Africane-Type Sesquiterpenoids from the Argentine Liverwort *Porella swartziana* and Their Antibacterial Activity

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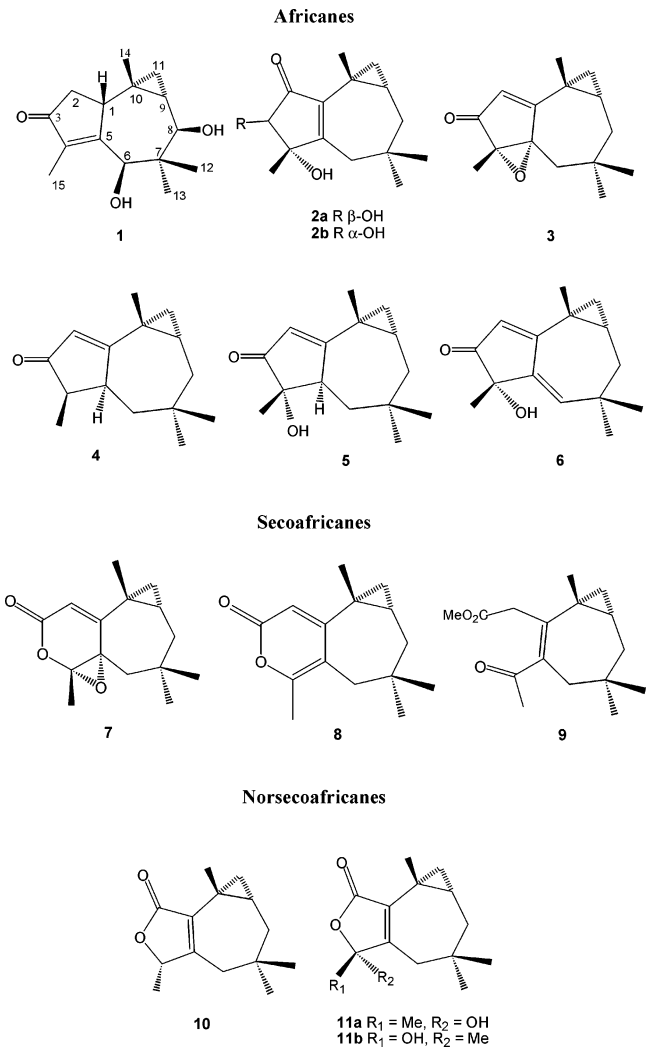
Seven africanes (**1**, **2a**,**b**, **3**−**6**), two of them new (**1**, **2a**), three secoafricanes (**7**−**9**), one of them new (**7**), and two norsecoafricanes (**10**, **11a**), one of them new (**10**), all of them swartzianin-type, have been isolated from an Argentine collection of the endemic liverwort *Porella swartziana*. The structures of the new compounds were established by extensive 1D and 2D NMR spectroscopic data. Absolute configurations of compounds **2a**, **2b**, and **10** were derived on the basis of CD spectra. The compounds were tested for activity against a variety of microbes, but none were found to exhibit significant antibacterial activity.

Most *Porella* species, belonging to the *Jungermanniales* (Hepaticae), are rich sources of sesqui- and diterpenoids,[1−11] some of which show interesting biological activities.[12] It has been reported that crude extracts of various *Porella* species show antimicrobial activity against Gram-positive bacteria.[13] The *Porella* species are divided into two groups, pungent and nonpungent. The former contain the strongly pungent sesquiterpene dialdehyde, polygodial as major component. The latter produce pinguisane, germacrane, and guaiane-type sesquiterpenoids as well as sacculatane-type diterpenoids. The stem-leafy liverwort *Porella swartziana* (Web.) Trev. belongs to the nonpungent group and grows endemically in the north of Argentina. A previous investigation from a Colombian collection reported the presence of africane-, guaiane-, and germacrane-type sesquiterpenoids.[14] The africane-type sesquiterpenoids are very rare in nature, and it has been suggested that they can be biosynthesized from humulene.[15] They have been found in the essential oil of *Lippia integrifolia*,[16] in the roots of *Senecio oxyriifolius*,[17] in a sapwood staining ascomycete fungus,[18] and in a marine invertebrate.[19,20] *P. swartziana* is widespread in rain forests of South American mountains ("yungas" region), and since occasionally different chemotypes are found for the same species of liverwort, depending on the location, we decided to analyze a *P. swartziana* collection from the north of Argentina, as part of a chemical investigation of South American liverworts.[21−25] We report in this paper the isolation and identification of 12 africane-type sesquiterpenoids: seven africanes (**1**, **2a**,**b**, **3**−**6**), two of them new (**1**, **2a**), three secoafricanes (**7**−**9**), one of them new (**7**), and two norsecoafricanes (**10**, **11a**), one of them new (**10**) (Chart 1). Complex mixtures of these compounds could be separated only by extensive use of HPLC in normal and reverse phase. Identification was accomplished by the use of 600 MHz NMR in mono- and bidimensional experiments, HR-MS, and comparison with spectroscopic data from previously identified compounds. Absolute configurations of compounds **2a**, **2b**, and **10** were derived on the basis of CD spectra and are as depicted. This method has been frequently employed, and some rules were derived to rationalize the observed values for the Cotton effect.[26−29] Particularly, it has been proved be a useful technique to establish the absolute stereostructures for γ-lactones and cyclic ketones, as described in the present paper.

Chart 1



Although it has been estimated that around 20 thousand plant species are used in traditional medicines, most

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Table 1. ^{1}H NMR Data of **1**, **2a**, **2b**, **7**, and **10** (600 MHz, $CDCl_3$)[a]

^{1}H	**1**	**2a**[b]	**2b**[b]	**7**	**10**
1	3.07 dd (7, 1)				
2α	2.56 d (18)			6.02 s	
2*β*	2.41 dd (18, 7)				
3		3.80 d (5.5)	4.16 s		
4					4.73 q (7)
6α	4.41 dd (9, 1.5)	2.35 d (15)	2.40 d (15)	1.25 d (14)	2.07 d (17.5)
6*β*		2.60 d (15)	2.54 d (15)	2.52 d (14)	2.34 dt (17.5, 1)
8α	4.21 td (4, 1.5)	0.69 dd (14.5, 11)	0.67 dd (11, 14)	0.50 dd (15, 12)	0.87−0.96[c]
8*β*		1.83 dd (14.5, 5)	1.83 dd (5, 14)	1.87 dd (15, 4)	2.04 ddd (13, 4, 1)
9	0.75 ddd (9, 5, 4.5)	0.85 m	0.85 m	0.99 dddd (12, 8, 5, 4)	0.87−0.96[c]
11α	0.84 dd (5.5, 4)	0.31 t (4.5)	0.22 t (4.4)	0.46 t (5)	0.38 t (4)
11*β*	0.70 dd (9, 4)	0.76 dd (8, 4.5)	0.72 dd (4.4, 8)	0.89 dd (8, 5)	0.87−0.96[c]
12	1.46 s	1.12 s	1.18 s	1.07 s	1.20 s
13	0.90 s	1.00 s	0.95 s	0.95 s	0.98 s
14	0.81 s	1.19 s	1.15 s	1.36 s	1.30 s
15	1.76 d (1)	1.45 s	1.30 s	1.80 s	1.34 d (7)
OH	4.27 d (9) (C-6)	4.58 d (5.5) (C-3)			
OH	2.30 d (4) (C-8)	3.72 s (C-4)			

[a] *J* values (in Hz) in parentheses. [b] Measured in C_3D_6O. [c] Overlapped signal.

species have not been thoroughly examined chemically or pharmacologically.[30] As no reports were available on the bioactivity of these africane-type compounds, we decided to test their antibacterial activity.

Results and Discussion

The air-dried plant material was extracted with ether and then methanol. The ether extract was analyzed by GC−MS, and africane-type sesquiterpenoids were detected. We carried out an exhaustive isolation of all africanes, even minor compounds, and investigated their antibacterial activity against Gram-positive and -negative bacteria. A combination of column chromatography on silica gel, Sephadex LH-20, and preparative HPLC of the ether extract furnished norsecoswartzianin (**11a**) as the major compound. Noteworthy is that this is not the major constituent from the Colombian collection of the same species in which caespitenone (**3**) accounted for 53.3% of the total africane content.[14] Caespitenone is also the major compound of *P. caespitans* var. *setigera* and has also been isolated from *P. japonica*. The ether extract of our collection also furnished minor amounts of the new africane-type sesquiterpenoids **1**, **2a**, **7**, and **10**, together with caespitenone (**3**), swartzianins B, C, and D (**4**−**6**), and secoswartzianins A and B (**8**, **9**) also found in the Colombian collection of *P. swartziana*, and compound **2b** previously obtained from *P. caespitans* var. *setigera*.[8]

Compound **1** showed a molecular ion peak at *m*/*z* 250. From its HREIMS spectrum, a molecular formula of $C_{15}H_{22}O_3$ has been deduced, accounting for five degrees of unsaturation. Additionally, in the ^{1}H NMR spectrum, a cyclopropane ring was suggested by the double doublets at *δ* 0.84 and 0.70 for H-11α and H-11*β*, respectively, as well as the signal at *δ* 0.75 (ddd, *J* = 9, 5, 4.5 Hz) for H-9 (Table 1). Four methyl signals, three singlets at *δ* 1.46, 0.90, and 0.81 as well as the doublet at *δ* 1.76, were found. As no olefinic protons were detected, a tetrasubstituted double bond was placed between C-4 and C-5. The C=C position was also identified by the homoallylic coupling (*J* = 1 Hz) between H-15 and H-1. The presence of an α,*β*-unsaturated ketone was deduced by the 1694 cm^{-1} absorption in the IR, the 238 nm band in the UV, and the signals at *δ* 210.5, 170.1, and 136.5 (Table 2) in the ^{13}C NMR spectrum. HMQC and HMBC data confirmed an africane-type sesquiterpenoid structure for compound **1**. Complete assignment of protons was achieved by COSY, HMQC, and HMBC (Table 3) spectra and comparison with spectral data of related compounds.[9−11,14] Africanes are rare in nature, and even rarer is the oxygenation in the seven-membered ring as in our present compound **1** with hydroxylation at C-6 and C-8. The α orientation of the cyclopropane ring in the africanes of *P. swarziana* has been strongly documented[14] and for compound **1** is also evident from the NOESY spectrum (Figure 1). The proposed configurations of C-1, C-6, C-8, C-9, and C-10 were also established by NOESY as well as by the observed coupling constants (*J*) in the ^{1}H NMR spectrum. Correlations were observed between H-8 and H-11*β*; H-9 and H-11*β*; H-15 and H-6 (α oriented); H-11α, H-11*β* and H-13; H-12 and the hydroxyl

Table 2. ^{13}C NMR Data of **1**, **2a**, **2b**, **7**, and **10** (150 MHz, $CDCl_3$)

^{13}C	**1**	**2a**[a]	**2b**[a]	**7**	**10**
1	41.4	140.5	139.24	165.8	129.6
2	39.8	203.9	203.48	120.6	173.5
3	210.5	76.3	82.54	160.9	
4	136.5	73.2	78.40	89.4	78.7
5	170.1	171.2	173.68	58.9	160.9
6	77.5	38.2	37.83	39.6	42.1
7	40.9	30.9	31.88	33.4	31.8
8	78.2	42.4	43.65	42.8	43.0
9	28.0	17.6	18.63	19.5	20.7
10	19.9	14.1	15.53	23.7	15.5
11	18.4	19.0	19.29	19.8	21.7
12	26.8	22.0	22.80	29.7	26.8
13	23.2	30.8	31.59	30.0	33.0
14	18.7	28.3	29.61	23.8	23.0
15	7.6	23.4	23.15	18.7	18.6

[a] Measured in C_3D_6O.

Table 3. HMBC Correlations of **1**, **7**, and **10**

1		**7**		**10**	
H	C	H	C	H	C
1	2*β*, 3, 4, 5, 6, 10, 11	2*β*	3, 5, 10	4	1, 5, 15
2*β*	1, 3, 10	6α	1, 5, 7, 8, 13	6α	1, 4, 5, 7, 8, 12, 13
2α	1, 3, 4, 5, 10	6*β*	1, 4, 5, 7, 13	6*β*	1, 2, 4, 5, 7, 8, 12, 13
6	1, 4, 5, 7, 8	8α	7, 9, 12	8α	6, 9
11α	8	8*β*	10	8*β*	6, 9, 10
11*β*	1, 8, 14	11α	10, 14	11α	8, 9, 14
12	6, 7, 8, 13	11*β*	8	11*β*	8, 9, 4
13	6, 7, 8, 12	12	5, 6, 7, 8, 13	12	6, 7, 8, 13
14	1, 9, 10, 11	13	6, 7, 8, 12	13	6, 7, 8, 12
15	1, 3, 4, 5, 10	14	1, 9, 10, 11	14	1, 9, 10, 11
OH (C-6)	7	15	4, 5	15	4, 5
OH (C-8)	7				

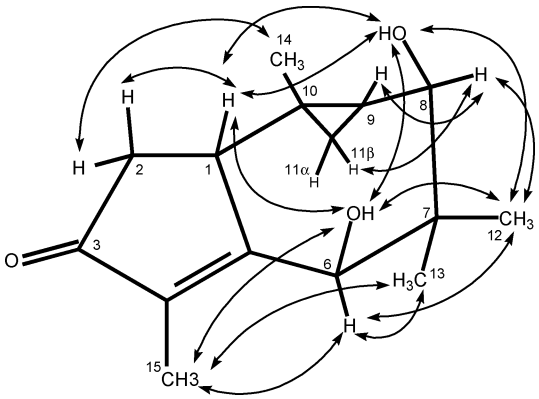

Figure 1. Partial NOEs observed for compound **1**.

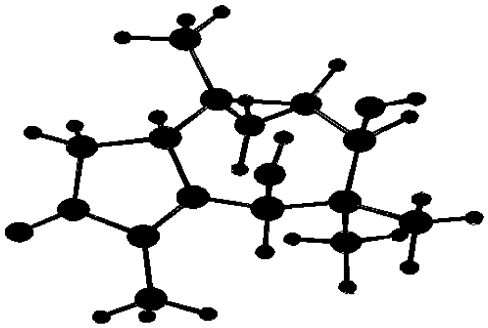

Figure 2. Minimum energy conformation of **1**.

protons at C-6 and C-8; and H-1 and protons of OH-6 and OH-8. Finally, protons of OH-6 and OH-8 were also correlated, indicating that the methyl groups at C-10, H-1, H-9, and the OH groups at C-6 and C-8 are all β oriented. The seven-membered ring conformation, as shown in Figure 1, is also supported by the observed coupling constants, $J_{6,8} = 1.5$ Hz (*W*-coupling between H-6 and H-8), indicating an equatorial arrangement of these hydrogens. An internal hydrogen bond is possible due to the *syn* orientation of the OH groups at C-6 and C-8. PCMODEL calculations[31] showed a dihedral angle of 174.21° for the fragment OH(6)−CH(6) with $J = 9$ Hz in the minimum energy conformer of **1** (Figure 2), in good agreement with the observed value of the coupling constant, indicating that the OH-6 is responsible for a stable H bonding between OH-6 and OH-8.

The EIMS spectrum of **2a** gave a molecular ion peak at *m*/*z* 250, and its HREIMS spectrum indicated a molecular formula of $C_{15}H_{22}O_3$ that accounted for five degrees of unsaturation. IR, UV, and NMR spectral features of **2a** suggested its close structural relation with the africane diol **2b** previously isolated from *Porella caespitans* var. *setigera*[8] and also reported in the present paper from our own collection of *P. swartziana*. The oily compounds **2a** and **2b** are epimers at C-3, a fact that was clear from the NMR data (spectral data of **2b** are included in Tables 1 and 2 for comparison). ^{1}H NMR spectra showed differences in the chemical shifts of H-3 and H-15 as well as in the multiplicity of the H-3 and OH-3 signals that are doublets ($J = 5.5$ Hz) in the spectrum of **2a** and singlets for compound **2b**. In the proton spectrum of **2a**, H-3 was 0.36 ppm upfield and H-15 was 0.17 ppm downfield compared with the same proton's signals in the ^{1}H NMR of **2b**. In compound **2b,** H-3 (β oriented) lies in a pseudoequatorial position that accounts for the observed deshielding. The MS spectra of **2a** and **2b** display differences only in peak intensities. Complete assignment of NMR spectra of **2a** was achieved by ^{1}H−^{1}H COSY, HMQC, and NOESY experiments (Tables 1 and 2 and Figure 3). Absolute configurations of **2b** and **2a**, as depicted, were established by their CD spectra. The n→π* transition of the carbonyl group gives rise to a weak absorption band at around 330 nm for α,β-unsaturated cyclopentenones. Cotton effects have been observed for this type of compounds at the mentioned wavelength.[27,28] No reports were found for the absolute configuration of **2b** even though the compound is known. Its CD spectrum showed a negative Cotton effect ($\Delta\epsilon_{336}$ −2.61), as can be predicted by the back octant rule.[29,32] In accord with the previously reported NOESY spectrum,[8] the CH_3-15 lies in the upper right (−) octant due to its pseudoaxial orientation, while the remaining substituents surrounding the carbonyl chromophore have symmetrical partners or lie close to the carbonyl plane and, therefore, exert low "weight" to the Cotton effect. Our new compound **2a** showed a similar CD spectrum with a negative Cotton effect ($\Delta\epsilon_{350}$ −0.68), as can be predicted by the back octant rule; therefore the CH_3-15 should lie in the upper right (−) octant and the configuration is as depicted. Therefore compounds **2a** and **2b** should belong to the same chiroptical series, and their configuration at C-4 should be identical.

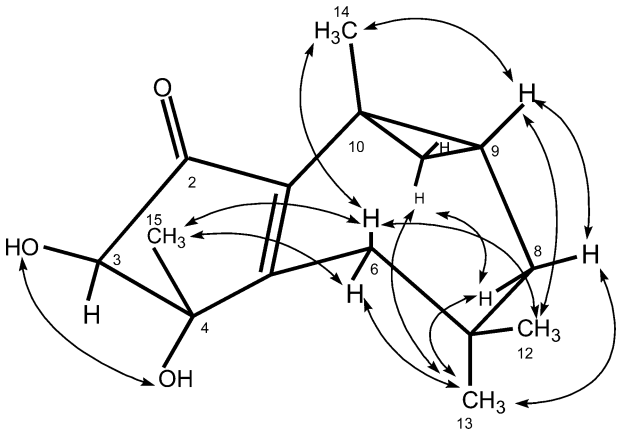

Figure 3. Partial NOEs observed for compound **2a**.

Compounds **3** (caespitenone), **4** (swartzianin B), **5** (swartzianin C), and **6** (swartzianin D) showed spectral features that were in good agreement with those of authentic samples previously isolated from the Colombian collection of the same species.[14]

The CIMS spectrum of compound **7** showed a quasi-molecular ion peak at *m*/*z* 249 (M+1), and its HREIMS spectrum indicated a molecular formula of $C_{15}H_{20}O_3$ that accounted for six degrees of unsaturation. The FTIR and UV spectra showed bands at 1735 and 1270 cm^{-1} and at λ_{max} 237 nm, respectively, corresponding to an α,β-unsaturated δ-lactone. The ^{13}C NMR spectrum (Table 2) disclosed an ester carbonyl signal at δ 160.9, two olefinic carbons at δ 165.8 and 120.6, and the signals at δ 89.4, 58.9 and δ 19.5, 23.7, 19.8, assignable to epoxide and cyclopropane rings, respectively. The ^{1}H NMR (Table 1) showed a singlet at δ 6.02 assigned to a vinyl proton and three signals at δ 0.99, 0.46, and 0.89 accounting for three cyclopropane protons. Spectral features indicated that **7** was closely related to the secoafricane secoswartzianin A (compound **8**), previously described from the Colombian *P. swartziana*[14] and also present in our own collection. In fact, **7** is the 4,5-epoxy analogue of **8**. The α orientation of the epoxy group, as depicted, is strongly supported by the observed correlations in the NOESY spectrum. The Dreiding model of the isomer with the epoxy group α oriented is precise in predicting the correlation peaks actually found between Me-14 and Me-15 and the ones observed for the seven-

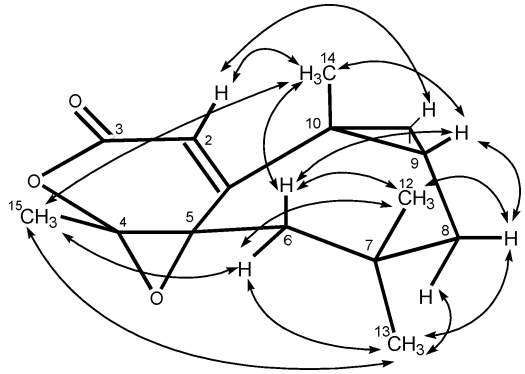

Figure 4. Partial NOEs observed for compound **7**.

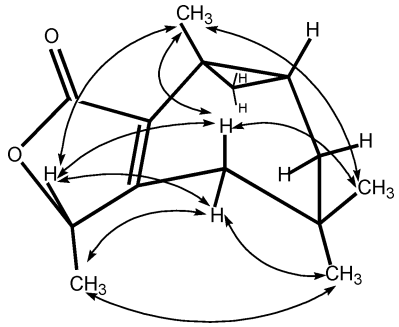

Figure 5. Partial NOEs observed for compound **10**.

membered ring protons. Total assignment was achieved by the ^{1}H−^{1}H COSY, HSQC, HMBC, and NOESY spectra (Tables 1−3 and Figure 4) and comparison with the corresponding spectral data of compound **8**.[14]

Compound **9** (secoswartzianin B) showed spectral features that were in good agreement with those of an authentic sample obtained from the Colombian collection of the same species.[14]

The EIMS spectrum of **10** gave a molecular ion peak at *m*/*z* 220, and its HREIMS spectrum indicated a molecular formula of $C_{14}H_{20}O_2$ that accounted for five unsaturations. The FTIR and the UV spectra showed bands at 1749 cm^{-1}and at λ_{max} 224 nm, respectively, corresponding to an α,β-unsaturated γ-lactone. The ^{13}C NMR spectrum (Table 2) displays an ester carbonyl signal at δ 173.5 together with two olefinic carbons at δ 129.6 and 160.9. The absence of vinylic protons and the presence of signals at δ 0.38 and 0.87−0.96 in the ^{1}H NMR spectrum (Table 1) suggested that the compound was a nor-γ-lactone with a cyclopropane ring and an endocyclic double bond conjugated with the carbonyl group. These data together with the presence of signals for four methyl groups confirmed a norafricane-type sesquiterpenoid structure for compound **10**. Comparison of the mentioned spectral data with those available for the previously known norafricane alcohols **11a**,**b**[14] suggested that **10** is the deoxy derivative of **11b**. Complete assignment of all carbons and protons of **10** was achieved by ^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY spectra (Tables 1−3 and Figure 5). The sesquiterpene lactones commonly found in some families of plants, particularly in species of the Asteraceae, occur in a few groups of carbon skeletons. ORD and CD[29] have been repeatedly employed to solve stereochemical problems in γ-lactones[26,27] of those common skeletal types, but no references were found regarding the absolute configuration of lactones with the structural characteristics of **10**. However the Cotton effect of the n→π* transition of the α,β-unsaturated γ-lactone chromophore of **10** ($\Delta\epsilon_{245}$ +1.46) could be detected and allowed us to propose the absolute configuration as depicted, employing the back octant rule.

The africane-type nor-lactone norsecoswartzianin (**11a**) had been previously described by Tori and co-workers,[14] and its NMR spectra showed two sets of very similar signals that suggest a 1:1 mixture of **11a** and **11b**, two epimers at C-4 as a consequence of isomerization of the hemiacetal in solution. However in crystalline form the compound exists in one isomeric form, as depicted in formula **11a**.

Antibacterial Effects of Africanes. Compounds **2b**, **3**−**6**, **8**−**10**, and **11a** were evaluated by the agar diffusion method against Gram-positive and -negative bacteria, showing a low activity. MICs of compounds **3**−**6**, **8**−**10**, and **11a** were determined by broth dilution assay against two strains of *Staphylococcus aureus*, indicating that none of the compounds are active at levels below 625 μg/mL. The activity of the tested compounds is considerably inferior to the commercial antibiotic penicillin G. Thus, while this study has suggested a basis for the traditional medicine uses of *Porella* species, the present results cannot satisfactorily explain the claimed effects.

Experimental Section

General Experimental Procedures. TLC was carried out on silica gel precoated glass plates (Kiesel gel 60 F254, Merck) with *n*-hexane−EtOAc (1:1, 2:1, and 4:1). Godin reagent followed by heating at 120 °C was used for detection. Column chromatography was carried out on silica gel 60 (70−230 mesh, Merck) and on Sephadex LH-20 (CH_2Cl_2−MeOH, 1:1). Preparative HPLC was performed by a Gilson pump system. Ultrasphere Beckman Silica, Beckman Cyano, and reversed-phase Beckman C_8 and C_{18} columns were used. GC−MS analysis was carried out on a Hewlett-Packard instrument. An HP-5MS (30m × 0.25 mm i.d. × 0.25 μm) column with temperature programming from 50 °C, then 50−280 °C at 5 °C min^{-1}, and finally isothermal at 280 °C for 15 min was used. The mass spectra including high-resolution mass spectra were recorded on a JEOL JMS AX-500 spectrometer (EIMS). Specific rotations were measured on a JASCO DIP-1000 polarimeter with $CHCl_3$ as solvent. The UV spectra were obtained on a Shimadzu UV-160A spectrophotometer. The CD spectra were recorded on a JASCO J-725 spectrometer. IR spectra were measured on a JASCO FT/IR-5300 spectrophotometer by the diffuse reflectance method. NMR spectra were recorded on a Varian Unity 200, a Varian Gemini 200 (200 MHz), or a Varian Unity 600 (600 MHz) spectrometer, in $CDCl_3$ or C_3D_6O as solvent.

Plant Material. *Porella swartziana* (whole plant) was collected in June 1996 at Cochuna, Tucumán Province, Argentina. A voucher specimen (A. Bardón 2) is on deposit at Abt. Systematische Botanik, Albrecht-von-Haller Institut, Göttingen, Germany.

Extraction and Isolation. The air-dried (whole plant) material (130 g) was extracted with Et_2O, followed by MeOH, at room temperature. Each extract was filtered, and the solvents were evaporated at reduced pressure. Small amounts of ether extract and CC fractions were analyzed by TLC and GC−MS, to detect the presence of africane derivatives. The Et_2O extract (3.4287 g) was chromatographed on silica gel with a gradient solvent system of *n*-hexane−EtOAc to give nine fractions.

Fraction 1 (439 mg) was chromatographed by preparative HPLC (Ultrasphere Beckman Silica column, *n*-hexane−EtOAc, 95:5) to afford caespitenone (**3**, 10.9 mg).

Preparative HPLC (Beckman CN, *n*-hexane−EtOAc, 95:5) of fraction 4 (82 mg) gave phytol and a mixture of sesquiterpenes. This mixture was purified to give secoswartzianin B (**9**, 6.7 mg) by preparative HPLC (Beckman CN, *n*-hexane−EtOAc, 97:3).

Fraction 5 (58 mg) was chromatographed by preparative HPLC (Beckman CN, *n*-hexane−EtOAc, 9:1) and rechromatographed on a Nucleosil 50-5 column with *n*-hexane−EtOAc, 7:3, to afford swartzianin B (**4**, 1 mg) and deoxynorsecoswartzianin (**10**, 3.4 mg).

Preparative HPLC (Beckman CN, *n*-hexane−EtOAc, 4:1) of fraction 6 (213 mg) gave swartzianin B (**4**, 10.4 mg), secoswartzianin A (**8**, 26.6 mg), and norsecoswartzianin (**11a**, 6.4 mg)

Fraction 7 (128 mg) was chromatographed on SiO_2 with a gradient solvent system of *n*-hexane−EtOAc, yielding three fractions (7-1, 7-2, and 7-3). The new compound **7** (4,5-epoxysecoswartzianin A, 1.2 mg) was obtained from fraction 7-1 by preparative HPLC (Ultrasphere Beckman Silica, *n*-hexane−EtOAc, 9:1). From fraction 7-3 stigmasterol (64 mg) and a new portion of norsecoswartzianin (**11a**, 13 mg) were obtained by preparative HPLC (Beckman CN, *n*-hexane−EtOAc, 4:1).

Fraction 8 (330 mg) was chromatographed on Sephadex LH-20 and SiO_2 to yield five fractions. Swartzianin C (**5**, 15.8 mg) was obtained from fraction 8-4 by preparative HPLC (Ultrasphere Beckman Silica, *n*-hexane−EtOAc, 4:1).

Fraction 9 (430 mg) was chromatographed on Sephadex LH-20 and silica gel using a $CHCl_3$−EtOAc gradient to afford six fractions. Norsecoswartzianin (**11a**, 4.4 mg) was obtained from fraction 9-3 by preparative HPLC (Beckman C_8, methanol−water, 9:1). Swartzianin D (**6**, 5.2 mg) was obtained from fraction 9-4 by HPLC (Beckman C_8, methanol−water, 9:1). Fraction 9-6 gave the new africane-type sesquiterpenoid **1** (2.0 mg), the known compound **2b** (5.8 mg), and the 3-epimer of this compound **2a** (1.1 mg) by preparative HPLC (Beckman C_{18}, methanol−water, 4:3).

The MeOH extract (3 g) was chromatographed on silica gel with a $CHCl_3$−EtOAc gradient to give six fractions. Swartzianin C (**5**, 2.6 mg) was obtained from fraction 4 by HPLC (silica, *n*-hexane−EtOAc, 4:1). From faction 5 new portions of norsecoswartzianin and stigmasterol were obtained by HPLC (silica, *n*-hexane−EtOAc, 7:3).

In Vitro Antibacterial Assays. The hole-plate diffusion on agar method was employed[33,34] against Gram-positive *Staphylococcus aureus* (ATCC 6538 P), *S. aureus* (F 7) that is a wild strain from an infectious case in Tucumán, Argentina (characterized and kept in culture collection), *Enterococcus faecalis* (ATCC 39212), *Lactobacillus paracasei* ssp. *paracasei* (CRL 75), *L. plantarum* (CRL 105), *L. plantarum* (CRL 358), *L. acidophillus* (ATCC 521), Gram-negative *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), and *Klebsiella pneumoniae* (F 350), a wild strain from an infectious case from Tucumán, Argentina (characterized and kept in culture collection). *Lactobacilli* strains were obtained from plants and milk cultures in the north of Argentina. Compounds were dissolved in EtOH, and 50 μL of a 1.5 mg/mL solution of the tested compounds was placed in 3 mm diameter holes in the agar plate containing an adequate dilution of the microorganisms. The culture was ready to use after three subcultures and further incubation of the cells for 6 h at 37 °C. Assays were carried out in LAPTg medium (1.5% peptone, 1% yeast extract, 1% glucose, 1% tryptone, 1% agar). The pH was adjusted to 6.8, and the medium was then sterilized by autoclaving for 20 min at 118 °C. The plates were incubated overnight at 37 °C. The activity was recorded by the diameter of the inhibition zone by means of three replicates. Penicillin 10U was used as the standard antibiotic for comparison.

The MIC values of compounds **3**−**6** and **8**−**11a** were determined by the broth microdilution method[35] in LAPTg medium. The inoculum was standardized by 0.5 McFarland scale that is indicative of 10^8 colony-forming units (CFU/mL). Dilutions of bacterial suspensions were made to obtain a 10^5 CFU/mL as bacterial standard. Bacterial counts were made to verify inocula conditions. The inoculum was then added to 0.1 mL of broth medium in polystyrene microplates containing the drug in a range of 5000 to 156.2 μg/mL. DMSO was employed as solvent. MIC is the lowest concentration that inhibits visible growth after incubation at 37 °C for 24 h.

Compound 1: amorphous; $[\alpha]^{25}_D$ +45.0° (*c* 1.00, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 238 (4.2) nm; CD (EtOH) $\Delta\epsilon_{252}$ +3.94, $\Delta\epsilon_{223}$ −1.48 (*c* 8.0 × 10^{-4}M); FTIR (KBr) ν_{max} 3323, 1694, 1630, 1381 cm^{-1}; ^1H and ^{13}C NMR, Tables 1 and 2; EIMS *m*/*z* 250 $[M]^+$ (2), 232 (58), 193 (27), 176 (77), 150 (100), 109 (56), 83 (57), 55 (38), 43 (65); HREIMS *m*/*z* 250.1564 $[M]^+$ (calcd for $C_{15}H_{22}O_3$, 250.1569).

Compound 2a: oil; $[\alpha]^{23}_D$ −40.0° (*c* 0.50, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 245 (4.1) nm; CD (EtOH) $\Delta\epsilon_{350}$ −0.68, $\Delta\epsilon_{252}$ +5.74, $\Delta\epsilon_{215}$ −8.58 (*c* 4 × 10^{-4} M); FTIR (KBr) ν_{max} 3353, 1706, 1640, 1376 cm^{-1}; ^1H and ^{13}C NMR, Tables 1 and 2; EIMS *m*/*z* 250 $[M]^+$ (11), 232 (17), 219 (18), 189 (17), 177 (17), 133 (22), 91 (39), 83 (53), 43 (100); HREIMS *m*/*z* 250.1581 $[M]^+$ (calcd for $C_{15}H_{22}O_3$, 250.1569).

Compound 7: oil; $[\alpha]^{24}_D$ −16.5° (*c* 0.55, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 237 (3.6) nm; CD (EtOH) $\Delta\epsilon_{256}$ −2.97, $\Delta\epsilon_{216}$ +2.35 (*c* 5.64 × 10^{-4}M); FTIR ν_{max} (KBr) 1735, 1458, 1270 cm^{-1}; ^1H and ^{13}C NMR, Tables 1 and 2; CIMS (isobutane) *m*/*z* 249 $[M + 1]^+$, 233 (11), 205 (5), 56 (100), 58 (87), 39 (81); HRCIMS *m*/*z* 249.1487 $[M + 1]^+$.

Compound 10: oil; $[\alpha]^{23}_D$ −50° (*c* 0.16, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 224 (4.0) nm; CD (EtOH) $\Delta\epsilon_{245}$ +1.46 (*c* 7.04 × 10^{-4}); FTIR ν_{max} (KBr) 1749, 1650 cm^{-1}; ^1H and ^{13}C NMR, Tables 1 and 2; EIMS *m*/*z* 220 $[M]^+$ (77), 205 (45), 191 (14), 177 (74), 176 (100), 133 (75),119 (67), 105 (87), 91 (96), 77 (83), 43 (99); HREIMS *m*/*z* 220.1467 $[M]^+$ (calcd for $C_{14}H_{20}O_2$, 220.1464).

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