Fernene Triterpenoids from the Lichen *Pyxine berteriana*

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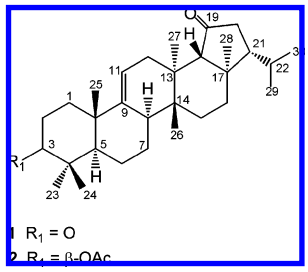
Two new fernene triterpenoids, fern-9(11)-en-3,19-dione (**1**) and 3β-acetoxyfern-9(11)-en-19-one (**2**), together with the known 3β-acetoxyfern-9(11)-en-19β-ol (**3**) and lichexanthone (**4**), have been isolated from the acetone extract of the lichen *Pyxine berteriana.* The structures of the new compounds were established on the basis of IR, extensive 1D and 2D NMR, and MS analyses. Although several fern-9(11)-enes have been isolated from lichens, compounds **1** and **2** are the first examples of naturally occurring fernene triterpenoids with a carbonyl function at C-19.

Lichens are symbiotic associations composed of at least a fungal partner, the mycobiont, and a photosynthetic partner, the photobiont.[1] These associations frequently produce characteristic secondary metabolites that are of fungal origin. Most are unique to lichens, and only a small number occur in non-lichenized fungi or higher plants.[2] Many of these lichen secondary compounds exhibit antibiotic, antitumor, antimutagenic, allergenic, antifungal, antiviral, enzyme inhibitory, and plant growth inhibitory properties.[2,3] Triterpenoids are widely distributed in lichens, being commonly present in genera such as *Nephroma* and *Pseudocyphellaria* as well as in different genera of the Physciaceae (e.g., *Dirinaria*, *Physcia*, and *Pyxine*) and the Parmeliaceae (e.g., *Parmelia* and *Evernia*).[3,4] A previous report on the secondary metabolites of *Pyxine berteriana* (Physciaceae) from Brazil indicated that it contained atranorin, lichexanthone, methyl pyxinate, and pyxinol, according to TLC analysis.[4] In the course of the search for new metabolites from the lichen *P. berteriana* (Fée) Imshaug we have isolated two new fernene triterpenoids, fern-9(11)-en-3,19-dione (**1**) and 3β-acetoxyfern-9(11)-en-19-one (**2**), together with the known 3β-acetoxyfern-9(11)-en-19β-ol (**3**) and lichexanthone (**4**), which is a chemical marker of a group of species in the genus *Pyxine*.[5,6] The structure elucidation of compounds **1** and **2** is described herein.



Compound **1** was obtained as a white, amorphous powder and showed a molecular ion at *m/z* 438.3485 in the HREIMS, indicative of a molecular formula of $C_{30}H_{46}O_2$. Its IR absorption bands at 1731 and 1708 cm^{-1} suggested the presence of two ketone groups contained in a cyclopentanone[7] and a cyclohexanone ring,[8] respectively. The assignment of ^{1}H and ^{13}C NMR spectroscopic data of **1** (Table 1) was based on DEPT, HSQC, HMBC, NOESY, and ^{1}H−^{1}H COSY spectra. A DEPT NMR experiment permitted differentiation of the 30 ^{13}C NMR resonances into eight methyl, eight sp^3 methylene, five sp^3 methine, and five sp^3 quaternary carbons, in addition to two carbonyls (δ_C 215.4 and 216.6) and a trisubstituted vinyl group resonating at δ_H 5.39 and at δ_C 117.1 and 148.9, characteristic of a $\Delta^{9,11}$ double bond.[9] Characteristic resonances in the ^{1}H and ^{13}C NMR spectra (Table 1) for six tertiary methyls [$\delta_{H/C}$ 0.77/15.6 (C-26), 0.91/15.8 (C-28), 0.97/17.0 (C-27), 1.04/24.3 (C-23), 1.12/21.7 (C-24), 1.30/24.2 (C-25)] and two secondary methyls [$\delta_{H/C}$ 0.88/22.8 (C-30), 0.99/22.2 (C-29)] indicated a fern-9(11)-ene-type pentacyclic triterpenoid skeleton.[9] In accordance with the COSY spectrum, the signal at δ_H 2.76 (H-2β) showed cross-peaks with the signals at δ_H 2.20 (H-1β), 2.23 (H-2α), and 1.64 (H-1α). On the basis of the HMBC and HSQC spectra, the signals at δ_H 2.76 and 2.23 (δ_C 35.1, H-2) showed cross-peaks with the signals at δ_C 40.4 (C-1), 216.6 (C-3), and 37.6 (C-10), establishing that C-3 corresponded to the carbonyl group at δ_C 216.6. Further correlations in the HMBC spectrum of the singlet at δ_H 1.30 (δ_C 24.2) with the signals at δ_C 37.6 (C-10), 40.4 (C-1), 46.4 (C-5), and 148.9 (C-9) allowed us to assign this methyl resonance to C-25. The NOESY correlations between H-5/CH$_3$-23 and CH$_3$-25/CH$_3$-24 in conjunction with HSQC data permitted assignment of the ^{1}H and ^{13}C resonances of CH$_3$-23 (δ_H 1.04, δ_C 24.3) and CH$_3$-24 (δ_H 1.12, δ_C 21.7). HMBC correlations of these methyl protons with the signal at δ_C 216.6 confirmed the assignment of this carbonyl group to C-3. The broad doublet at δ_H 2.09 (δ_C 38.8) was assigned to H-8 on the basis of the cross-peaks with the signals at δ_H 1.41 and 1.70 (H-7) in the COSY spectrum, while correlations of the signal at δ_H 2.09 with H-5 (1.72) and CH$_3$-27 (δ_H 0.97) in the NOESY spectrum confirmed its α-orientation. We assigned the chemical shift of C-18 at δ_C 64.6 on the basis of the HMBC correlations of CH$_3$-27 (δ_H 0.97) and CH$_3$-28 (δ_H 0.91) to C-18. The NOESY cross-peaks between H-18 (δ_H 2.18) and CH$_3$-26 (δ_H 0.77) showed that H-18 had a β-orientation and indicated *trans*-fusion of the D/E ring. On the other hand, H-18 correlated in the HMBC spectrum with the signals at δ_C 36.3 (C-13), 43.0 (C-17), and the carbonyl group at δ_C 215.4. This observation together with the presence of a singlet for H-18 in the ^{1}H NMR spectrum suggested that C-19 corresponded to the carbonyl group at δ_C 215.4. Further HMBC correlations of H-20β (δ_H 2.38) to C-17, C-19, and C-21 confirmed the presence of a carbonyl group at C-19. On the basis of the NOESY spectrum, the signal at δ_H 1.45 (δ_C 55.2, H-21) showed cross-peaks with H-18β, H-20β, and CH$_3$-26, confirming the β-orientation of H-21, and hence the α-orientation of the isopropyl chain. Thus, this natural product corresponds to fern-9(11)-en-3,19-dione.

Compound **2** was obtained as a white, amorphous powder. Its HREIMS showed a molecular ion at *m/z* 482.3782, indicative of a molecular formula of $C_{32}H_{50}O_3$ and eight unsaturation degrees. Three of these were due to the presence of two carbonyl groups

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Table 1. NMR Spectroscopic Data (500 MHz, $CDCl_3$) of Compounds **1** and **2**[a]

	1		**2**	
position	δ_C, mult.	δ_H (J in Hz)	δ_C, mult.	δ_H (J in Hz)
1	40.4, CH_2	1.64, m (H-1α) 2.20, m (H-1β)	39.0, CH_2	1.40, m (H-1α) 1.93, dt (13.6, 3.4) (H-1β)
2	35.1, CH_2	2.23, m (H-2α) 2.76, td (14.6, 5.6) (H-2β)	24.6, CH_2	1.66, m
3	216.6, qC		80.9, CH	4.48, dd (9.0, 6.7)
4	48.0, qC		38.1, qC	
5	46.4, CH	1.72, m	44.5, CH	1.38, m
6	19.2, CH_2	1.40 (H-6α), 1.75 (H-6β), m	18.8, CH_2	1.61, 1.76, m
7	17.8, CH_2	1.41, 1.70, m	17.8, CH_2	1.27, 1.63, m
8	38.8, CH	2.09, bd (13.6)	39.0, CH	2.02, bd
9	148.9, qC		150.1, qC	
10	37.6, qC		37.6, qC	
11	117.1, CH	5.39, m	116.4, CH	5.32, m
12	35.0, CH_2	2.51, ddd (17.9, 5.6, 1.5) (H-12α), 1.68, m (H-12β)	35.1, CH_2	2.49, ddd (17.8, 5.4, 1.8) (H-12α), 1.66, m (H-12β)
13	36.3, qC		36.3, qC	
14	37.3, qC		37.3, qC	
15	28.9, CH_2	1.40, m	28.9, CH_2	1.36, m
16	35.8, CH_2	1.65, m (H-16β), 1.82, dt (13.2, 3.4) (H-16α)	35.8, CH_2	1.64, m (H-16β), 1.83, dt (13.2, 3.3) (H-16α)
17	43.0, qC		43.1, qC	
18	64.6, CH	2.18, s	64.7, CH	2.17, s
19	215.4, qC		215.5, qC	
20	42.4, CH_2	1.77, dd (18.9, 10.3) (H-20α), 2.38, dd (18.9, 8.2) (H-20β)	42.4, CH_2	1.77, dd (18.9, 10.0) (H-20α), 2.38, dd (18.9, 8.0) (H-20β)
21	55.2, CH	1.45, m	55.2, CH	1.44, m
22	30.2, CH	1.64, m	30.2, CH	1.63, m
23	24.3, CH_3	1.04, s	27.4, CH_3	0.84, s
24	21.7, CH_3	1.12, s	16.1, CH_3	0.94, s
25	24.2, CH_3	1.30, s	25.2, CH_3	1.08, s
26	15.6, CH_3	0.77, s	15.6, CH_3	0.73, s
27	17.0, CH_3	0.97, s	16.9, CH_3	0.98, s
28	15.8, CH_3	0.91, s	15.8, CH_3	0.91, s
29	22.2, CH_3	0.99, d (6.6)	22.3, CH_3	0.98, d (6.5)
30	22.8, CH_3	0.88, d (6.6)	22.8, CH_3	0.88, d (6.5)
CH_3COO			170.9, qC	
CH_3COO			21.3, CH_3	2.05, s

[a] Assigned by a combination of $^1H-^1H$ COSY, NOESY, HSQC, and HMBC experiments.

(one band at 1732 cm^{-1} in the IR consistent with both a cyclopentanone ring and an ester group at δ_C 170.9 and 215.5 in the ^{13}C NMR spectrum) and one trisubstituted double bond [δ_H 5.32 and δ_C 116.4 and 150.1]. The assignment of ^1H and ^{13}C NMR spectroscopic data of **2** (Table 1) was based on DEPT, HSQC, HMBC, NOESY, and $^1H-^1H$ COSY spectra. The ^{13}C NMR spectrum showed 32 resonances, of which 30 were attributed to a fern-9(11)-ene-type pentacyclic triterpene skeleton and two to an acetyl group (δ_H 2.05; δ_C 21.3 and 170.9).[9] A DEPT NMR experiment showed the presence of nine methyl, eight sp^3 methylene, six sp^3 methine (one oxygenated), and five sp^3 quaternary carbons, in addition to the carbonyl and olefinic carbons. Analysis of the ^{13}C NMR spectroscopic data of **2** (Table 1) revealed structural similarity to those of 3β-acetoxyfern-9(11)-en-19β-ol (**3**),[9] except for the presence of a ketone functionality (δ 215.5 ppm) and the absence of the hydroxy group at C-19 (δ_C 71.2 ppm, δ_H 4.23 ppm). This observation, along with the HMBC correlations from H-18 (δ_H 2.17) to C-13, C-17, C-28, and the carbonyl signal at δ_C 215.5, indicated the presence of a C-19 ketone functionality. Further HMBC correlations from H-20 to C-17, C-21, C-22, and the signal at δ_C 215.5 together with comparison of ^1H and ^{13}C NMR data of **2** with those of rings C, D, and E of compound **1** confirmed that both compounds shared the same ketone functionality at C-19 and differed in the substituent at C-3. The NMR data of compound **2** showed close resemblance with those of rings A and B and CH_3-23, CH_3-24, and CH_3-25 of 3β-acetoxyfern-9(11)-en-19β-ol (**3**).[9] On the basis of the HMBC and HSQC spectra, the signals at δ_H 1.40 and 1.93 (δ_C 39.0, H-1) showed cross-peaks with the signals at δ_C 24.6 (C-2), 25.2 (CH_3-25), 37.6 (C-10), and 44.5 (C-5), whereas the signal at δ_H 4.48 (δ_C 80.9, H-3) correlated with the signals at δ_C 16.1 (CH_3-24), 24.6 (C-2), 27.4 (CH_3-23), and 38.1 (C-4). These data together with the HMBC correlations of the methyl singlet at δ_H 2.05 (δ_C 21.3, $C\underline{H}_3COO$) with δ_C 80.9 (C-3) and 170.9 ($CH_3\underline{C}OO$) established the position of the acetoxy group at C-3. NOESY correlations between H-3 and δ_H 0.84 (CH_3-23), 1.38 (H-5α), and 1.40 (H-1α) confirmed the β-orientation of the acetoxy group. The NOESY cross-peaks between H-5/CH_3-23 and CH_3-25/CH_3-24 in conjunction with HSQC data allowed assignment of the ^1H and ^{13}C NMR resonances of CH_3-23 (δ_H 0.84, δ_C 27.4), CH_3-24 (δ_H 0.94, δ_C 16.1), and CH_3-25 (δ_H 1.08, δ_C 25.2). As a consequence, compound **2** is identified as 3β-acetoxyfern-9(11)-en-19-one.

Compounds **1** and **2** are the first naturally occurring examples of fernene-type triterpenoids containing an unprecedented ketone function at C-19. Previously, two semisynthetic derivatives with a carbonyl group at C-19, fern-9(11)-en-19-one and fern-7-en-19-one, were obtained by CrO_3 oxidation of two fernene triterpenoids isolated from the rhizomes of *Davallia solida*.[7] Compound **1** is the second example of a natural fernene-type triterpenoid with two ketone groups. Previously, fern-9(11)-en-3,12-dione has been isolated from the lichen *Xanthoria resendei*.[8]

Compound **3** was isolated as a white, amorphous powder and identified as 3β-acetoxyfern-9(11)-en-19β-ol by comparison of the NMR and EIMS data with those reported previously.[9] Lichexanthone (**4**) was isolated as a yellow, amorphous powder and identified by EIMS and ^1H NMR data and comparison with published data.[3]

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 343 polarimeter. IR spectra were recorded on a Nicolet Magna-550 FT-IR spectrometer. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker AM 500 spectrometer. EIMS data were recorded on a Shimadzu QP-5000 mass spectrometer. HREIMS were obtained on a VG ZAB T4 mass spectrometer. Analytical HPLC was carried out on a Gilson 506C HPLC chromatograph using a reversed-phase analytical column (Phenomenex Hypersil; 5 μm pore size, 250 × 4.6 mm). The samples were eluted with a two-solvent system at a rate of 1 mL min^{-1}. Solvent A was 1% aqueous

orthophosphoric acid−MeOH (7:3), and solvent B was MeOH. The gradient started with 0% B and increased to 58% B within 15 min, then to 100% B within 15 min, followed by 100% B for 10 min. Compounds were detected using a 170 photodiode array detector set at 245 nm, operated in series with Unipoint System software, recording the absorption spectrum in the range 200−400 nm. TLC was performed on precoated Si gel F254 (cyclohexane−EtOAc (9:1)) and detected by spraying with H_2SO_4 (5% EtOH).

Lichen Material. Thalli of *P. berteriana* were collected on *M. azedarach* by one of the authors (M.T.A.) at Glew, Buenos Aires Province, Argentina, on October 7, 2000. A voucher specimen (39315) was identified by M.T.A. and preserved at the Herbarium of the Faculty of Exact and Natural Sciences (BAFC), Buenos Aires, Argentina.

Extraction and Isolation. The lichen (1.05 g wet weight) was cleaned, cut into small pieces, and extracted in acetone (100 mL) at room temperature. The acetone extract was evaporated under reduced pressure to give a residue (103 mg), which was subjected to silica gel column chromatography using cyclohexane and cyclohexane−EtOAc mixtures (99:1 and 98:2) as eluents to give the pure triterpenoids **1** (8.0 mg), **2** (3.6 mg), and **3** (2.9 mg) and lichexanthone (**4**) (14.6 mg). Compounds **1** and **2** showed one peak in their HPLC chromatograms at t_R 41.4 and 37.5 min, respectively.

Fern-9(11)-en-3,19-dione (1): white, amorphous powder; $[\alpha]^{20}_D$ −9.3 (*c* 0.33, $CHCl_3$); IR (KBr) ν_{max} 2964, 2936, 2669, 1731, 1708, 1469, 1382, 1110 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 438 $[M]^+$, 423, 405; HREIMS *m*/*z* 438.3485 (calcd for $C_{30}H_{46}O_2$, 438.3498).

3β-Acetoxyfern-9(11)-en-19-one (2): white, amorphous powder; $[\alpha]^{20}_D$ +6.7 (*c* 0.15, $CHCl_3$); IR (KBr) ν_{max} 2948, 2854, 1732, 1451, 1376, 1246, 1027 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 482 $[M]^+$, 467, 407; HREIMS *m*/*z* 482.3782 (calcd for $C_{32}H_{50}O_3$, 482.3760).

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Supporting Information Available: Spectroscopic data of new compounds **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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