Published in final edited form as:
J Nat Prod. 2009 October ; 72(10): 1902–1904. doi:10.1021/np900442y.

Fernene Triterpenoids from the Lichen *Pyxine berteriana*

Marta S. Maier[*,†], **María L. Rosso**[†,‡], **Alejandra T. Fazio**[†,‡], **Mónica T. Adler**[†], and **María D. Bertoni**[‡]

UMYMFOR (CONICET–UBA) and Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, and PROPLAME-PRHIDEB-Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Pabellón 2, Ciudad Universitaria, 1428 Buenos Aires, Argentina

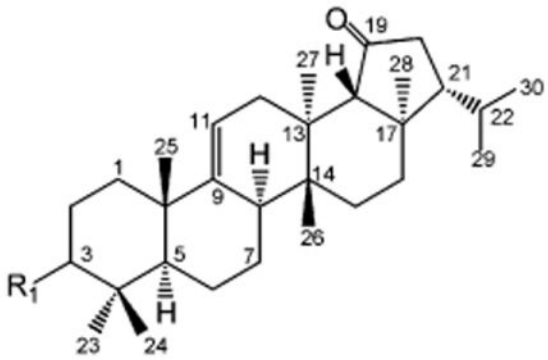
Abstract

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.

[*]Corresponding author. Tel and/Fax: +54 11 4576-3385. maier@qo.fcen.uba.ar.
[†]UMYMFOR (CONICET–UBA) and Departamento de Química Orgánica.
[‡]PROPLAME-PRHIDEB-Departamento de Biodiversidad y Biología Experimental.

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.



1 R_1 = O
2 R_1 = β-OAc

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 ^1H and ^{13}C NMR spectroscopic data of **1** (Table 1) was based on DEPT, HSQC, HMBC, NOESY, and ^1H–^1H 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 ^1H 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 ^1H 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 ^1H 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 (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. ^{1}H 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}; ^{1}H 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}; ^{1}H 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).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the University of Buenos Aires (Grant X124) and CONICET (PIP 5509). Mass spectrometry was provided by the Washington University Mass Spectrometry Resource, an NIH Research Resource (Grant No. P41RR0954). M.A.F. thanks the University of Buenos Aires for a Doctoral fellowship. M.S.M. and M.T.A. are Research Members of the National Research Council of Argentina (CONICET).

References and Notes

1. Nash, TH, III. Lichen Biology. Nash, TH., III, editor. Cambridge University Press; New York: 1996. p. 1-7.Chapter 1
2. Elix, JA. Lichen Biology. Nash, TH., III, editor. Cambridge University Press; New York: 1996. p. 176-178.Chapter 9
3. Huneck, S.; Yoshimura, I. Identification of Lichen Substances. Springer Verlag; Berlin: 1996. p. 208-209.
4. Huneck S, Morales Mendez A, Kalb K. J Hattori Bot Lab. 1987; 62:331–338.
5. Kathirgamanathar S, Ratnasooriya WD, Baekstrom P, Andersen RJ, Karunaratne V. Pharm Biol. 2006; 44:217–220.
6. Kalb, K. Brasilianische Flechten. 1. Die Gattung Pyxine, Bibliotheca Lichenologica. Vol. Band 24. J Cramer: Stuttgart; 1987. p. 20-21.
7. Tanaka Y, Kitajima J, Ageta H. Nat Med. 1998; 52:409–413.
8. González AG, Martín JD, Pérez C. Phytochemistry. 1974; 13:1547–1549.
9. Wilkins AL, Elix JA. Aust J Chem. 1990; 43:623–627.

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)	39.0, CH_2	1.40, m (H-1α)
		2.20, m (H-1β)		1.93, dt (13.6, 3.4) (H-1*β*)
2	35.1, CH_2	2.23, m (H-2α)	24.6, CH_2	1.66, m
		2.76, td (14.6, 5.6) (H-2*β*)		
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-6*β*), 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-12*β*)	35.1, CH_2	2.49, ddd (17.8, 5.4, 1.8) (H-12α), 1.66, m (H-12*β*)
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-16*β*), 1.82, dt (13.2, 3.4) (H-16α)	35.8, CH_2	1.64, m (H-16*β*), 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-20*β*)	42.4, CH_2	1.77, dd (18.9, 10.0) (H-20α), 2.38, dd (18.9, 8.0) (H-20*β*)
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_3*C*OO			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.