhamnopyranosyl]-19-*O*-α-L-rhamnopyranoside.

The HRFABMS of the bitter compound **2** was consistent with the formula $C_{32}H_{54}O_{11}$, accounting for six degrees of unsaturation. The ^{13}C NMR spectrum of compound **2** (Table 7) showed 32 resonances assigned to six methyl groups, seven methylenes (one olefinic and one oxygenated), 15 methines (two olefinic and nine oxygenated), three quaternary carbons, and one disubstituted olefinic carbon. Extensive analysis of 1D and 2D NMR spectra and comparison with literature data allowed us to identify the aglycone moiety of **2** as cleroda-3,14-dien-6,13-diol, a common aglycone of diterpenoid glycosides from ferns belonging to the Gleicheniaceae family.[1–4] The presence of two sugar units was evident from the anomeric proton resonances at δ 4.40 (1H, d, $J_{1'',2''}$ = 7.7 Hz) and 4.33 (1H, d, $J_{1',2'}$ = 7.8 Hz) in the ^{1}H NMR spectrum of **2**. They were identified as β-D-quinovose and β-D-glucose by comparison of the NMR data with those of compounds **1a** and **1c**. Long-range correlations between the anomeric proton of glucose and C-13 (δ 81.9) of the aglycone, as well as between the anomeric proton of quinovose and C-6 (δ 87.1) of the aglycone were observed in the HMBC spectrum of **2** (Supporting Information, Table E). The location of the sugar units was further confirmed by NOESY correlations (Supporting Information, Figure D) observed between the anomeric proton of glucose (δ 4.33) and both H-14 and CH_3-

Table 5. ^{13}C NMR Data of Compounds **1h**, **1k**–**n** (150 MHz, MeOH-d_4), **1o** and **1p** (150 MHz, $CDCl_3$)

C	1h	1k	1l	1m	1n	1o	1p
1	40.7	40.7	40.6	40.7	40.7	39.1	39.2
2	18.9	19.1	19.1	19.1	19.1	17.7	17.8
3	36.6	37.8	37.8	37.8	37.8	36.3	36.6
4	39.7	38.6	38.6	38.6	38.6	36.9	37.5
5	58.2	58.0	58.0	58.0	58.1	56.6	56.5
6	19.4	19.8	19.8	19.8	19.8	19.2	19.3
7	43.5	43.5	43.5	43.5	43.5	42.5	42.6
8	73.9	73.9	74.3	73.8	73.9	73.0	73.0
9	60.9	60.9	60.9	60.9	60.9	59.1	59.2
10	40.3	40.4	40.3	40.3	40.4	39.0	39.0
11	20.4	20.6	20.4	20.4	20.4	18.4	18.5
12	47.2	46.6	47.3	47.2	47.3	45.7	45.7
13	81.0	82.1	80.9	81.1	81.2	80.2	80.3
14	143.8	144.5	143.7	143.8	143.5	141.2	141.3
15	116.0	116.1	116.1	116.0	116.2	116.2	116.1
16	22.8	22.6	22.6	22.8	22.9	21.9	21.8
17	30.9	31.0	30.9	30.9	30.9	30.5	30.5
18	27.7	28.3	28.3	28.3	28.3	27.4	27.6
19	64.9	71.5	71.5	71.5	71.5	67.1	71.2
20	16.6	16.4	16.4	16.4	16.4	15.6	15.7
$CH_3\underline{C}$=O					172.6		
$\underline{CH_3}C$=O					21.0		
13-*O*-	Rha	Glc	Rha	Rha	Rha	Rha	Rha
1′	96.0	99.5	96.7	96.0	96.2	94.2	94.2
2′	83.6	75.3	72.4	83.6	79.6	77.8	77.8
3′	72.3	78.3	72.5	72.3	75.1	71.3	71.3
4′	74.6	71.7	73.8	74.6	71.5	71.7	71.7
5′	69.5	77.6	69.8	69.5	69.5	66.0	66.0
6′	18.0	62.8	18.0	18.0	18.0	17.4	17.4
2′-*O*- or 19-*O*-	Qui	Rha	Rha	Qui	Qui	Qui	Qui
1″	106.6	101.8	101.8	106.6	106.3	101.8	101.8
2″	75.6	72.4	73.4	75.6	75.3	71.4	71.4
3″	77.6	72.7	72.6	77.6	77.6	72.3	72.3
4″	76.7	73.9	73.9	76.8	76.9	73.4	73.4
5″	73.3	69.9	69.6	73.3	73.2	69.9	70.0
6″	18.2	18.0	18.0	18.2	18.2	17.2	17.2
19-*O*-Rha							
1‴				101.8	101.8		97.7
2‴				72.4	72.4		69.9
3‴				72.6	72.7		69.3
4‴				73.9	73.9		71.0
5‴				69.8	69.8		66.4
6‴				18.0	18.0		17.4

16 of the aglycone, as well as between the anomeric proton of quinovose (δ 4.40) and H-6 of the aglycone. Additional NOESY correlations were detected between CH_3-20 and both CH_3-17 and CH_3-19 of the aglycone. The structure of **2** was therefore established as 3,14-clerodadiene 6α-*O*-*β*-D-quinovopyranosyl-13α-*O*-*β*-D-glucopyranoside.

Major diterpenoid glycosides were tasted for their bitterness. It is noteworthy that compound **1i** is the only one among those tasted that does not carry a sugar moiety at C-13 and, correspondingly, is the only nonbitter compound, indicating that glycosilation at C-13 could be a requirement for bitterness in labdane- and clerodane-type diterpenoid glycosides.

Identification of the known compound afzelin was accomplished by spectroscopic analysis and comparison with published data.[10] This compound has been previously isolated from Gleicheniaceous ferns of the genus *Dicranopteris*,[1] this being the first report on the isolation from a *Gleichenia* species.

Experimental Section

General Experimental Procedures. Optical rotations were determined with a JASCO P-1030 digital polarimeter. The IR spectra were measured on a Shimadzu FT/IR-8400S spectrometer by the diffuse reflectance method. NMR spectra were recorded at 600 MHz for ^1H and 150 MHz for ^{13}C on a Varian Unity 600. Chemical shifts are expressed in ppm relative to TMS. Low- and high-resolution mass spectra were measured on a JEOL JMS AX-500 spectrometer. TLC was carried out using silica gel precoated aluminum plates. Spots were visualized by spraying the plates with Godin[11] reagent followed by heating at 120 °C. For column chromatography, Si gel 60 (70–230 mesh) was employed. Preparative HPLC was carried out on C_{18} and C_8 columns (Luna; 5 μm, 10 × 250 mm), using a refractive index detector. X-ray diffraction data were measured with a Mac Science MXC18 diffractometer using copper radiation Mo Kα (λ = 0.71073 Å). All diagrams and calculations were performed using maXus.

Plant Material. *Gleichenia quadripartita* was collected near Rio Villegas village, Rio Negro Province, Argentina, in February 2005. A voucher specimen (LIL 607333) was deposited at the herbarium of Fundación Miguel Lillo, Tucumán, Argentina.

Extraction and Isolation. The air-dried fronds (32.2 g) were ground and extracted successively with Et_2O and MeOH. A part (3.5 g) of the bitter-tasting MeOH extract (5.55 g) was further processed by column chromatography on Si gel using a $CHCl_3$−MeOH gradient to afford five bitter fractions. Fraction 1 (210.6 mg) was processed by HPLC (C_{18}, MeOH−H_2O, 4:1, 2.0 mL/min) to give **1b** (2.0 mg) and two fractions, A and B. Further purification of fraction A by RP-HPLC (C_{18}, MeOH−H_2O, 62:38, 2.0 mL/min) gave afzelin (7.2 mg), **1i** (29.7 mg), **1d** (13.2 mg), and **1e** (2.1 mg). RP-HPLC of fraction B (C_{18}, MeOH−H_2O, 3:1, 2.0 mL/min) afforded **1g** (17.0 mg). Processing of fraction 2 (343.9 mg) by HPLC (C_{18}, MeOH−H_2O, 76:24, 2.0 mL/min) gave **1c** (25.2 mg) and **2** (13.1 mg) along with fractions C−E. HPLC of fraction C (C_{18}, MeOH−H_2O, 3:2, 2.5 mL/min) gave **1j** (5.6 mg) and **1a** (3.0 mg). Further purification of fraction D by HPLC (C_8, MeOH−H_2O, 3:1, 2.0 mL/min) afforded **1f** (2.5 mg). Fraction E was also purified by RP-HPLC (C_8, MeOH−H_2O, 66:34, 2.5 mL/min) to give **1l** (40.5 mg). Fraction 3 (287.3 mg) was processed by RP-HPLC (C_{18}, MeOH−H_2O, 72:28, 2.0 mL/min) to afford fractions F−H. HPLC of fraction F (C_8, MeOH−H_2O, 65:35, 2.0 mL/min) gave **1k** (27.3 mg). Purification of fraction G by HPLC (C_8, MeOH−H_2O, 66:34, 2.5 mL/min) afforded **1h** (87.6 mg). Further processing of fraction H by HPLC (C_8, MeOH−H_2O, 7:3, 2.0 mL/min) gave **1n** (3.5 mg). Finally, fraction 5 (340 mg) was further purified by HPLC (MeOH−H_2O, 68:32, 2.5 mL/min) to afford **1m** (63.6 mg).

***ent*-14-Labden-3*β*,8*β*-diol 13α-[*O*-*β*-D-quinovopyranosyl-(1→2)-α-L-rhamnopyranoside] (1a):** colorless crystals; $[\alpha]_D^{21}$ –43.2 (*c* 1.00, MeOH); IR (neat) ν_{max} 3389, 1449, 1412, 1375, 1115, 1066, 1011, 979, 914; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; HRFABMS *m*/*z* (rel int) 639.3746 (9.2) $[M + Na]^+$ (calcd for $C_{32}H_{56}O_{11}Na$, 639.3722).

***ent*-14-Labden-8*β*-ol 13α-*O*-*β*-D-glucopyranoside (1b):** amorphous solid; $[\alpha]_D^{22}$ –29.7 (*c* 1.00, MeOH); IR (neat) ν_{max} 3387, 1373, 1074, 1031, 1017, 924, 911; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; HRFABMS *m*/*z* (rel int) 493.3161 (7.1) $[M + Na]^+$ (calcd for $C_{26}H_{46}O_7Na$, 493.3143); EIMS *m*/*z* (rel int) 452 (1) $[M - H_2O]^+$, 434 (1) $[M - 2H_2O]^+$, 290 (47) $[M - H_2O - 162]^+$, 191 (87) $[M - H_2O - CH_2CH_2CCH_3OGlc\text{-}CH{=}CH_2]^+$, 81 (100).

***ent*-14-Labden-8*β*,19-diol 13α-*O*-*β*-D-glucopyranoside (1c):** white solid; $[\alpha]_D^{22}$ –27.1 (*c* 1.00, MeOH); IR (neat) ν_{max} 3405, 1644, 1076, 1021; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; HRFABMS *m*/*z* (rel int) 509.3100 (53.1) $[M + Na]^+$ (calcd for $C_{26}H_{46}O_8Na$, 509.3092).

***ent*-14-Labden-8α,19-diol 13α-*O*-*β*-D-glucopyranoside (1d):** amorphous solid; $[\alpha]_D^{20}$ –31.0 (*c* 1.00, MeOH); IR (neat) ν_{max} 3389, 1456, 1387, 1083, 1035, 934; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; HRFABMS *m*/*z* (rel int) 509.3089 (17.8) $[M + Na]^+$ (calcd for $C_{26}H_{46}O_8Na$, 509.3092).

***ent*-14-Labden-8*β*,18-diol 13α-*O*-*β*-D-glucopyranoside (1e):** amorphous solid; $[\alpha]_D^{22}$ –35.0 (*c* 1.00, MeOH); IR (neat) ν_{max} 3395, 1446, 1372, 1075, 1035; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; HRFABMS *m*/*z* (rel int) 509.3101 (12.4) $[M + Na]^+$ (calcd for $C_{26}H_{46}O_8Na$, 509.3092).

***ent*-14-Labden-8α,18-diol 13α-*O*-*β*-D-glucopyranoside (1f):** amorphous solid; $[\alpha]_D^{20}$ –37.3 (*c* 1.00, MeOH); IR (neat) ν_{max} 3374, 1389, 1076, 1035, 937; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; HRFABMS *m*/*z* (rel int) 509.3111 (18.4) $[M + Na]^+$ (calcd for $C_{26}H_{46}O_8Na$, 509.3092).

***ent*-14-Labden-8*β*,19-diol 13α-*O*-α-L-rhamnopyranoside (1g):** white solid; $[\alpha]_D^{20}$ –81.7 (*c* 1.00, MeOH); IR (neat) ν_{max} 3394, 1450, 1376, 1053, 1033, 982, 913; ^1H NMR data, see Table 3; ^{13}C NMR data, see Table 2; HREIMS *m*/*z* (rel int) 470.3256 (0.3) M^+ (calcd for

Table 6. ^{1}H NMR Data of Compounds **1m**, **1n** (600 MHz, MeOH-d_4), and **1p** (600 MHz, $CDCl_3$)[a]

H	1m	1n	1p
1β	1.75–1.70*	1.79 br d (13.0)	1.72 br d (12.6)
1α	0.92 td (12.9, 3.6)	0.91 td (13.0, 2.7)	0.90 td (12.6, 3.3)
2a	1.60–1.52*	1.59–1.51*	1.53–1.47*
2b	1.43–1.35*	1.42–1.34*	1.46–1.38*
3α	1.80 br d (13.5)	1.79 br d (13.0)	1.78 br d (15.1)
3β	1.01–0.92*	1.00–0.94*	1.02–0.96*
5β	0.99 dd (NC, 2.2)	1.00 dd (NC, 2.0)	0.97 dd (12.1, 2.2)
6a	1.65 td (13.5, 3.0)	1.65 td (13.7, 3.3)	1.62–1.52*
6b	1.58–1.52*	1.58–1.50*	1.62–1.52*
7α	1.74 dt (13.5, 3.0)	1.73 dt (13.7, 3.3)	1.75 dt (13.5, 3.3)
7β	1.41–1.34*	1.42–1.36*	1.42 td (13.5, 4.7)
9β	0.72 br s	0.73 br t (3.0)	0.74 br t (3.0)
11a	1.52 tt (12.9, 4.1)	1.58–1.49*	1.46–1.38*
11b	1.35–1.30*	1.40–1.32*	1.31 br t (13.5)
12a	1.64–1.58*	1.65–1.52*	1.63 td (13.5, 4.8)
12b	1.58–1.52*	1.65-.152*	1.58–1.50*
14	5.84 dd (17.4, 11.0)	5.84 dd (17.4, 11.2)	5.70 dd (17.6, 10.7)
15 *cis*	5.26 dd (11.0, 1.1)	5.23 dd (11.2, 1.2)	5.20 dd (10.7, 1.1)
15 *trans*	5.21 dd (17.4, 1.1)	5.22 dd (17.4, 1.2)	5.16 dd (17.6, 1.1)
16	1.36 s	1.36 s	1.34 s
17	1.11 s	1.12 s	1.15 s
18	0.98 s	0.979 s	1.00 s
19a	3.84 d (9.3)	3.85 d (9.2)	3.81 d (9.2)
19b	3.16 d (9.3)	3.16 d (9.2)	3.23 d (9.2)
20	0.96 s	0.975 s	0.94 s
13-*O*-Rha			
1′	5.18 d (1.6)	5.09 d (1.9)	5.03 d (1.4)
2′	3.69 dd (1.6, 3.6)	3.91 dd (1.9, 3.6)	3.82 dd (1.4, 3.0)
3′	3.76 dd (3.6, 9.4)	5.01 dd (3.6, 9.8)	5.20 dd (3.0, 9.9)
4′	3.33 t (9.4)	3.60 t (9.8)	4.88 t (9.9)
5′	3.78 dq (9.4, 6.3)	3.88 dq (9.8, 6.0)	3.97 dq (9.9, 6.3)
6′	1.22 d (6.3)	1.25 d (6.0)	1.15 d (6.3)
2′-*O*-Qui			
1″	4.34 d (7.2)	4.17 d (7.7)	4.41 d (8.0)
2″	3.26 dd (7.2, 9.1)	3.21 dd (7.7, 9.2)	5.03 dd (8.0, 9.9)
3″	3.29 t (9.1)	3.26 t (9.2)	5.16 t (9.9)
4″	2.98 t (9.1)	2.97 t (9.2)	4.78 t (9.9)
5″	3.28 dq (9.1, 6.3)	3.24 dq (9.2, 6.3)	3.50 dq (9.9, 6.2)
6″	1.26 d (6.3)	1.25 d (6.3)	1.18 d (6.2)
19-*O*-Rha			
1‴	4.58 d (1.8)	4.58 d (1.6)	4.65 d (1.6)
2‴	3.78 dd (1.8, 3.6)	3.78 dd (1.6, 3.4)	5.22 dd (1.6, 3.6)
3‴	3.62 dd (3.6, 9.6)	3.62 dd (3.4, 9.3)	5.26 dd (3.6, 9.9)
4‴	3.36 t (9.6)	3.36 t (9.3)	5.06 t (9.9)
5‴	3.57 dq (9.6, 6.3)	3.58 dq (9.3, 6.2)	3.84 dq (9.9, 6.0)
6‴	1.26 d (6.3)	1.26 d (6.2)	1.22 d (6.0)
C$\underline{H}_3$CO		2.09 s	2.15, 2.13, 2.06, 2.05, 2.03, 2.02, 2.01, 1.99, s

[a] NC: coupling constant not calculated because of overlapping resonances. * = overlapped resonances.

$C_{26}H_{46}O_7$, 470.3245); EIMS *m*/*z* (rel int) 470 (0.3) $[M]^+$, 452 (6) $[M - H_2O]^+$, 434 (7) $[M - 2H_2O]^+$, 306 (43) $[M - 146 - H_2O]^+$, 81 (100).

***ent*-14-Labden-8β,19-diol 13α-*O*-[β-D-quinovopyranosyl-(1→2)-α-L-rhamnopyranoside] (1h):** amorphous solid; $[\alpha]_D^{21}$ –49.0 (*c* 1.00, MeOH); IR (neat) ν_{max} 3396, 1377, 1109, 1066, 1019, 976, 917; ^{1}H NMR data, see Table 4; ^{13}C NMR data, see Table 5; HRFABMS *m*/*z* (rel int) 639.3730 (83.7) $[M + Na]^+$ (calcd for $C_{32}H_{56}O_{11}Na$, 639.3722).

***ent*-14-Labden-8α,13α-diol 19-*O*-β-D-glucopyranoside (1i):** needle-like solid; $[\alpha]_D^{22}$ –39.5 (*c* 1.00, MeOH); IR (neat) ν_{max} 3374, 1159, 1125, 1068, 1014; ^{1}H NMR data, see Table 3; ^{13}C NMR data, see Table 2; HRFABMS *m*/*z* (rel int) 509.3076 (7.6) $[M + Na]^+$ (calcd for $C_{26}H_{46}O_8Na$, 509.3092).

***ent*-14-Labden-8β,13α-diol 19-*O*-β-D-glucopyranoside (1j):** amorphous solid; $[\alpha]_D^{20}$ –33.2 (*c* 1.00, MeOH); IR (neat) ν_{max} 3391, 1454, 1415, 1371, 1175, 1100, 1076, 1024, 915; ^{1}H NMR data, see Table 3; ^{13}C NMR data, see Table 2; HRFABMS *m*/*z* (rel int) 509.3102 (14.3) $[M + Na]^+$ (calcd for $C_{26}H_{46}O_8Na$, 509.3092).

***ent*-14-Labden-8β-ol 13α-*O*-β-D-glucopyranosyl-19-*O*-α-L-rhamnopyranoside (1k):** amorphous solid; $[\alpha]_D^{20}$ –47.2 (*c* 1.00, MeOH); IR (neat) ν_{max} 3395, 1450, 1412, 1376, 1073, 1052, 984, 912; ^{1}H NMR data, see Table 4; ^{13}C NMR data, see Table 5; HRFABMS *m*/*z* (rel int) 655.3687 (19.5) $[M + Na]^+$ (calcd for $C_{32}H_{56}O_{12}Na$, 655.3671).

***ent*-14-Labden-8β-ol 13α-*O*-α-L-rhamnopyranosyl-19-*O*-α-L-rhamnopyranoside (1l):** amorphous solid; $[\alpha]_D^{20}$ –87.1 (*c* 1.00, MeOH); IR (neat) ν_{max} 3398, 1374, 1126, 1052, 982, 913; ^{1}H NMR data, see Table 4; ^{13}C NMR data, see Table 5; HRFABMS *m*/*z* (rel int) 639.3730 (13.3) $[M + Na]^+$ (calcd for $C_{32}H_{56}O_{11}Na$, 639.3722).

***ent*-14-Labden-8β-ol 13α-*O*-[β-D-quinovopyranosyl-(1→2)-α-L-rhamnopyranosyl]-19-*O*-α-L-rhamnopyranoside (1m):** amorphous solid; $[\alpha]_D^{21}$ –76.6 (*c* 1.00, MeOH); IR (neat) ν_{max} 3389, 1448, 1412, 1374, 1122, 1073, 979, 915; ^{1}H NMR data, see Table 6; ^{13}C NMR data, see Table 5; HRFABMS *m*/*z* (rel int) 785.4316 (53.1) $[M + Na]^+$ (calcd for $C_{38}H_{66}O_{15}Na$, 785.4301).

***ent*-14-Labden-8β-ol 13α-*O*-[β-D-quinovopyranosyl-(1→2)-3′-*O*-acetyl-α-L-rhamnopyranosyl]-19-*O*-α-L-rhamnopyranoside (1n):** amorphous solid; $[\alpha]_D^{21}$ –56.9 (*c* 1.00, MeOH); IR (neat) ν_{max} 3416, 1723, 1448, 1412, 1374, 1251, 1122, 1051, 980; ^{1}H NMR data, see Table 6; ^{13}C NMR data, see Table 5; HRFABMS *m*/*z* (rel int) 827.4423 (9.1) $[M + Na]^+$ (calcd for $C_{40}H_{68}O_{16}Na$, 827.4406).

***ent*-14-Labden-8β-ol-19-acetyl 13α-*O*-[2″,3″,4″-tri-*O*-acetyl-β-D-quinovopyranosyl-(1→2)-3′,4′-di-*O*-acetyl-α-L-rhamnopyranoside] (1o):** amorphous solid; $[\alpha]_D^{20}$ –34.5 (*c* 1.00, $CHCl_3$); IR (neat) ν_{max} 3550, 1756, 1374, 1245, 1224, 1071, 1038, 924, 755; ^{1}H NMR

Table 7. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) Data of Compound **2** in MeOH-d_4

C	δ_C	H	δ_H (J in Hz)
1	18.9	1a	1.60–1.54*[a]
		1b	1.50 m
2	27.6	2	1.98 br s
3	122.7	3	5.16 br s
4	145.9		
5	45.4		
6	87.1	6β	3.48 dd (10.8, 4.6)
7	36.6	7α	1.63–1.55*
		7β	2.07, *m*
8	35.5	8β	1.59–1.53*
9	39.0		
10	47.1	10β	1.32–1.26*
11	33.0	11a	1.42–1.36*
		11b	1.28–1.23*
12	35.5	12	1.46–1.37*
13	81.9		
14	144.6	14	5.85 dd (17.7, 11.0)
15	115.9	15 *trans*	5.21 dd (17.7, 1.2)
		15 *cis*	5.19 dd (11.0, 1.2)
16	22.8	16	1.32 s
17	16.1	17	0.81 d (6.3)
18	23.8	18	1.85 d (1.4)
19	16.8	19	1.08 s
20	18.5	20	0.72 s
13-*O*-Glc			
1′	99.3	1′	4.33 d (7.8)
2′	75.4	2′	3.13 dd (7.8, 9.3)
3′	78.7	3′	3.33 t (9.3)
4′	71.7	4′	3.29 t (9.3)
5′	77.6	5′	3.25 ddd (9.3, 2.3, 5.7)
6′	62.9	6a′	3.84 dd (11.8, 2.3)
		6b′	3.66 dd (11.8, 5.7)
6-*O*-Qui			
1″	104.3	1″	4.40 d (7.7)
2″	75.6	2″	3.20 dd (7.7, 9.3)
3″	77.9	3″	3.25 t (9.3)
4″	77.0	4″	2.97 t (9.3)
5″	72.8	5″	3.21 dq (9.3, 6.2)
6″	18.3	6″	1.23 d (6.2)

[a] * = overlapped resonances.

data, see Table 4; ^{13}C NMR data, see Table 5; HRFABMS *m/z* (rel int) 891.4374 (42.4) $[M + Na]^+$ (calcd for $C_{44}H_{68}O_{17}Na$, 891.4355).

***ent*-14-Labden-8β-ol 13α-*O*-[2″,3″,4″-tri-*O*-acetyl-β-D-quinovopyranosyl-(1→2)-3′,4′-di-*O*-acetyl-α-L-rhamnopyranosyl]-19-*O*-2‴,3‴,4‴-tri-*O*-acetyl-α-L-rhamnopyranoside (1p):** white solid; $[\alpha]_D^{21}$ –51.0 (*c* 1.00, $CHCl_3$); IR (neat) ν_{max} 3543, 1755, 1372, 1244, 1222, 1077, 1044, 982, 925, 756; ^{1}H NMR data, see Table 6; ^{13}C NMR data, see Table 5; HRFABMS *m/z* (rel int) 1121.5151 (12.1) $[M + Na]^+$ (calcd for $C_{54}H_{82}O_{23}Na$, 1121.5146).

3,14-Clerodadien 6α-*O*-β-D-quinovopyranosyl-13α-*O*-β-D-glucopyranoside (2): white solid; $[\alpha]_D^{20}$ –38.5 (*c* 1.00, MeOH); IR (neat) ν_{max} 3402, 1446, 1411, 1375, 1068, 1036, 1009; ^{1}H NMR and ^{13}C NMR data, see Table 7; HRFABMS *m/z* (rel int) 637.3577 (15.5) $[M + Na]^+$ (calcd for $C_{32}H_{54}O_{11}Na$, 637.3565).

X-ray Crystallographic Analysis of 1a. $C_{32}H_{56}O_{11}$, $M_r = 616.789$, monoclinic, $P2_1$, $a = 11.136(2)$ Å, $b = 11.655(3)$ Å, $c = 15.148(4)$ Å, $V = 1965.9(9)$ Å^3, $Z = 2$, $D_x = 1.042$ Mg m^{-3}, $\mu = 0.077$ mm^{-1}, $T = 298$ K, absorption correction: cylinder, $\theta_{max} = 26.08°$, 5634 measured reflections, 3473 independent reflections, 2269 observed reflections, refinement on F^2, $R = 0.0755$, wR(ref) = 0.2417, wR(gt) = 0.2097, $S = 1.023$, 3473 reflections, 452 parameters, only coordinates of H atoms refined, $(\Delta/\sigma)_{max} = 0.000$, $\Delta\rho_{max} = 0.204$ e Å^3, $\Delta\rho_{min} = -0.197$ e Å^3, extinction correction: SHELXL, extinction coefficient = 0.051(8).

Acid Hydrolysis of Glycosides 1a–n and 2. Hydrolysis of glycosides was carried out employing a previously described method.[7] For the separation of sugars a Shodex Rspak NH2P-50 4E column was used. The absolute configuration of sugars was determined using chiral detection in a Shodex OR-1 detector.

Acetylation of 1h. A mixture of compound **1h** (10 mg), Ac_2O (1.0 mL), and pyridine (1.0 mL) was stirred overnight at room temperature. The reaction mixture was then dried, and the product was purified by RP-HPLC (C_{18}, MeOH–H_2O, 9:1, 2.0 mL/min) to give the hexaacetate **1o** (12.4 mg).

Acetylation of 1m. The reaction was carried out in the same way as for **1h**. The product was purified by RP-HPLC (C_{18}, MeOH–H_2O, 85:15, 2.0 mL/min) to give the octaacetate **1p** (10.3 mg).

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Supporting Information Available: ^{1}H and ^{13}C, ^{1}H–^{1}H COSY, HMQC, HMBC, and NOESY NMR spectra; EI- and FABMS of compound **1a–p** and **2**, X-ray data of **1a**, figures of partial NOESY correlations for compounds **1a**, **1c–f**, and **2**, as well as HMBC tables for all new compounds. These materials are available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Raja, D. P.; Manickam, V. S.; de Britto, A. J.; Gopalakrishnan, S.; Ushioda, T.; Satoh, M.; Tanimura, A.; Fuchino, H.; Tanaka, N. *Chem. Pharm. Bull.* **1995**, *43*, 1800–1803.
(2) Wada, H.; Shimizu, Y.; Hakamatsuka, T.; Tanaka, N.; Cambie, R. C.; Braggins, J. E. *Aust. J. Chem.* **1998**, *51*, 171–173.
(3) Siddiqui, H. L.; Munesada, K.; Suga, T. *J. Chem. Soc., Perkin Trans.* **1992**, *1*, 781–785.
(4) Aoki, T.; Ohro, T.; Hiraga, Y.; Suga, T.; Uno, M.; Ohta, S. *Phytochemistry* **1997**, *46*, 839–844.
(5) Murakami, T.; Tanaka, N. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Grisebach, H., Kirby, G. W., Tamm, Ch., Eds.; Springer: Vienna, 1988; *Vol. 54*, pp 1–353.
(6) Munesada, K.; Siddiqui, H. L.; Suga, T. *Phytochemistry* **1992**, *31*, 1533–1536.
(7) Socolsky, C.; Salvatore, A.; Asakawa, Y.; Bardón, A. *Arkivoc* **2003**, *2003*, 347–355.
(8) Socolsky, C.; Muruaga, N.; Bardón, A. *Chem. Biodiversity* **2005**, *2*, 1105–1108.
(9) Crystallographic data for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the director, CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).
(10) Pauli, G. F. *J. Nat. Prod.* **2000**, *63*, 834–838.
(11) Godin, P. *Nature* **1954**, *174*, 134.

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