UNSATURATED FATTY ACID LACTONES FROM THE FUNGUS *OPHIOSTOMA PILIFERUM*

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Dedicated with respect to Dr. Arnold Brossi on the occasion of his 70th birthday.

Abstract ------- Three new hydroxylated lactones piliferolide A, B, and C (**3-5**), derived from oleic and palmitic acid have been isolated from liquid cultures of *Ophiostoma piliferum*, a fungus associated with aspen. Their structures, including absolute configuration, have been determined from spectroscopic data and by chemical studies.

Unsaturated fatty acid lactones have been shown to play a regulating role in various stages of the life cycle of certain fungi.[1~3] For example, it has been shown that the C_{18} lactone (**1**), called psiA (psi = precocious sexual inducer), plays a role in the induction of sporulation in *Aspergillus nidulans*[1,2] and that the C_{10} lactone (**2**) induces the production of aerial mycelium and the antibiotic leukaemomycin in *Streptomyces griseus*.[3] We report herein the isolation and structure determination of three new hydroxylated unsaturated fatty acid lactones (**3**, **4**, and **5**) produced by the fungus *Ophiostoma piliferum* (Fr) H. P. Sydow (= *Ceratocystis pilifera*). *O. piliferum* is a blue stain fungus which causes staining of aspen logs and chips.[4] We have recently shown that the growth of *O. piliferum* is strongly inhibited by metabolites of the fungus *Sporomiella similis* Khan and Cain.[5]

RESULTS AND DISCUSSION

The fungus was grown in still culture for eight weeks on a medium of 2% potato dextrose broth. The culture broth was separated from the mycelium and extracted with EtOAc. The crude metabolites were separated by flash chromatography over silica gel to give, in order of elution, compounds (**3**, **4**, and **5**).

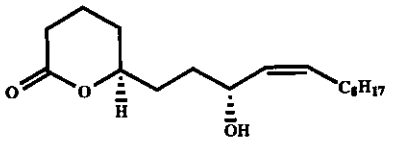
Compound (**3**), for which we suggest the name piliferolide A, has the molecular formula $C_{18}H_{32}O_3$ as determined by high resolution mass spectrometry (hrms), indicating three sites of unsaturation. The molecular weight was confirmed by chemical ionization mass spectrometry (cims). The ir spectrum shows hydroxyl (3520 cm^{-1}) and γ-lactone (1775 cm^{-1}) absorptions. The ^{13}C nmr spectrum confirms the presence of eighteen carbons, and shows a lactone carbonyl (δ 177.3), a 1,2-disubstituted double bond (δ 133.0, 132.3) and two oxygenated methine carbons (δ 81.0, 73.2). The ATP (attached proton test) spectrum also indicates the presence of a methyl group and twelve methylene carbons (see Table 1).

The ^1H nmr spectrum of **3** shows the presence of a saturated γ-lactone, substituted at C-4 [δ 2.52, 2H, dd, J=6.8, 9.0 Hz; 2.32, 1H, ddt, J=12.0, 6.8, 6.8 Hz; 1.85, 1H, m; and 4.48, 1H, m].[6] In addition, it shows two *trans* related olefinic proton signals at δ 5.65 (dt, J=16.0, 7.0 Hz) and 5.45 (ddt, J=16.0, 2.0, 7.0 Hz), and a quartet at δ 4.08 (J=7.0 Hz), indicative of a proton geminal to an allylic hydroxyl group.[1,2] The resulting partial structure (CH(OH)-CH=CH) was confirmed by the 2D ^1H-^1H COSY-45° spectrum. The proton resonance at δ 4.08 (H-11) shows cross peaks with the proton at δ 5.45 (H-12) and 1.45 (H-10) while the proton at δ 5.45 has cross peaks at δ 4.08 (H-11), 5.65 (H-13) and 2.05 (H-14). Similarly, the proton at δ 5.65 shows cross peaks with the proton resonances at δ 5.45 (H-12) and 2.05 (H-14).

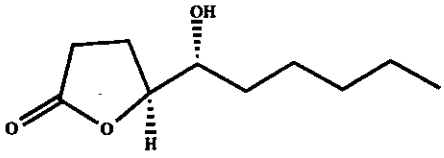
Further insight into the structure of **3** was obtained from the mass spectrum (Table 2). In addition to showing a peak at *m/z* 278.2248 ($C_{18}H_{30}O_2$, calcd 278.2245) due to the loss of H_2O, it shows fragment peaks at *m/z* 239.1644 ($C_{14}H_{23}O_3$, calcd 239.1647) and 225.1491 ($C_{13}H_{21}O$, calcd 225.1490) resulting from allylic and vinylic cleavage across the C_{14}-C_{15} and C_{13}-C_{14} bonds.[1,7] Other important fragment ions appear at m/z 199.1333 ($C_{11}H_{19}O_3$, calcd 199.1334) and 127.1122 ($C_8H_{15}O$, calcd 127.1122) arising from characteristic α-cleavage of the bonds on each side of the hydroxyl bearing carbon (C-11). It also exhibits a major fragment peak at *m/z* 85.0302 ($C_4H_5O_2$, calcd 85.0289) due to the γ-lactone moiety.[8]

Treatment of **3** with trimethylsilyl chloride yielded the ether (**3a**) the hrms of which confirmed the location of the hydroxyl group at C-11. The spectrum shows a base peak at *m/z* 199.1519 ($C_{11}H_{23}OSi$, calcd 199.1518) resulting from cleavage of the C_{10}-C_{11} bond.[1,9] Another prominent fragment ion peak appears at *m/z* 271.1733 ($C_{14}H_{27}O_3Si$, calcd 271.1729) resulting from cleavage of the C_{11}-C_{12} bond.[1,9]

It remained to ascertain the stereochemistry at C-4 and C-11 of **3**. The absolute configuration at C-11 was assigned from the CD spectrum of the *p*-chlorobenzoate (**3a**) obtained from **3**.[10] The CD spectrum exhibits a positive Cotton effect (Δε +1.53, *c*=0.3, CH_3CN) at 246 nm, establishing the *S*-configuration at C-11.[1,10] The absolute configuration at C-4 was assigned on the basis of empirical rules for the CD spectra of saturated



1



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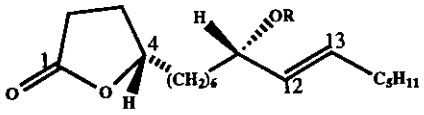
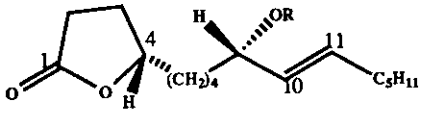
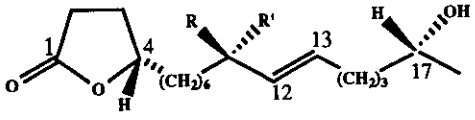


3 R=H
3a R=Si(CH$_3$)$_3$
3b R= *p*-ClC$_6$H$_4$CO



4 R=H
4a R=*p*-ClC$_6$H$_4$CO



5 R=H, R'=OH
5a R,R'=carbonyl

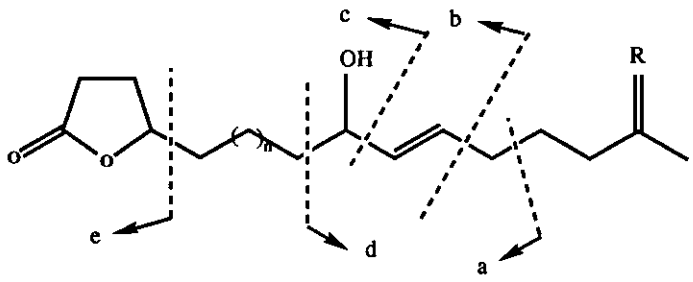
Table 1

^{13}C Nmr data for compounds (**3**,**4**, and **5**).

Carbon	**3**	**4**	**5**
1	177.3	176.8	177.2
2	28.9	28.9	28.9
3	28.0	28.1	28.1
4	81.0	80.9	81.0
5	35.6	35.6	35.6
6	25.2[a]	25.3	25.2[a]
7	29.3[b]	25.3	29.3[b]
8	29.3[b]	37.1	29.4[b]
9	25.4[a]	73.1	25.4[a]
10	37.4	132.9	37.3
11	73.2	132.5	73.1
12	133.0	32.2	133.5
13	132.3	31.4	131.7
14	32.2	28.9	32.2
15	31.4	22.5	29.7[b]
16	29.4[b]	14.1	38.8
17	22.5		68.1
18	14.1		23.6

Signals with the same superscript in the same column are interchangable.

Table 2



Mass spectra of **3**,**4**, and **5**

ion	**3** n=4, R=2H	**4** n=2, R=2H	**5** n=4, R=aOH, bH
M^+	296.2346($C_{18}H_{32}O_3$) (4)	268.2040($C_{16}H_{28}O_3$) (1)	312 ($C_{18}H_{32}O_4$) (12)
M^+-H_2O	278.2248($C_{18}H_{30}O_2$) (14)	250.1937($C_{16}H_{26}O_2$) (4)	294.2201($C_{18}H_{30}O_3$) (4)
M^+-$2H_2O$	-	-	276.2094($C_{18}H_{28}O_2$) (4)
M^+-$2H_2O$+CH_3	-	-	261.1862($C_{17}H_{25}O_2$) (2)
a	239.1644($C_{14}H_{23}O_3$) (2)	211.1340($C_{12}H_{19}O_3$) (0.5)	239.1653($C_{14}H_{23}O_3$) (1)
b	225.1491($C_{13}H_{21}O_3$) (68)	197.1181($C_{11}H_{17}O_3$) (9)	225.1499($C_{13}H_{21}O_3$) (6)
b-H_2O	207.1386($C_{13}H_{19}O_2$) (34)	179.1073($C_{11}H_{15}O_2$) (10)	207.1389($C_{13}H_{19}O_2$) (4)
c	199.1333($C_{11}H_{19}O_3$) (6)	171.1022($C_9H_{15}O_3$) (3)	199.1341($C_{11}H_{19}O_3$) (16)
c-H_2O	181.1227($C_{11}H_{17}O_3$) (5)	153.0920($C_9H_{13}O_2$) (5)	181.1232($C_{11}H_{17}O_2$) (13)
d	127.1122($C_8H_{15}O$) (64)	127.1129($C_8H_{15}O$) (23)	143.1073($C_8H_{15}O_2$) (2)
d-H_2O	109.1016(C_8H_{13}) (58)	109.1026(C_8H_{13}) (18)	125.0969($C_8H_{13}O$) (63)
d-$2H_2O$	-	-	107.0863(C_8H_{11}) (31)
e	85.0302($C_4H_5O_2$) (32)	85.0310($C_4H_5O_2$) (17)	85.2980($C_4H_5O_2$) (38)
base	57.0369(C_3H_5O)	57.0371(C_3H_5O)	55.0568(C_4H_7)

γ-lactones.[11,12] The CD spectrum of the dihydro derivative of **3** shows a positive Cotton effect (Δε +3.21, *c*=0.52, CH_3CN) at 212 nm, establishing the *S*-configuration at C-4. The structure of piliferolide A is thus 11(*S*),12(*E*)-hydroxyoctadecen-4(*S*)-olide (**3**).

Piliferolide B (**4**), oil, $[\alpha]_D$ -18.2° (*c*=0.17, $CHCl_3$), displays intense absorption at 3460 cm^{-1} (hydroxyl) and 1775 cm^{-1} (γ-lactone) in its ir spectrum. Its hrms shows a molecular ion peak at *m/z* 268.2040 corresponding to the molecular formula $C_{16}H_{28}O_3$ (calcd 268.2038). The fragmentation pattern of **4** was found to be very similar to that of piliferolide A (Table 2). The molecular ion is shifted to lower molecular weight by 28 daltons, indicating that the compound in hand is a bisnor homolog of **3**. The ^{13}C nmr spectrum confirms the presence of 16 carbon atoms. ATP experiments reveal the presence of one methyl, ten methylene, four methine and one quaternary carbon. The ^1H- and ^{13}C-nmr (Table 1) confirm the presence of the -CH(OH)-CH=CH- (*trans*) moiety as well as a γ-lactone substituted at C-4.[1,2,6]

Further structural features are revealed by the hrms of **4**. The presence of an allylic hydroxyl group at C-9 is clearly demonstrated by the spectrum, which shows fragment ion peaks at *m/z* 171.1022 ($C_9H_{15}O_3$, calcd 171.1021) and 127.1125 ($C_8H_{15}O$, calcd 127.1122) due to the cleavage of C_8-C_9 and C_9-C_{10} bonds (Table 1). The absolute configuration at C-9 and C-4 was again established by the CD spectra of the *p*-chlorobenzoate (**4a**) and dihydro derivative (**4b**) of piliferolide B. The positive Cotton effect shown by **4a** (Δε +1.03, *c*=0.6, CH_3CN, at 246 nm) and **4b** (Δε +0.36, *c*=0.75, CH_3CN, at 212 nm) is indicative of the *S*-configuration at both centers.[10-12] Thus, piliferolide B is 9(*S*),10(*E*)-hydroxyhexa decen-4(*S*)-olide (**4**). Piliferolide C (**5**), oil, $[\alpha]_D$ -10.8° (*c*=0.13, $CHCl_3$) shows an intense absorption for an hydroxyl group (3425 cm^{-1}) and a γ-lactone (1770 cm^{-1}) in its ir spectrum. Its structure was assigned by comparison of hrms, ^1H and ^{13}C nmr spectral data with those of **3**. The only difference between the compounds was found to reside in the side chain. The spectra of **5** reveal the presence of an additional secondary hydroxyl group (δ_H 3.78, m; δ_C 68.1).

The hrms of **5** is consistent with this assignment. The M^+ ion was not detected in the hrms but this does show intense peaks for M^+-H_2O (*m/z* 294.2201; $C_{18}H_{30}O_3$, calcd 294.2194), M^+-$2H_2O$ (*m/z* 276.2094; $C_{18}H_{28}O_3$, calcd 276.2089) and M^+-$2H_2O$+Me (m/z 261.1826, $C_{17}H_{25}O_2$, calcd 261.1854). The other fragments are comparable with those of **3** (Table 2). The molecular weight was confirmed by cims. The fragment ion peaks at *m/z* 143.1073 ($C_8H_{15}O_2$, calcd 143.1071) and 239.1653 ($C_{14}H_{23}O_3$, calcd 239.1647) indicate the location of the second hydroxyl group at C-15, C-16 or C-17. The presence of a characteristic downfield doublet for a methyl group on oxygenated carbon at δ 1.17 (J= 6.5 Hz) in the ^1H-nmr spectrum located the group at C-17. This was confirmed by the ^{13}C-nmr spectrum (CH_3 signal at δ 23.6).[13]

The *S*-configuration at C-17 was determined by the Horeau method.[14] The allylic hydroxyl group in **5** was selectively oxidized with freshly precipitated MnO_2/CH_2Cl_2.[15,16] The reaction of the resulting ketone (**5a**) with racemic 2-phenylbutanoic anhydride gave 2-phenylbutanoic acid the optical rotation of which indicated the *S*-configuration at C-17 in **5a** and hence in **5** itself. Assuming that the configurations at C-4 and C-11 are as in **3**, compound (**5**) is identified as 11(*S*),17(*S*),12(*E*)-dihydroxyoctadecen-4(*S*)-olide.

Although similar hydroxy lactones have been reported previously from natural sources,[1,2,17] the present paper constitutes the first report of such compounds in the genus *Ophiostoma*. Compounds (**3**, **4**, and **5**) have not been reported previously.

EXPERIMENTAL

Ft-ir spectra were recorded on a Nicolet 7199 FTIR interferometer. Uv spectra were obtained on a Hewlett Packard 8450A Diode Array spectrophotometer and optical rotations were determined with a Perkin Elmer 241 polarimeter. CD spectra were recorded on a JASCO Optical Rotatory Dispersion SS-20-2 recorder. Hrms were recorded on an AEIMS-50 mass spectrometer. Cims were recorded on an AEIMS-12 mass spectrometer with ammonia as reagent gas. Nmr spectra (^1H and ^{13}C) were obtained on Bruker WM-360 or Varian Unity 500 multinuclear spectrometers. Chemical shifts are referenced to residual hydrogen (7.2 ppm) or the carbon (77.0 ppm) resonance of $CDCl_3$. Flash chromatography was performed on silica gel 230-400 mesh, General Intermediates of Canada. Analytical tlc was carried out on E. Merck precoated aluminium sheets of silica gel 60F-254 (0.2 mm thickness). Tlc plates were visualized using iodine vapor or 5% phosphomolybdic acid in 5% sulfuric acid. All solvents were distilled prior to use. Skellysolve B (SKB) refers to Skelly Oil Company petroleum ether, b.p. 62-70°.

Isolation Of Metabolites.

The culture of *Ophiostoma piliferum* (strain NOF 1772) was obtained from Dr. Y. Hiratsuka, Forestry Canada, Northern Forestry Centre, Edmonton. Thirty 2.5-liter Fernbach flasks each containing 1 liter of 2% aqueous potato dextrose broth were inoculated with *ca.* 10 ml of a mycelial suspension of the fungus and the still cultures were kept at room temperture (20-25°C) for 8 weeks. The mycelium was separated by filtration, the filtered broth was concentrated to about 6 liters under reduced pressure (waterbath 35-40°C) and then extracted by stirring with EtOAc for 12 h (3 x 1.5 l). The organic extract was dried over Na_2SO_4 and the solvent was removed to afford crude extract (1.7 g). The crude extract was subjected to flash chromatography over silica gel

using gradient elution (0-100% EtOAc in SKB) to give, in order of increasing polarity, compounds **3-5**.

Piliferolide A (3). The fractions eluted with EtOAc : SKB (3:7) were evaporated and purified by preparative tlc [EtOAc : SKB (6:4)] to afford compound (**3**); oil (22 mg), $[\alpha]_D^{21}$ -23° (*c*=0.1, $CHCl_3$,); ir ν_{max} ($CHCl_3$): 3520, 2925, 2855, 1775, 1180 cm^{-1}; hrms: see Table 2; ^{1}H-nmr (360 MHz, $CDCl_3$): δ 5.65 (1H, dt, J=16.0, 7.0 Hz, H-13), 5.45 (1H, ddt, J=16.0, 2.0, 7.0 Hz, H-12), 4.48 (1H, m, H-4), 4.08 (1H, q, J=7.0 Hz, H-11), 2.52 (2H, dd, J=6.8, 9.0 Hz, H-2), 2.32 (1H, ddt, J=12.0, 6.8, 6.8 Hz, H_a-3), 1.85 (1H, m, H_b-3), 1.2-1.7 (21H, m), 0.88 (3H, t, J=6.2 Hz, H-18); ^{13}C-nmr (75 MHz, $CDCl_3$): see Table 1.

Piliferolide B (4). The eluate obtained from the column with EtOAc : SKB (4:6) was further purified by preparative tlc using EtOAc : SKB (6:4) as mobile phase (two fold development) to give compound (**4**) as a colorless oil (3.8 mg), $[\alpha]_D^{20}$ -18.2° (*c*=0.17, $CHCl_3$); ir ν_{max} ($CHCl_3$): 3460, 2925, 2855, 1775, and 1180 cm^{-1}; hrms: see Table 2; ^{1}H-nmr (360 MHz, $CDCl_3$): δ 5.62 (1H, dt, J=15.5, 7.0 Hz, H-11), 5.42 (1H, ddt, J=15.5, 7.0, 2.0 Hz, H-10), 4.46 (1H, m H-4), 4.04 (1H, q, J=7.0 Hz, H-9), 2.52 (2H, dd, J=7.0, 8.8 Hz, H-2), 2.32 (1H, ddt, J=12.5, 6.5, 6.5 Hz, H_a-3), 1.85 (1H, m, H_b-3), 1.2-1.7 (17H, m), 0.85 (3H, t, J=6.3 Hz, H-16); ^{13}C-nmr (125 MHz, $CDCl_3$): see Table 1.

Piliferolide C (5). Evaporation of fractions eluted with EtOAc:SKB (9:1) gave an oily material which was purified by preparative tlc, using MeOH : EtOAc (1:49). Compound (**5**) was obtained as a colorless oil (3.0 mg), $[\alpha]_D^{21}$ -10.8° (*c*=0.13, $CHCl_3$); ir ν_{max} ($CHCl_3$): 3425, 2925, 2855, 1770, 1180 cm^{-1}; cims (NH_3): *m/z* 330$(M+18)^+$(40), 312$(M)^+$(17); hrms: see Table 2; ^{1}H-nmr (360 MHz, $CDCl_3$): δ 5.62 (1H, dt, J=16.0, 7.0 Hz, H-13), 5.45 (1H, ddt, J=16.0, 7.0, 2.0 Hz, H-12), 4.45 (1H, m, H-4), 4.05 (1H, q, J=7.0 Hz, H-11), 3.78 (1H, m, H-17), 2.52 (2H, dd, J=6.5, 9.5 Hz, H-2), 2.30 (1H, ddt, J=12.0, 6.2, 6.2 Hz, H_a-3), 1.82 (1H, m, H_b-3), 1.7-1.2 (19H, m), 1.17 (3H, d, J=6.0 Hz, H-18); ^{13}C-nmr (125 MHz, $CDCl_3$): see Table 1.

Determination of Absolute Configuration at C-17 of 5. Compound **5** (3 mg) was added to a suspension of manganese dioxide in CH_2Cl_2 (5 ml). The mixture was stirred for 45 min, then filtered and the precipitate washed with fresh solvent. The combined filtrates were concentrated to afford **5a**: ir ν_{max} ($CHCl_3$) 3480, 2930, 2850, 1775, 1670, and 1180 cm^{-1}. Compound (**5a**) (2.0 mg) was added to a solution of racemic 2-phenylbutanoic anhydride in dry pyridine (0.4 ml). The resulting mixture was allowed to stand for 10 h. 0.1 M NaOH was then added dropwise to pH 9 and the solution was extracted with $CHCl_3$. The aqueous layer was acidified to pH 3 using 1 M HCl and the acidic layer was extracted with C_6H_6 (10 ml). The extract was concentrated to 1.0 ml. The optical rotation of the 2-phenylbutanoic acid was negative, establishing the *S*-configuration at C-17.

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