Antibacterial Prenylated Acylphloroglucinols from the Fern *Elaphoglossum yungense*

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Seven new prenylated acylphloroglucinols, yungensins A−G, were isolated from the diethyl ether extract of the scaly rhizomes and roots of an Argentine collection of the fern *Elaphoglossum yungense*. All the compounds contained a geranyl group attached to the filicinic acid-type residue. The diethyl ether extract and yungensins A−F displayed antibacterial effects against *Staphylococcus aureus* and *Pseudomonas aeruginosa* with MICs ranging from 10 to 200 μg/mL. All tested acylphloroglucinols, except for yungensin D, caused bacteriolysis of *S. aureus* at MBC or higher doses (50−200 μg/mL). At 10 μg/mL, the ether extract as well as yungensins A, B, and D−F altered the biofilm production in both microoganisms.

The genus *Elaphoglossum*, recently relocated in the family Dryopteridaceae,[1] comprises around 600 species and is widely spread in tropical and subtropical regions. *E. piloselloides*, *E. gayanum*, *E. yungense*, *E. crassipes*, *E. lorentzii*, and *E. lindbergii* grow in the northwest of Argentina.[2] As part of our ongoing phytochemical investigations on ferns of the genus *Elaphoglossum*, we recently reported on the acylphloroglucinols of Argentine collections of *E. piloselloides* and *E. gayanum*.[3,4] The compounds from the former showed strong molluscicidal activity against the schistosomiasis vector snail *Biomphalaria peregrina*.[3,4]

Many species of the genus *Dryopteris* (Dryopteridaceae) contain acylphloroglucinols in their rhizomes and scales.[5] Powdered rhizomes and extracts from these plants have been used in the past as vermifuges for humans as well as for animals.[6−8] Although their use in herbal medicine is regarded as obsolete today, their strong biological activity has brought attention to acylphloroglucinols. So far, a wide variety of bioactive acylphloroglucinols have been isolated from *Hypericum* species[9−11] as well as from the ferns of the genus *Dryopteris*[12,13] and *Elaphoglossum*.[3,4]

Continuing our search for bioactive fern constituents,[3,4,14−16] we now report the isolation, identification, and antibacterial evaluation of seven new prenylated acylphloroglucinols from an Argentine collection of the fern *E. yungense* de la Sota. It is noteworthy that all the compounds contain a geranyl residue attached to the filicinic acid-type ring. The antibacterial activity of the acylphloroglucinols along with the diethyl ether extract of the fern was determined against a Gram (+) and a Gram (−) bacterium. In addition, we investigated the effects of the isolated compounds on biofilm formation of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Biofilms are complex communities of bacteria embedded in a self-produced matrix and attached to inert or living surfaces.[17] These microorganisms are more resistant to antibiotics and to the immunologic system than planktonic cells;[18] therefore, studies on the effects of natural compounds on biofilm-producing pathogenic bacteria, particularly *S. aureus* and *P. aeruginosa*, are important for the discovery of new substances to control microbial pathogenicity.

Results and Discussion

The scaly rhizomes and roots of *E. yungense* were air-dried, ground, and extracted with Et_2O. Fractionation of the resulting extract by column chromatography over SiO_2 followed by normal-phase HPLC of the acylphloroglucinol-containing fractions led to the isolation of the new compounds **1**−**7**, which were named yungensins A−G, respectively. On TLC, acylphloroglucinols are readily identified by their UV absorbance at 254 nm and characteristic reddish-orange or yellow spots with the Godin reagent.[19]

The molecular formula of yungensin A (**1**), $C_{35}H_{44}O_8$, was deduced from its positive HREIMS, which showed a molecular ion peak at *m/z* 592.3027 (calcd 592.3037). Its ^{1}H NMR spectrum in acetone-d_6 (Table 1) showed a signal at a very low field (δ 18.58 ppm), suggesting the presence of an enolizable β-triketone system. This evidence together with the ^{13}C NMR signal at δ 49.9 revealed the presence of a filicinic acid ring prenylated at C-4.[20] Signals for a methylene bridge (AB system) were also observed at δ 3.54 (d, J = 15.4 Hz) and 3.51 (d, J = 15.4 Hz). The ^{1}H and ^{13}C NMR spectra of **1** (Tables 1 and 2) resembled those of hyperbrasilol B, an antibacterial acylphloroglucinol previously isolated from *Hypericum brasiliense* (Hypericaceae),[20] indicating that the structure of compound **1** consisted of a prenylated filicinic acid-type ring connected to a chromene-type ring through a methylene bridge. Nevertheless, the acyl moieties of **1** were not isobutanoyls and the C-4 prenyl chain was longer than that in hyperbrasilol B. Analysis of the ^{1}H, COSY, HSQC, and HMBC NMR spectra of acylphloroglucinol **1** showed that the acyl group attached to the filicinic acid-type ring was an acetyl residue and that a butanoyl group was attached to the chromene-type ring at C-8′. The prenyl moiety at C-4 was identified as geranyl (δ 2.76, 1H, dd, J = 13.3, 9.0 Hz, H-9a; δ 2.59, 1H, dd, J = 13.3, 6.6 Hz, H-9b; δ 4.66, 1H, br t, J = 7.0 Hz, H-10; δ 1.66−1.56, 4H, overlapping signals, H-12 and H-13; δ 4.85, br s, H-14; δ 1.58, 3H, s, H-16; δ 1.46, 3H, s, H-17; δ 1.38, 3H, s, H-18). On the basis of the foregoing evidence, the structure of yungensin A (**1**) was established as 2-{[5,7-dihydroxy-2,2-dimethyl-8-butanoyl-6-chromenyl]methyl}-3,5-dihydroxy-4-methyl-4-(3,7-dimethyl-2,6-octadienyl)-6-acetyl-2,5-cyclohexadien-1-one. This compound has a stereogenic center at C-4 whose absolute configuration could not be determined.

Yungensin B (**2**) was obtained as a yellow oil. The molecular formula was deduced to be $C_{37}H_{48}O_8$ from the $[M]^+$ ion peak at *m/z* 620.3340 (calcd 620.3350) in its HREIMS spectrum. The 1D NMR data for this compound (Tables 1 and 2) were similar to those of yungensin A, except for the presence of signals due to the acyl moiety attached to the chromene-type ring, corresponding to a hexanoyl unit (δ 3.16, 1H, ddd, J = 16.1, 8.4, 6.6 Hz, H-2″a; δ 3.09, 1H, ddd, J = 16.1, 8.4, 6.6 Hz, H-2″b; δ 1.71, 2H, m, H-3″; δ 1.43−1.35, 4H, overlapping signals, H-4″ and H-5″; δ 0.92, 3H, t, J = 7.1 Hz, H-6″). The location of the hexanoyl moiety at C-8′ was evident from the long-range H−C correlation between 7′-OH and C-1″. Thus, the structure of **2** was identified as 2-{[5,7-

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Chart 1

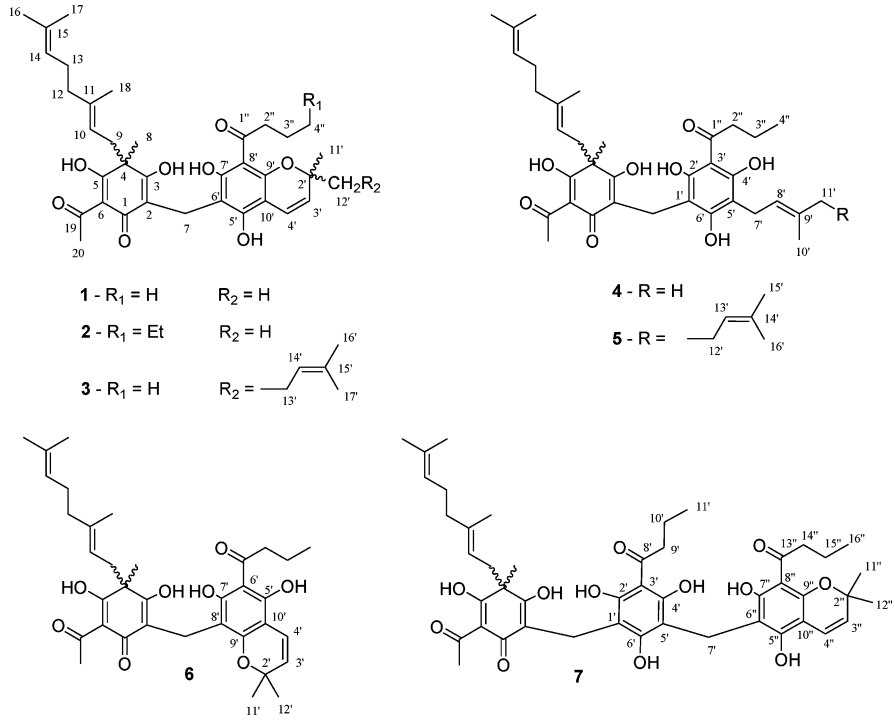

Table 1. ^{1}H NMR Data of Compounds **1**−**3** and **6** (acetone-d_6, 600 MHz)

	δ [ppm], multiplicity, J [Hz]			
H	**1**	**2**	**3**	**6**
7	3.54, d, J = 15.4 3.51, d, J = 15.4	3.54, d, J = 15.4 3.51, d, J = 15.4	3.54, d, J = 15.6 3.51, d, J = 15.6	3.60, d, J = 15.6 3.55, d, J = 15.6
8	1.53, s	1.53, s	1.53, s	1.56, s
9	2.76, dd, J = 13.3, 9.0 2.59, dd, J = 13.3, 6.6	2.76, dd, J = 13.2, 9.2 2.59, dd, J = 13.2, 6.6	2.76, dd, J = 13.2, 9.2 2.59, dd, J = 13.2, 6.4	2.77, dd, J = 13.3, 9.0 2.61, dd, J = 13.3, 7.1
10	4.66, br t, J = 7.0	4.66, br t, J = 7.4	4.66, br t, J = 7.5	4.68, br t, J = 7.9
12	1.66−1.56[a]	1.66−1.56[a]	1.68−1.56[a]	1.62−1.52[a]
13	1.66−1.56[a]	1.66−1.56[a]	1.68−1.56[a]	1.62−1.52[a]
14	4.85, br s	4.85, br t, J = 7.4	4.86, br s	4.82, br t, J = 7.1
16	1.58, s	1.58, s	1.58, s	1.54, s
17	1.46, s	1.46, s	1.48, s	1.44, s
18	1.38, s	1.38, s	1.38, s	1.39, s
20	2.69, s	2.69, s	2.69, s	2.70, s
3′	5.57, d, J = 10.0	5.57, d, J = 10.0	5.55, d, J = 10.1	5.64, d, J = 9.8
4′	6.62, d, J = 10.0	6.63, d, J = 10.0	6.69, d, J = 10.1	6.67, d, J = 9.8
11′	1.52, s	1.52, s	1.48, s	1.66, s
12′	1.52, s	1.52, s	1.88, ddd, J = 14.0, 11.2, 5.4 1.78, ddd, J = 14.0, 11.2, 5.4	1.54, s
13′			2.19, m 2.13, m	
14′			5.14, tt, J = 7.1, 1.4	
16′			1.64, d, J = 1.4	
17′			1.57, s	
2″	3.13, ddd, J = 15.8, 8.0, 6.9 3.07, ddd, J = 15.8, 8.0, 6.9	3.16, ddd, J = 16.1, 8.4, 6.6 3.09, ddd, J = 16.1, 8.4, 6.6	3.15, dt, J = 16.2, 7.4 3.08, dt, J = 16.2, 7.4	3.16, ddd, J = 17.2, 8.2, 6.3 3.07, ddd, J = 17.2, 8.2, 6.3
3″	1.72, sext.d, J = 7.3, 1.6	1.71, m	1.73, sext., J = 7.4	1.69, sext.d, J = 6.3, 1.1
4″	1.01, t, J = 7.3	1.43−1.35[a]	1.01, t, J = 7.4	0.98, t, J = 7.4
5″		1.43−1.35[a]		
6″		0.92, t, J = 7.1		
3-OH	9.95, s	9.95, s	9.95, s	9.14, s
5-OH	18.58, s	18.58, s	18.58, s	18.58, s
5′-OH	11.41, s	11.41, s	11.40, s	14.19, s
7′-OH	16.27, s	16.28, s	16.29, s	11.76, s

[a] Overlapping signals.

dihydroxy-2,2-dimethyl-8-hexanoyl-6-chromenyl]methyl}-3,5-dihydroxy-4-methyl-4-(3,7-dimethyl-2,6-octadienyl)-6-acetyl-2,5-cyclohexadien-1-one.

The HREIMS of yungensin C (**3**) indicated the molecular formula $C_{40}H_{52}O_8$ (*m*/*z* 660.3663, calcd 660.3664). The NMR data of **3** (Tables 1 and 2) showed close similarity with those of yungensin A (**1**), with the exception of the signals due to a 4-methyl-3-pentenyl group. The HMBC spectrum of yungensin C showed cross-peaks between the signals assigned to H-12′ and both C-2′ and C-3′, indicating the position of the chain at C-2′. The foregoing evidence allowed us to establish the structure of **3** as 2-{[5,7-dihydroxy-2-methyl-2-(4-methyl-3-pentenyl)-8-butanoyl-6-chromenyl]methyl}-

Table 2. ^{13}C NMR Data of Compounds **1**−**7** (acetone-d_6, 150 MHz)

	δ [ppm]						
C	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1	188.7	188.7	188.7	188.7	188.7	188.9	189.2
2	114.2	114.2	114.2	114.5	114.5	114.1	114.1
3	171.0	171.0	171.0	170.6	170.6	170.4	170.6
4	49.9	49.9	49.9	49.9	49.9	49.8	50.1
5	199.2	199.2	199.2	199.1	199.1	199.0	199.3
6	111.1	111.1	111.1	111.2	111.2	111.1	111.1
7	17.0	17.0	17.0	17.6	17.5	17.2	17.7
8	23.1	23.1	23.1	23.0	23.0	22.9	23.0
9	38.9	38.9	38.9	38.9	38.9	39.0	39.1
10	117.9	117.9	117.9	117.8	117.8	117.7	117.8
11	140.5	140.5	140.5	140.7	140.7	140.8	140.6
12	40.2	40.2	40.2	40.3	40.3	40.3	40.2
13	27.3	27.3	23.1	27.4	27.4	25.8	27.4
14	124.8	124.8	124.8	124.8	124.8	124.7	124.7
15	131.6	131.6	131.7	131.7	131.7	131.7	131.7
16	25.8	25.8	25.8	25.8	25.8	27.4	25.7
17	17.6	17.6	17.6	17.6	17.7	17.5	17.5
18	16.1	16.1	16.1	16.1	16.1	16.1	15.9
19	203.6	203.6	203.6	203.6	203.6	203.7	203.7
20	29.1	29.1	29.1	29.1	29.1	29.2	29.2
1′				106.5	106.5		107.0
2′	79.2	79.2	82.0	160.1	160.1	81.5	160.8
3′	125.9	125.9	124.5	105.8	105.8	125.7	105.4
4′	117.6	117.6	118.2	160.1	160.1	117.2	159.8
5′	160.0	160.0	160.0	108.8	108.7	160.8	106.2[a]
6′	107.0	107.0	106.9	160.1	160.1	107.1	159.3
7′	162.4	162.4	162.3	22.4	22.3	161.3	16.6
8′	105.0	105.0	104.1	123.4	123.2	106.1	208.6
9′	156.5	156.5	156.8	132.8	136.8	155.8	46.5
10′	104.5	104.5	104.9	18.0	16.3	102.7	18.5
11′	28.0	28.1	26.9	25.8	40.4	28.1	14.1
12′	28.0	28.0	42.2		27.3	27.5	
13′			23.9		125.0		
14′			124.8		131.7		
15′			132.2		25.8		
16′			25.8		17.6		
17′			17.6				
1″	207.5	207.7	207.4	207.7	207.7	207.9	
2″	46.4	44.5	46.5	46.5	46.5	47.0	79.3
3″	19.1	25.4	19.0	18.8	18.8	18.5	126.5
4″	14.2	32.4	14.2	14.2	14.2	14.2	117.3
5″		23.3					158.9
6″		14.2					106.9[a]
compound **7**: 7″: 162.3, 8″: 105.4, 9″: 156.6, 10″: 104.2, 11″: 28.0, 12″: 25.7, 13″: 207.7, 14″: 46.6, 15″: 9.0, 16″: 14.1							

[a] Signals are exchangeable.

Table 3. ^{1}H NMR Data of Compounds **4**, **5**, and **7** (acetone-d_6, 600 MHz)

	δ [ppm], multiplicity, *J* [Hz]		
H	**4**	**5**	**7**
7	3.56, d, *J* = 15.6	3.57, d, *J* = 15.6	3.60, d, *J* = 15.6
	3.53, d, *J* = 15.6	3.54, d, *J* = 15.6	3.55, d, *J* = 15.6
8	1.54, s	1.54, s	1.54, s
9	2.76, dd, *J* = 13.0, 9.0	2.76, dd, *J* = 13.3, 9.0	2.74, dd, *J* = 13.6, 9.5
	2.60, dd, *J* = 13.0, 7.1	2.60, dd, *J* = 13.3, 6.9	2.58, dd, *J* = 13.6, 7.1
10	4.68, br t, *J* = 8.3	4.66, br t, *J* = 8.3	4.62, br s
12	1.64−1.58[a]	1.66−1.58[a]	1.57−1.44[a]
13	1.64−1.58[a]	1.66−1.58[a]	1.57−1.44[a]
14	4.86, br s	4.86, br s	4.76, br s
16	1.59, s	1.59, s	1.52, s
17	1.47, s	1.47, s	1.40, s
18	1.37, s	1.37, s	1.29, br s
20	2.69, s	2.69, s	2.71, s
7′	3.40, dd, *J* = 16.0, 6.5	3.42, dd, *J* = 16.0, 6.6	3.80, s
	3.35, dd, *J* = 16.0, 6.5	3.38, dd, *J* = 16.0, 6.6	
8′	5.12, tt, *J* = 6.5, 1.2	5.15, tquart., *J* = 6.6, 1.4	
9′			3.16, quart., *J* = 7.3
10′	1.76, s	1.78, s	1.71, sext., *J* = 7.3
11′	1.66, d, *J* = 1.2	1.98, dd, *J* = 8.2, 6.6	0.99, t, *J* = 7.3
12′		2.08, dd, *J* = 8.2, 6.6	
13′		5.08, tt, *J* = 6.6, 1.2	
15′		1.62, d, *J* = 1.2	
16′		1.56, s	
2″	3.12, t, *J* = 7.3	3.12, t, *J* = 7.3	
3″	1.70, sext., *J* = 7.3	1.70, sext., *J* = 7.3	5.60, d, *J* = 10.0
4″	0.97, t, *J* = 7.3	0.97, t, *J* = 7.3	6.64, d, *J* = 10.0
11″			1.51, s
12″			1.52, s
14″			3.12, dd, *J* = 7.8, 6.6
15″			1.72, sext., *J* = 7.3
16″			1.01, t, *J* = 7.3
3-OH			9.65, s
5-OH	18.56, s	18.56, s	18.59, s
7″-OH			16.37, s

[a] Overlapping signals.

3,5-dihydroxy-4-methyl-4-(3,7-dimethyl-2,6-octadienyl)-6-acetyl-2,5-cyclohexadien-1-one. The absolute configuration of C-4 and C-2′ could not be defined.

The HREIMS spectrum of yungensin D (**4**) exhibited a molecular ion peak at *m*/*z* 594.3197, indicating the molecular formula $C_{35}H_{46}O_8$ (calcd 594.3194). The ^{1}H and ^{13}C NMR data (Tables 3 and 2, respectively) suggested the presence of the same filicinic acid-type ring as in yungensins A−C linked via a methylene bridge to an acylphloroglucinol-type ring. The 1D NMR data of **4** resembled those of drummondin F, an antibacterial compound previously isolated from *Hypericum drummondii*,[21] indicating the presence of a prenyl-substituted acylphloroglucinol-type ring. The prenyl side chain attached to the acylphloroglucinol-type ring was identified as 3-methyl-2-butenyl by analysis of the 2D NMR data of **4**. The HMBC spectrum showed cross-peaks between H-7′ and C-4′, C-5′, and C-6′, indicating the location of this moiety at C-5′. Therefore, the structure of yungensin D was established as 2-{[2,4,6-trihydroxy-5-(3-methyl-2-butenyl)-3-butanoylphenyl]methyl}-3,5-dihydroxy-4-methyl-4-(3,7-dimethyl-2,6-octadienyl)-6-acetyl-2,5-cyclohexadien-1-one.

The molecular formula of yungensin E (**5**) was established as $C_{40}H_{54}O_8$ by analysis of its HREIMS data (*m*/*z* 662.3821). The ^{1}H and ^{13}C NMR data of this compound resembled those of acylphloroglucinol **4**, with the exception of the prenyl moiety attached to C-5′. Analysis of the ^{1}H ^{1}H COSY, HSQC, and HMBC spectra of **5** allowed identification of the prenyl side chain at C-5′ as geranyl. A NOESY correlation between H-8′ and H-11′ clearly indicated the *E* configuration of the double bond at C-8′. Accordingly, the structure of compound **5** was established as 2-{[2,4,6-trihydroxy-5-(3,7-dimethyl-2,6-octadienyl)-3-butanoylphenyl]methyl}-3,5-dihydroxy-4-methyl-4-(3,7-dimethyl-2,6-octadienyl)-6-acetyl-2,5-cyclohexadien-1-one.

Yungensin F (**6**) has the same molecular formula as compound **1**; hence, these compounds are isomers. The only significant difference involved the chemical shifts of the signals assigned to the OH protons of the chromene-type ring in the ^{1}H NMR spectrum at δ 14.19 (OH-5′) and 11.76 (OH-7′), indicating that the cyclization occurs at the hydroxy group adjacent to the methylene bridge, as previously stated for similar compounds.[20] Further evidence to support the proposed structure was obtained from the HMBC spectrum of **6** that showed a cross-peak between the 5′-OH proton and both C-6′ and C-1″, unambiguously confirming the location of the OH group at C-5′. Thus, compound **6** is 2-{[5,7-dihydroxy-2,2-dimethyl-6-butanoyl-8-chromenyl]methyl}-3,5-dihydroxy-4-methyl-4-(3,7-dimethyl-2,6-octadienyl)-6-acetyl-2,5-cyclohexadien-1-one.

The molecular formula of yungensin G (**7**), $C_{46}H_{56}O_{12}$, was obtained from its HRFABMS, which showed a molecular ion peak $[M]^+$ at *m*/*z* 800.3770 (calcd 800.3773). Its ^{1}H NMR spectrum showed evidence of two methylene bridges (δ 3.60, d, *J* = 15.6 Hz; δ 3.55, d, *J* = 15.6 Hz; and δ 3.80, 2H, s), indicating that this acylphloroglucinol has three ring systems. The chemical shift of the signals assigned to CH_2-7 is characteristic of a methylene group located between a hexadienone and an aromatic ring, while the signal at δ 3.80 indicates that the other methylene bridge connects two aromatic rings.[12] Comparison of the 1D NMR data of this

Table 4. Antibacterial Activity of Acylphloroglucinols **1**−**6** and the Et_2O Extract of *E. yungense* against *Staphylococcus aureus* ATCC 6738 and *Pseudomonas aeruginosa* ATCC 27853

	MIC/MBC [μg/mL]		% growth inhibition at 10 μg/mL	
sample	*S. aureus*	*P. aeruginosa*	*S. aureus*	*P. aeruginosa*
diethyl ether extract	200/>200	200/200	80	68
1	10/50	10/50	99	99
2	10/100	10/50	99	99
3	50/100	50/50	92	87
4	100/>200	50/100	83	86
5	100/200	100/200	87	87
6	50/100	50/50	93	86

compound with those of yungensins A−F indicated that one of the rings was the same prenylated filicinic acid of the previously described compounds, the second was an acylphloroglucinol-type ring, and the third was a dimethylchromene-type ring. The acyl groups were identified as an acetyl and two butanoyl residues, whose positions were determined by analysis of the HMBC spectrum of **7**. The ring locations and the assignments of their carbons were accomplished by analysis of the HMBC correlations of the methylene bridges' protons with the ring carbons. On the basis of the foregoing evidence, the structure of yungensin G (**7**) was established as depicted. This is the first tricyclic acylphloroglucinol isolated from an *Elaphoglossum* fern species.

As previously indicated for yungensin A, the absolute configuration of yungensins B−G at C-4 could not be assessed.

As observed before for other *Elaphoglossum* ferns, all the acylphloroglucinols from *E. yungense* have the same acyl residue (acetyl) attached to C-6 of the filicinic acid-type ring.[4] Furthermore, acylphloroglucinols from *Elaphoglossum* ferns are structurally more related to *Hypericum* acylphloroglucinols than to *Dryopteris* acylphloroglucinols.

Based on our unambiguous assignments of the ^{13}C NMR signals at δ 189 (C-1) and 199 (C-5) for yungensins A−F, which rely on HMBC correlations, we suggest that the previously reported ^{13}C NMR assignments for C-1 and C-5 of drummondins A−F[21,22] and sarothralens B, C, and D,[23] as well as hyperbrasilol B and related compounds,[11,20] should be interchanged.

Antibacterial Activity. The MIC values of acylphloroglucinols **1**−**6** against *S. aureus* and *P. aeruginosa*, compiled in Table 4, ranged from 10 to 200 μg/mL. Nevertheless, absorbance measurements performed at 10 μg/mL indicated that all compounds caused considerable bacterial growth inhibition compared to the control. The MBCs of yungensins A−F were in all cases equal or higher than MIC values for both microorganisms.

Contact of acylphloroglucinols **1**−**3**, **5**, and **6** with the *S. aureus* culture at MBC or higher doses produced a decrease in optical density, suggesting bacteriolysis.[24,25] In *P. aeruginosa*, the antimicrobial activity did not involve bacteriolysis, indicating that acylphloroglucinols **1**−**6** display different mechanisms of action against *S. aureus* (Gram +) and *P. aeruginosa* (Gram −).

It was previously reported that medicinal plant extracts, as well as some natural products, that displayed antibiotic activity against Gram (+) lacked activity against Gram (−) bacteria.[26,27] For this reason it is worth pointing out that yungensins A−F displayed activity not only against Gram (+) but against Gram (−) bacteria as well. This is the first time that bacteriolytic activity against Gram (+) and bactericidal activity against Gram (−) bacteria are reported for acylphloroglucinols.

The presence of a chromene-type ring seems to accentuate the bactericidal and bacteriolytic effects of acylphloroglucinols since compounds **1**−**3** and **6** are more active against both microorganisms than compounds **4** and **5**.

Biofilm Assay. At 10 μg/mL, samples **1**, **2**, **5**, and **6** produced an increase of the amount of biofilm of *S. aureus* and *P. aeruginosa* compared to the control (Figure 1), despite the great reduction in growth. The increase in biofilm production might be part of a defense mechanism against the antibiotic effect of acylphloroglucinols.[28]

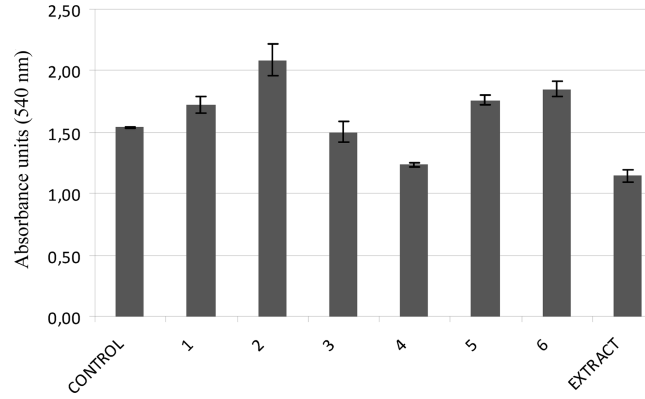

Figure 1. Effect of the Et_2O extract of *E. yungense* and of pure acylphloroglucinols (**1**−**6**) on *S. aureus* biofilm production. The error bars indicate standard deviation (SD).

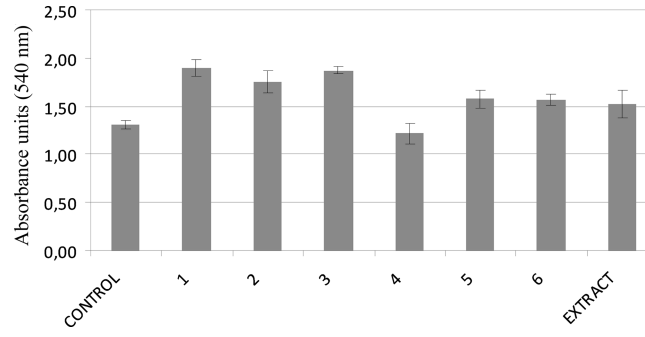

Figure 2. Biofilm production by *P. aeruginosa* cultures grown in LB broth untreated and treated with natural products **1**−**6** and the Et_2O extract of *E. yungense*. The error bars indicate standard deviation (SD).

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a Jasco P-1030 polarimeter. Infrared spectra were measured on a Shimadzu FT/IR-8400S spectrophotometer by the diffuse reflectance method. Low- and high-resolution MS spectra were recorded on a JEOL JMS AX-500 instrument. NMR spectra were measured in acetone-d_6 at 600 MHz for ^1H and 150 MHz for ^{13}C on a Varian Unity 600 spectrometer. Column chromatography was carried out over silica gel 60 (70−230 mesh, Merck), using an *n*-hexane−EtOAc gradient followed by acetone as eluents. The elution of the column was monitored by TLC on aluminum precoated plates F_{254}. The spots on the plates were visualized under UV light and further spraying of the plates with Godin reagent.[19] HPLC separations were performed on a Gilson apparatus, using a silica gel column (Chemcopak; Chemcosorb 5 Si-U, 5 μm, 250 × 10 mm i.d.), and ultraviolet and refractive index detectors in parallel. Mixtures of HPLC grade *n*-hexane and EtOAc were used to elute the samples (in some cases, 1% HOAc was added).

Plant Material. *E. yungense* was collected in Yacuchina, Tucumán, Argentina, in December 2008. The plant material was identified by Lic. Marcela Hernández de Terán, and a voucher specimen (LIL 609964) was deposited at the Herbarium of the Fundación Miguel Lillo, Tucumán, Argentina.

Extraction and Isolation. The air-dried, scaly rhizomes and roots of *E. yungense* (130 g) were powdered and extracted at room temperature with Et_2O. The crude extract (5.8 g) was fractionated by column chromatography over silica gel to give three acylphloroglucinol-containing fractions. A portion (200 mg) of fraction 1 (3.0 g) was processed by normal-phase HPLC (*n*-hexane−EtOAc, 98:2, 3.0 mL/min) to afford compounds **1** (19 mg), **2** (8 mg), and **3** (5.7 mg). Fraction

2 (228 mg) was exhaustively processed by NPHPLC, affording compounds **6** (28 mg) and **7** (1 mg). Processing of fraction 3 (415 mg) by NPHPLC (*n*-hexane−EtOAc, 8:2, 1% HOAc, 3.0 mL/min) led to the isolation of compounds **4** (21 mg) and **5** (13 mg).

Yungensin A (1): yellow oil; $[\alpha]_D^{21.9}$ +60.6 (*c* 1.0, $CHCl_3$); IR ν_{max}^{neat} cm^{-1} 3163, 2710, 2646, 1641, 1601, 1564; ^1H NMR data (500 MHz, acetone-d_6) in Table 1; ^{13}C NMR data (125 MHz, acetone-d_6) in Table 2; HREIMS, 70 eV, *m*/*z* 592.3027 (calcd for $C_{35}H_{44}O_8$ 592.3037).

Yungensin B (2): yellow oil; $[\alpha]_D^{23.1}$ +94.0 (*c* 1.0, $CHCl_3$); IR ν_{max}^{neat} cm^{-1} 3163, 2710, 2648, 1641, 1601; ^1H NMR data (600 MHz, acetone-d_6) in Table 1; ^{13}C NMR data (150 MHz, acetone-d_6) in Table 2; HREIMS, 70 eV, *m*/*z* 620.3340 (calcd for $C_{37}H_{48}O_8$ 620.3350).

Yungensin C (3): yellow oil; $[\alpha]_D^{21.7}$ +131.4 (*c* 1.0, $CHCl_3$); IR ν_{max}^{neat} cm^{-1} 3169, 2719, 2648, 1639, 1603; ^1H NMR data (600 MHz, acetone-d_6) in Table 1; ^{13}C NMR data (150 MHz, acetone-d_6) in Table 2; HREIMS, 70 eV, *m*/*z* 660.3663 (calcd for $C_{40}H_{52}O_8$ 660.3664).

Yungensin D (4): colorless oil; $[\alpha]_D^{23.8}$ +73.6 (*c* 1.0, $CHCl_3$); IR ν_{max}^{neat} cm^{-1} 3352, 3157, 2729, 2650, 1639, 1610, 1583; ^1H NMR data (600 MHz, acetone-d_6) in Table 3; ^{13}C NMR data (150 MHz, acetone-d_6) in Table 2; HREIMS, 70 eV, *m*/*z* 594.3197 (calcd for $C_{35}H_{46}O_8$ 594.3194).

Yungensin E (5): colorless oil; $[\alpha]_D^{23.8}$ +61.5 (*c* 1.0, $CHCl_3$); IR ν_{max}^{neat} cm^{-1} 3360, 3153, 2729, 2648, 1637, 1610, 1582; ^1H NMR data (600 MHz, acetone-d_6) in Table 3; ^{13}C NMR data (150 MHz, acetone-d_6) in Table 2; HREIMS, 70 eV, *m*/*z* 662.3821 (calcd for $C_{40}H_{54}O_8$ 662.3820).

Yungensin F (6): yellow oil; $[\alpha]_D^{22.1}$ +84.4 (*c* 1.0, $CHCl_3$); IR ν_{max}^{neat} cm^{-1} 3242, 2731, 2660, 1639, 1606, 1543; ^1H NMR data (600 MHz, acetone-d_6) in Table 1; ^{13}C NMR data (150 MHz, acetone-d_6) in Table 2; HREIMS, 70 eV, *m*/*z* 592.3037 (calcd for $C_{35}H_{44}O_8$ 592.3037).

Yungensin G (7): colorless oil; $[\alpha]_D^{22.7}$ −5.3 (*c* 1.0, $CHCl_3$); IR ν_{max}^{neat} cm^{-1} 3230, 2629, 1637, 1608, 1562; ^1H NMR data (600 MHz, acetone-d_6) in Table 3; ^{13}C NMR data (150 MHz, acetone-d_6) in Table 2; HRFABMS, *m*/*z* 800.3770 (calcd for $C_{46}H_{56}O_{12}$ 800.3773).

Determination of MIC and MBC by the Broth Microdilution Method. The test was performed in sterile 96-well microplates. Different concentrations of the samples (1, 5, 10, 50, 100, and 200 μg/mL) were tested against *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 6738. The inoculum (150 μL) containing (1−2) × 10^6 cfu was added to each well. Luria−Bertani (LB) and Möeller−Hinton (MH) media were used for *P. aeruginosa* and *S. aureus*, respectively. A number of wells were reserved in each plate for sterility control (no inoculum added), inoculum viability (no substance added), and solvent effect (the final concentration of DMSO did not exced 2.5%). Plates were incubated at 37 °C during 24 h. Growth was detected as turbidity (600 nm) relative to an uninoculated well using a microtiter plate reader (Power Wave XS2, Biotek, Winooski, VT, USA). Minimal inhibitory concentration (MIC) was defined as the lowest concentration of the tested sample showing no visible bacterial growth after incubation (OD lower than 0.05 at 600 nm). To determine the minimal bactericidal concentration (MBC), an aliquot (10 μL) of each incubated well showing no visible growth was subcultured in appropriate media (MH or LB). MBC was defined as the lowest concentration that showed no visible growth on the agar medium (99.9% killed). The assay was carried out in three replicates for each compound. Ciprofloxacin (50 μg/mL) was used as positive control.

Biofilm Experiment.[29] The effect of acylphloroglucinols **1**−**6** and the Et_2O extract of *E. yungense* on the biofilm production by *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 6738 was evaluated at 10 μg/mL. The test was performed by the crystal violet staining method. The amount of biofilm produced was measured by discarding the medium, rinsing the wells with H_2O (×3), and staining bound cells with crystal violet (0.1% w/v) for 20 min, followed by washing with H_2O. The dye was solubilized in 180 μL of absolute EtOH, and absorbance at 540 nm was determined using a microtiter plate reader. Eight replicates were used for each sample, and each experiment was performed three times.

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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