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Biotransformation of Salpichrolides A, C, and G by Three Filamentous Fungi

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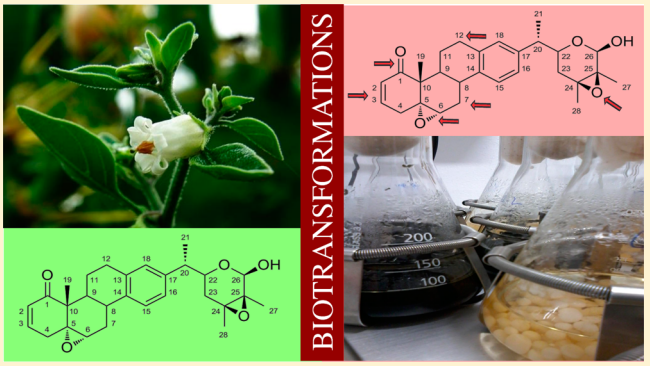
Supporting Information

ABSTRACT: Incubation of salpichrolide A (**1**) with *Rhizomucor miehei* produced hydroxylation in rings B and C (C-7 and C-12) and led to C-5−C-6 epoxide opening, while incubation of salpichrolides C (**2**) and G (**3**) with *R. miehei* led to epoxide opening at the C-24−C-25 and C-5−C-6 positions, respectively. Biotransformation of salpichrolide A (**1**) with *Cunninghamella elegans* produced stereoselective hydroxylated, oxidized, and reduced derivatives in different positions of the A, B, and C rings and C-5−C-6 epoxide opening. In addition, selective epoxide opening at the C-5−C-6 or C-24−C-25 positions was obtained from the incubation of salpichrolide A (**1**) with *Curvularia lunata*.



Withanolides are steroidal lactones built on an ergostane skeleton of 28 carbons functionalized at carbons 1, 22, and 26, commonly known as the withanolide skeleton. These compounds are generally polyoxygenated, and this profusion of oxygen functions has led to several natural modifications of the carbocyclic skeleton and of the side-chain, resulting in compounds with complex structural features. These compounds have been isolated, largely, but not exclusively, from genera belonging to the plant family Solanaceae. Their chemistry and occurrence have been the subject of several reviews.[1−3]

The genus *Salpichroa* Miers is a small group of about 16 South American species. A family of aromatic D-ring withanolides and related ergostane derivatives has been isolated from *S. origanifolia* and *S. tristis* var. *lehmannii*,[4−9] and many of these compounds exhibit interesting biological activities such as antifeedant[10−12] and cancer chemopreventive effects.[13] In particular, salpichrolide A (**1**) showed a selective antiproliferative activity toward hormone-dependent ER(+) breast cell lines when compared to ER(−) negative cell lines.[14]

Biotransformation methods with filamentous fungi can be used as an attractive alternative when compared to the traditional chemical methods that search for new highly oxygenated and bioactive compounds with a large diversity of biological properties. These fungi have the ability to introduce hydroxy groups, regio- and stereoselectively, in molecular positions difficult to achieve by chemical means. Although there have been many studies on the biotransformation of steroid and terpenoid compounds,[15−20] only a few reported compounds with the withanolide skeleton have been investigated in this manner, such as the biohydroxylation of withaferin A to give 12-hydroxy and 15-hydroxy derivatives by *Cunninghamella elegans*[21,22] and *C. echinulata*.[23] From the present work, we report the microbial transformation of the natural withanolides salpichrolides A (**1**),[4] C (**2**),[5] and G (**3**)[7] isolated from *S. origanifolia*; the biotransformation of **1** with filamentous fungi *Rhizomucor miehei*, *Cunninghamella elegans*, and *Curvularia lunata*; and the biotransformation of **2** and **3** with *R. miehei*. Biocatalysis of these natural compounds has not been described so far.

RESULTS AND DISCUSSION

The biotransformation of salpichrolide A (**1**) with *R. miehei* for 7 days resulted in the recovery of the substrate (4.6%) and metabolites **4** (0.3%), **5** (4%), **6** (2%), **7** (0.8%), and **8** (1.2%) (Figure 1). Metabolite **4** gave a molecular formula of $C_{28}H_{32}O_6$, indicating that the microorganism inserted an oxygen atom into the substrate (**1**) and two hydrogen atoms were lost. The ^{1}H NMR spectrum of compound **4** (Table 1) was closely related to that of **1**. The main differences observed between compounds **1** and **4** comprised the presence of the signals corresponding to a methylene group adjacent to a carbonyl group (δ 3.53 and 2.29) coupled with the signal corresponding to H-9 (δ 2.68) and the deshielding of the H-18 signal from δ 6.90 for compound **1** to δ 7.85 for compound **4**.[4] The ^{13}C NMR spectrum of **4** (Table 2) showed the expected chemical shift

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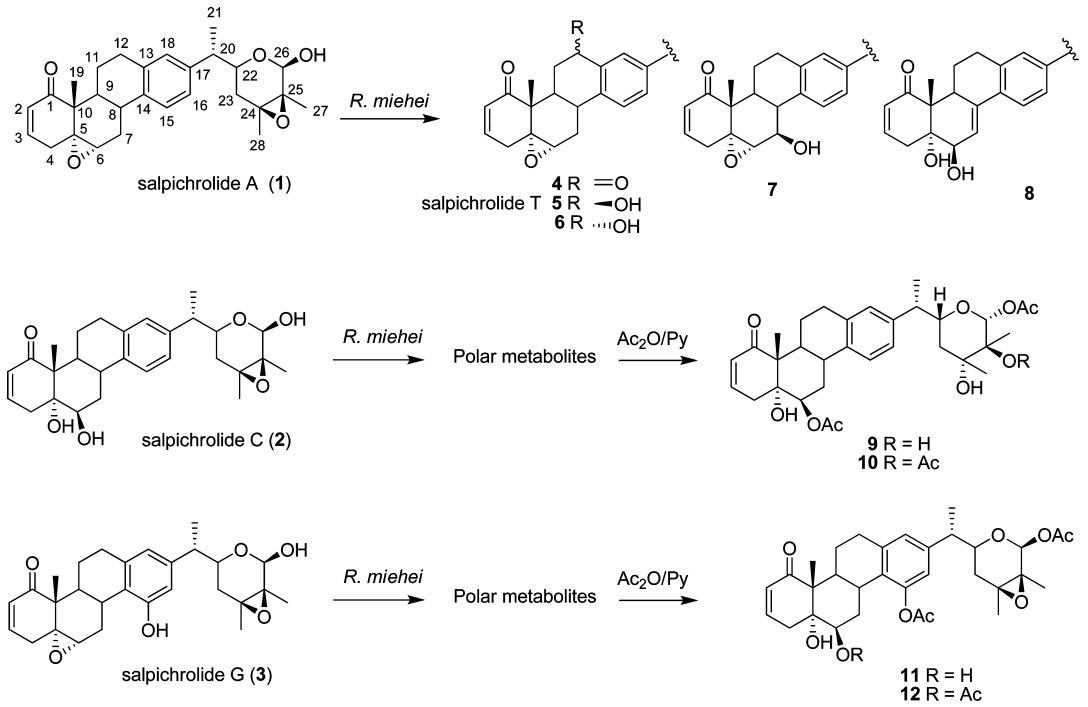

Figure 1. Derivatives obtained by biotransformation of salpichrolides A (**1**), C (**2**), and G (**3**) with *Rhizomucor miehei*.

Table 1. ^{1}H NMR Data of Compounds 4, 6−8, and 10 in $CDCl_3$[a]

position	4	6	7	8	10
2	6.03 dd (10.1, 2.5)	6.01 dd (10.1, 2.5)	6.01 ddd (10.2, 2.7, 0.8)	6.00 dd (10.2, 2.3)	5.89 dd (10.2, 2.2)
3	6.78 ddd (10.1, 5.1, 2.5)	6.78 ddd (10.1, 5.1, 2.5)	6.76 ddd (10.2, 5.0, 2.3)	6.80 ddd (10.2, 5.3, 2.3)	6.53 ddd (10.2, 5.2, 2.2)
4α	1.95 dd (19.7, 5.1)	1.93 m	1.99 ddd (19.7, 5.0, 0.8)	2.35 dd (19.9, 5.3)	2.09 m
4β	3.17 dt (19.7, 2.5)	3.14 dt (19.5, 2.5)	3.19 dt (19.8, 2.7)	3.45 dt (19.9, 5.3)	2.86 m
6	3.26 d (4.9)	3.24 d (4.9)	3.10 brs	3.98 d (5.3)	4.91 t (2.6)
7α	2.03 dd (15.3, 10.9)	1.93 m	4.06 brd (8.6)	6.40 dd (5.3, 1.9)	1.93 m
7β	2.83 m	2.72 m			2.36 dt (14.6, 3.4)
8	2.96 m	2.60 m	2.63 dd (12.3, 8.6)		2.87 m
9	2.68 m	2.64 m	2.07 m	2.98 brd (12.2)	2.16 m
11α	3.53 dd (15.0, 2.5)	2.78 m	2.57 m	2.53 m	2.45 m
11β	2.29 dd (15.0, 14.1)	1.53 m	1.37 m	1.58 m	1.35 m
12α			2.90 ddd (15.5, 12.2, 4.5)	3.08 ddd (16.8, 13.3, 4.4)	2.94 m
12β		4.81 t (2.9)	2.73 m	2.89 dt (16.8, 3.2)	2.72 m
15	7.28 m	7.16 d (8.2)	7.78 d (8.2)	7.64 d (8.3)	7.08 d (8.1)
16	7.42 dd (8.3, 2.1)	7.11 dd (8.2, 1.9)	7.02 dd (8.3, 1.6)	7.05 dd (8.3, 1.4)	6.93 dd (8.1, 1.5)
18	7.85 d (2.1)	7.18 d (1.9)	6.92 d (1.6)	6.99 brs	6.87 brs
19	1.43 s	1.37 s	1.42 s	1.29 s	1.29 s
20	2.83 m	2.76 m	2.74 m	2.77 q (6.7)	2.81 m
21	1.27 m	1.23 d (7.1)	1.24 d (7.2)	1.26 d (7.2)	1.21 d (7.1)
22	3.84 ddd (11.3, 5.4, 2.8)	3.88 ddd (11.1, 6.4, 2.5)	3.86 ddd (11.2, 5.8, 2.6)	3.87 ddd (11.2, 5.9, 2.4)	3.67 m
23α	1.85 dd (14.6, 12.6)	1.89 m	1.83 dd (14.4, 2.6)	1.87 dd (14.4, 2.5)	1.51 m
23β	1.52 m	1.58 m	1.58 m	1.59 m	1.51 m
26	4.97 d (10.1)	4.95 brs	4.99 d (9.8)	4.99 d (9.4)	5.58 s
27	1.38 s	1.357 s	1.39 s	1.40 s	1.39 s
28	1.35 s	1.365 s	1.36 s	1.37 s	1.27 s
OH-26	3.41 d (10.1)		3.43 d (9.8)	3.43 m	
OH-24					5.04 s
CH_3CO-6					2.083 s
CH_3CO-25					1.99 s
CH_3CO-26					2.077 s

[a]Chemical shifts (δ) downfield from TMS. *J* couplings (in parentheses) in Hz. Run at 400.13 MHz.

corresponding to a keto group assigned at C-12 (δ 198.5). The position of a keto group at C-12 was supported by the analysis of the ^{13}C NMR data of **4** and **1**[4] [β-effects at C-11 (+15.7) and C-13 (+6.8)] and confirmed by HMBC correlations from C-12

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Table 2. ^{13}C NMR Data of Compounds 4, 6−8, 10−12, and 14−16 in Cl_3CD[a]

position	4[b]	6[b]	7[b]	8[b]	10[b]	11[b]	12[b]	14[c]	15[c]	16[c]
1	201.3	202.8	202.4	203.4	203.5	204.2	203.4	202.7	75.6	73.0
2	128.7	129.2	129.3	128.3	129.3	129.4	129.4	129.4	74.3	73.2
3	142.4	143.0	142.6	143.6	140.7	141.0	140.2	141.9	72.6	70.90
4	33.5	33.9	33.3	33.7	35.6	35.5	35.4	72.5	32.4	30.4
5	64.3	64.9	65.4	77.3	76.5	77.9	76.5	64.3	63.9	62.1
6	57.8	59.1	63.8	73.0	75.9	75.7	76.8	59.1	55.2	55.0
7	29.3	30.1	70.7	114.9	30.8	34.1	30.2	30.2	30.3	30.4
8	32.4	29.8	42.7	131.5	33.3	31.6	32.5	33.4	31.5	31.4
9	35.9	33.2	33.6	37.4	38.3	41.2	40.6	37.0	35.8	36.1
10	48.3	48.6	48.8	50.4	52.7	53.4	53.2	47.9	39.7	39.8
11	41.1	33.6	26.2	27.2	26.2	24.7	24.8	25.2	22.3	21.7
12	198.5	68.4	31.0	30.8	31.0	32.4	32.4	30.8	30.8	30.8
13	144.4	138.1	138.0	138.4	137.8	141.1	140.9	137.9	136.6	138.8
14	141.9	137.3	137.0	138.4	137.7	130.3	129.7	137.1	139.2	136.4
15	126.3	126.7	127.7	124.5	125.9	150.0	150.0	126.6	126.6	126.6
16	133.6	128.3	126.1	126.3	125.7	120.5	120.5	125.7	126.0	126.3
17	141.9	141.8	141.3	144.0	140.2	141.8	142.0	140.1	140.7	140.7
18	126.3	130.1	129.0	129.7	129.4	127.0	127.3	128.9	128.8	128.7
19	15.2	15.5	15.3	16.4	14.9	14.8	14.7	16.5	15.8	15.5
20	43.0	43.6	43.3	43.6	43.3	42.8	42.7	43.3	43.4	43.0
21	17.3	17.9	17.5	17.6	17.3	17.3	17.3	17.6	17.7	17.7
22	67.2	67.7	67.8	67.8	76.3	71.0	70.9	67.8	67.8	70.9
23	33.9	34.4	34.0	34.3	40.3	34.4	34.4	33.9	34.1	34.5
24	64.9	64.9	65.2	65.1	73.1	62.0	62.0	65.2	65.1	62.0
25	63.7	63.9	64.0	64.0	89.0	61.1	61.1	64.0	64.0	61.1
26	91.5	92.0	92.1	92.0	93.9	92.0	91.9	92.0	92.0	92.0
27	16.5	16.9	16.9	16.9	12.2	16.9	16.9	16.9	17.0	16.9
28	18.6	19.1	19.1	19.1	24.3	18.5	18.5	19.1	19.2	18.5
CH_3CO-2										169.2
CH_3CO-2										21.5
CH_3CO-3										169.9
CH_3CO-3										21.6
CH_3CO-6					170.3		170.1			
CH_3CO-6					21.7		21.7			
CH_3CO-15						169.4	169.1			
CH_3CO-15						21.6	21.4			
CH_3CO-25					172.9					
CH_3CO-25					22.2					
CH_3CO-26					169.7	170.7	170.7			170.6
CH_3CO-26					21.4	21.5	21.5			21.5

[a]Chemical shifts (δ) downfield from TMS. [b]Run at 100.03 MHz. [c]Run at 125.03 MHz.

to H_2-11 and H-18 (see Supporting Information). Therefore, metabolite **4** is the 12-oxo derivative of substrate **1**. The spectroscopic data of metabolite **5** were identical to those described for salpichrolide T (**5**), previously isolated from the aerial parts of *S. tristis* var. *lehmannii*.[9] Metabolite **6** revealed a molecular formula of $C_{28}H_{34}O_6$ by HRESITOFMS with an extra oxygen atom as compared to substrate **1**. Its ^1H NMR spectrum (Table 1) showed a signal at δ 4.81 (t, J = 2.9 Hz) corresponding to a carbynolic proton. The position of this hydroxylation was established by the analysis of ^{13}C NMR data of **1**[4] and **6**, particularly by the β-effect of C-11 (+8.2), suggesting that the hydroxy group is located at C-12 (Table 2). This assumption was confirmed by the cross-correlation peak from H-12 to C-8 (δ 29.8), C-13 (δ 138.1), and C-18 (δ 130.1) in the HMBC experiment (see Supporting Information). The α-orientation of the hydroxy group at C-12 was deduced from the value of the coupling constant between H-12 and H_2-11 (t, J = 2.9 Hz) and confirmed by comparison with the values obtained for its epimer **5** (dd, J = 10.8, 5.0 Hz).[9] Metabolite **7** gave a molecular formula of $C_{28}H_{34}O_6$, indicating that an oxygen atom was inserted into substrate **1** by the microorganism. The ^1H NMR spectrum of **7** (Table 1) showed a signal at δ 4.06 assigned to a carbynolic proton. The location of the hydroxy group was established at the C-7 position through the correlation between the signal at δ 4.06 and H-6 (δ 3.10) and H-8 (δ 2.63) in the $^1H-^1H$ COSY spectrum and confirmed by the analysis of the ^{13}C NMR spectrum (Table 2) compared with that of substrate **1**, where an α-effect of C-7, a β-effect of C-6 and C-8, and a γ-effect of C-9 were observed. The orientation of the hydroxy group at C-7 was established by a NOESY experiment and by the multiplicity and coupling constant values. The NOESY spectrum was inconclusive because only NOE effects between H-7 and H-6 (δ 3.10) and H-15 (δ 7.78) were observed. However, the multiplicity and coupling constant values of H-6 (δ 3.10, brs) and H-7 (δ 4.06, brd, J = 8.4 Hz) agreed with a β-orientation of the

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Table 3. ^{1}H NMR Data of Compounds 11, 12, and 14−16 in Cl_3CD[a]

position	11[b]	12[b]	14[c]	15[c]	16[c]
1				3.83 brs	3.82 brd (11.9)
2	5.87 dd (10.2, 2.8)	5.89 dd (10.2, 2.4)	5.96 d (10.1)	4.25 brs	5.22 brs
3	6.56 ddd (10.2, 5.2, 2.2)	6.51 ddd (10.2, 5.2, 2.3)	6.66 dd (10.1, 4.5)	4.07 brs	5.01 brs
4α	3.18 dt (19.4, 2.8)	2.84 dt (19.5, 2.6)	3.66 d (4.5)	2.66 m	2.51 dd (15.4, 3.7)
4β	2.04 ddd (19.4, 5.2, 2.2)	2.05 m		1.22 m	1.43 brd (15.4)
6	3.67 t (2.8)	4.80 t (2.8)	3.31 d (4.9)	2.88 d (3.3)	2.84 d (4.7)
7α	1.80 m	1.82 m	1.81 dd (15.0, 11.1)	2.57 m	2.58 m
7β	2.57 m	2.68 m	2.69 m	1.83 m	1.81 m
8	3.07 t (11.6)	2.95 brt (12.5)	2.76 m	2.81 m	2.78 m
9	2.11 m	2.16 m	2.01 td (11.7, 2.2)	2.21 brt (12.0)	2.21 td (12.3, 1.6)
11α	2.52 brd (12.0)	2.51 brd (12.0)	2.41 dq (12.5, 2.2)	1.82 m	1.81 m
11β	1.14 m	1.16 m	1.27 m	1.22 m	1.18 m
12α	2.92 td (16.3, 4.0)	2.95 m	2.84 td (12.8, 4.3)	2.70 m	2.68 m
12β	2.70 dt (16.3,2.7)	2.71 m	2.68 m	2.70 m	2.68 m
15			7.05 d (8.1)	7.00 d (8.0)	7.01 d (8.0)
16	6.57 d (1.4)	6.54 d (1.5)	6.92 brd (8.1)	6.91 brd (8.0)	6.88 brd (8.0)
18	6.76 d (1.4)	6.77 d (1.5)	6.82 brs	6.81 brs	6.78 brs
19	1.31 s	1.288 s	1.46 s	1.19 s	1.12 s
20	2.60 m	2.60 q (6.8)	2.68 m	2.66 m	2.59 m
21	1.13 d (7.1)	1.13 d (7.1)	1.16 d (7.2)	1.18 d (7.5)	1.13 d (5.6)
22	3.89 ddd (11.3, 6.3, 2.4)	3.89 ddd (11.3, 6.3, 2.2)	3.78 ddd (11.3, 5.8, 2.5)	3.79 ddd (10.9, 5.8, 2.5)	3.93 ddd (11.5, 6.2, 2.2)
23a	1.81 m	1.81 m	1.77 dd (14.4, 2.3)	1.77 m	1.81 m
23b	1.53 m	1.51 m	1.51 dd (14.4, 11.2)	1.50 dd (14.2, 11.4)	1.48 m
26	6.01 s	6.00 s	4.91 d (9.9)	4.91 d (5.6)	6.00 s
27	1.25 s	1.25 s	1.31 s	1.31 s	1.24 s
28	1.30 s	1.294 s	1.29 s	1.29 s	1.28 s
OH-26			3.41 d (9.9)	3.50 m	
OH-1					2.63 d (11.9)
CH_3CO-2					2.04 s
CH_3CO-3					2.02 s
CH_3CO-6		2.09 s			
CH_3CO-15	2.22 s	2.15 s			
CH_3CO-25					
CH_3CO-26	1.98 s	1.99 s			1.98 s

[a]Chemical shifts (δ) downfield from TMS. J couplings (in parentheses) in Hz. [b]Run at 400.13 MHz. [c]Run at 500.13 MHz.

hydroxy group at C-7, with a H-7/H-6 dihedral angle near 90° and a H-7/H-8 dihedral angle near 160°. Accordingly, metabolite **7** was assigned as the 7β-hydroxy derivative of salpichrolide A (**1**). Finally, the last metabolite isolated (**8**) gave a molecular formula of $C_{28}H_{34}O_6$ by HRESITOFMS, confirming the insertion of an oxygen atom into substrate **1** by the microorganism. The ^{1}H NMR spectrum of **8** was consistent with the typical 5α,6β-diol system of salpichrolide C (**2**)[5] (Table 1). In addition, it showed a double doublet at δ 6.40 corresponding to an olefinic proton, which showed a correlation with the H-6 signal (δ 3.98) in the COSY experiment. In the ^{13}C NMR spectrum (Table 2), two vinyl carbons were evident at δ 114.9 and 131.5, suggesting a double bond at the C-7−C-8 positions. The resonances at δ 77.3 (C-5) and δ 73.0 (C-6) were consistent with a 5α,6β-diol system. Therefore, the structure of compound **8** was determined as the 7-ene derivative of salpichrolide C (**2**).

Biotransformation of salpichrolide C (**2**) with *R. miehei* for 7 days resulted in the recovery of 32% of substrate (**2**) and a mixture of polar metabolites that was acetylated to give **9** (25.0 mg, 8%) and **10** (4.4 mg, 1.4%) (Figure 1). The spectroscopic data of **9** were identical to those of a diacetoxy derivative obtained from salpichrolide A (**1**) by treatment with aqueous sulfuric acid in THF and subsequent acetylation.[7] The molecular formula of compound **10**, $C_{34}H_{42}O_{10}$, indicated that this compound is a triacetoxy derivative. Inspection of the 1D and 2D NMR spectroscopic data (Tables 1 and 2) indicated that compound **10** is related closely to those of the diacetoxy derivative **9**. The location of the third acetyl group (δ_H 1.99, δ_C 172.9, and δ_C 22.2) was established from the resonance shift of C-25 from δ 76.0 for compound **9** to δ 89.0 for compound **10**. The correlations observed in the HMBC spectrum for H-26 (δ 5.58), H_2-23 (δ 1.51), and H_3-27 (δ 1.39) with C-25 were used to confirm this inference (see Supporting Information). The configurations of C-24, C-25, and C-26 were determined as 24*R*, 25*S*, and 26*R* by the cross-correlation peaks observed between H-22 (δ 3.67) and the resonance corresponding to H-26 and H_3-28 (δ 1.27) in the NOESY experiment (see Supporting Information). The change of the configuration at C-26 of the compounds obtained by biotransformation of **9** and **10** was because the 24,25-hydroxy lactol moiety in the side-chain exists as a mixture of epimers at C-26 and the acetylation of this mixture produces only the acetylated derivative 26*R*.[7] Thus, compound **10** was assigned as the 25-acetoxy derivative of **9**. Therefore, the microorganism produced the side-chain epoxide opening of salpichrolide C (**2**).

Biotransformation of salpichrolide G (**3**) with *R. miehei* for 7 days resulted in recovery of 15% of substrate (**3**) and a mixture

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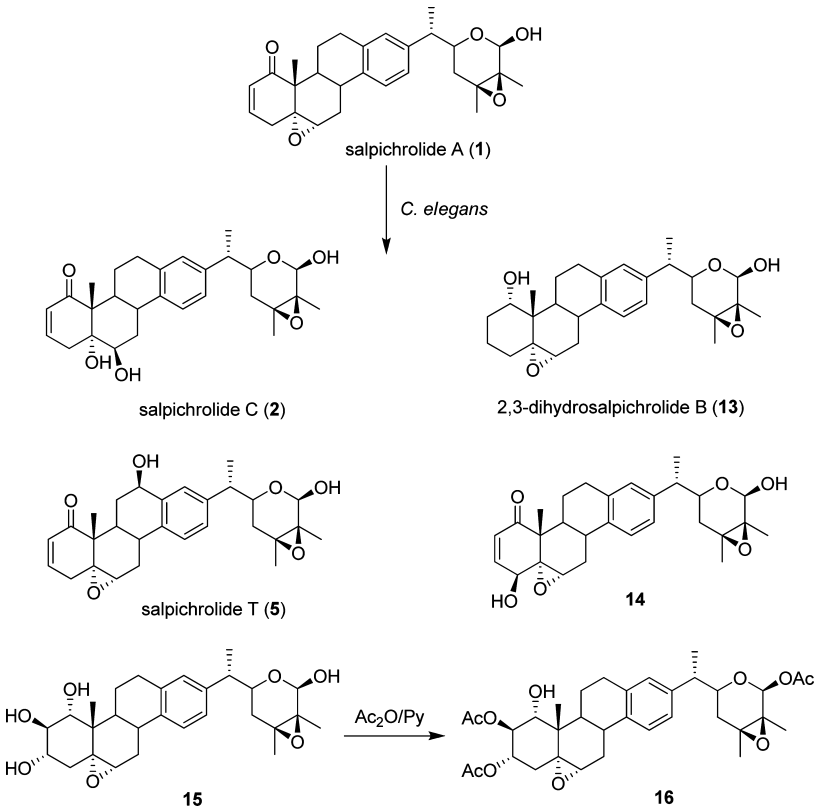

Figure 2. Derivatives obtained by biotransformation of salpichrolide A (**1**) with *Cunninghamella elegans*.

of polar metabolites that was acetylated to give compounds **11** (5.6 mg, 3%) and **12** (5.2 mg, 2.7%) (Figure 1). The molecular formula of compound **11** ($C_{32}H_{40}O_9$) was compatible with the initial insertion of an oxygen atom and two hydrogen atoms on substrate **3** by the microorganism; subsequently, a diacetoxy derivative was originated by acetylation. The NMR spectroscopic data of **11** (Tables 2 and 3) showed a typical 1-oxo-2-ene-5α,6β-dihydroxy system in rings A and B, indicating opening of the epoxide at the C-5−C-6 positions.[5] The two acetyl groups were evident from the signals at δ 2.22 and 1.98 in the ^{1}H NMR spectrum and the signals at δ 21.5, 21.6, 169.4, and 170.7 in the ^{13}C NMR spectrum. The location of acetyl groups was established at C-15 and C-26 by the resonance shift of C-15 from δ 154.9 for salpichrolide G (**3**) to δ 150.0 for compound **11** and that for H-26 from δ 5.00 for **3** to δ 6.01 for compound **11**. The acetyl group at C-26 was confirmed by the cross-correlation peak between the signal at δ 1.98 and the signal corresponding to C-26 at δ 92.0 in the HMBC spectrum. The molecular formula of compound **12** ($C_{34}H_{42}O_{10}$) indicated that this compound is a triacetoxy derivative. Its ^{1}H and ^{13}C NMR spectra showed signals similar to those of compound **11** (Tables 2 and 3). The main differences observed between compounds **11** and **12** were an additional singlet signal at δ 2.09 assigned to an acetoxy group and the deshielding of the signals corresponding to the C-6 position from δ_H 3.67 and δ_C 75.7 for compound **11** to δ_H 4.80 and δ_C 76.5 for compound **12**. Accordingly, compound **12** was determined as the 6-acetoxy derivative of compound **11**.

Biotransformation of salpichrolide A (**1**) with *C. elegans* for 7 days resulted in recovery of 4.6% of substrate **1** and three derivatives for which the spectroscopic data were found to be identical to those of salpichrolide C (**2**),[5] salpichrolide T (**5**),[9] and 2,3-dihydrosalpichrolide B (**13**).[12] The metabolites **14** (0.3%) and **15** (2%) were also obtained (Figure 2). The molecular formula of compound **14** ($C_{28}H_{34}O_6$) confirmed that the microorganism had inserted an oxygen atom into the molecule of substrate **1**. Its ^{1}H NMR spectrum showed characteristic olefinic proton signals of H-2 and H-3 at δ 5.96 (d, J = 10.1 Hz) and δ 6.66 (dd, J = 10.1, 4.5 Hz), respectively, and the signal corresponding to a carbynolic proton at δ 3.66 (d, J = 4.5 Hz) (Table 3). The correlation found between H-3 and the signal at δ 3.66 in the COSY spectrum suggested that the hydroxy group is located at C-4. This assumption was confirmed by the HMBC correlation between the signal of H-4 and C-2 at δ 129.4, C-3 at δ 141.9, C-5 at δ 64.3, and C-10 at δ 47.9 and between the signal of H-3 and C-10 and C-4 at δ 72.5 (see Supporting Information). The orientation of the hydroxy group at C-4 was determined by comparison of the multiplicity and coupling constant value between the H-4 signal and those of withangulatin G, with a 1-oxo-2-en-4β-hydroxy-5α,6β-dihydroxy system in the A/B rings.[24] Thus, metabolite **14** was found to be the 4β-hydroxy derivative of salpichrolide A (**1**). The HRESITOFMS of compound **15** showed a molecular ion corresponding to a molecular formula of $C_{28}H_{38}O_7$, indicating the presence of two oxygen atoms more than in substrate **1**. The ^{1}H NMR spectrum of metabolite **15** showed the absence of the olefinic resonances at high-frequency shifts, with a signal at δ 3.79 assigned to H-22, a signal at δ 4.91 assigned at H-26, and three broad singlet signals at δ 3.83, 4.25, and 4.07 corresponding to three additional carbynolic protons. In the HSQC spectrum, these signals correlated with the signals at δ_C 75.6, 74.3, and 72.6, respectively, confirming the presence of three oxygenated secondary carbons in ring A. To facilitate the structural elucidation of compound **15**, this was acetylated to give the triacetyl derivative **16**. The ^{1}H NMR spectrum of **16** exhibited three singlet signals of acetoxy groups at δ 2.04, 2.02,

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and 1.98 and three signals of deshielded carbynolic protons at δ 6.00 assigned to H-26, δ 5.22, and δ 5.01. The signal at δ 3.82 was assigned to H-1 by the cross-correlation peaks observed between the signal at δ 3.82 and the signals at δ 73.2 (C-2), δ 62.1 (C-5), δ 39.8 (C-10), and δ 15.5 (C-19) in the HMBC experiment. A detailed examination of the ^{1}H−^{1}H COSY spectrum of compound **16** led to the identification of the spin system XABCX$_2$, consisting of OH-1 (δ 2.63, d, J = 11.9 Hz), H-1 (δ 3.82, brd, J = 11.9 Hz), H-2 (δ 5.22, brs), H-3 (δ 5.01, brs), and H$_2$-4 (δ 2.51, dd, J = 15.4, 3.7 Hz and δ 1.43, brd, J = 15.4 Hz), suggesting the presence of a 1-hydroxy-2,3-diacetoxy system in ring A. This assumption was confirmed by the HMBC spectrum (see Supporting Information). The orientation of the C-1, C-2, and C-3 substituents was determined by the analysis of the coupling constants and by a NOESY experiment. The NOE observed between H-1 and H-2, H-11α (δ 1.81), and H$_3$-19 (δ 1.12) indicated an α-orientation of the hydroxy group at C-1. The multiplicity of the signals assigned to H-2 (δ 5.22, brs) and H-3 (δ 5.01, brs) was consistent with a β-orientation of the acetoxy group at C-2 and an α-orientation of the acetoxy group at C-3 for the most stable conformer of the proposed structure (MMFF94 force field in Macromodel). This assumption was confirmed by the relevant correlations observed in the NOESY spectrum of H-2 with H-1, H-3, and OH-1 and of H-3 with H-2, H-4α, and H-4β (see Supporting Information).Thus, compound **16** was assigned as the 2β,3α,26-triacetoxy derivative of metabolite **15** isolated from biotransformation of salpichrolide A (**1**) with *C. elegans*. Accordingly, this metabolite **15** was elucidated as the 1α,2β,3α-trihydroxy derivative of **1**.

Biotransformation of salpichrolide A (**1**) with *C. lunata* for 7 days resulted in recovery of 24% of the substrate and the isolation of two metabolites, for which the spectroscopic data were found to be identical to those of salpichrolide C (**2**, 6.4%) and salpichrolide M[8] (**17**, 1.3%).

The incubation times in all biotransformations were established based on the control of the reactions by TLC. The full and unambiguous proton and carbon NMR assignments for all compounds were confirmed using combinations of COSY, HSQC, HMBC, and NOESY experiments. High-resolution mass spectrometric measurements were in agreement with the structures proposed.

R. miehei and *C. elegans* are two filamentous fungi that have been used for biohydroxylation of diterpene and triterpene substrates.[25−27] Hence, the formation of products **5**, **7**, and **14** most likely would involve P-450 cytochrome monooxygenase found in *R. miehei* and *C. elegans*, and it is probably responsible for biohydroxylation of substrate **1** in C-1, C-4, and C-7 (Figure 4). In the literature, extensive reference is made to the fact that filamentous fungi, as well as conducting biohydroxylations, can act as "enzyme bags", catalyzing a variety of transformations due to the presence of dehydrogenases, oxidases, reductases, or epoxide hydrolase, among other enzymes. Thus, metabolite **4** may be formed from **5** by oxidation, through the action of an oxidase in *R. miehei*. An oxidation reaction of alcohols has been reported with whole cells of *R. miehei*.[28,29] With other filamentous fungi, regioselective oxidation of the hydroxy group has also been found in one of the hydroxy groups of *ent*-manoyl oxides.[30,31] The reduction of the keto group in derivative **4**, by action of a dehydrogenase present in *R. miehei*, would lead to compound **6** with the *S*-configuration for the 12-hydroxy derivative obtained (Figure 4). Most reductions carried out with yeasts and many microorganisms are consistent with

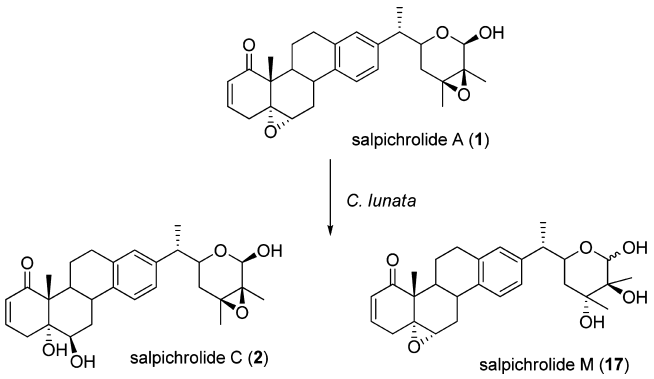

Figure 3. Derivatives obtained by biotransformation of salpichrolide A (**1**) with *Curvularia lunata*.

Prelog's rule.[32] Hydrogen transfer occurs from the *re* face of a prochiral ketone, yielding *S*-configurated alcohols.[33−35]

The action of epoxide hydrolases present in *R. miehei*, *C. elegans*, and *C. lunata* could account for the stereoselective opening of epoxy groups in substrates **1**−**3** to give dihydroxy derivatives **2**, **9**, **11**, and **17** (Figure 5).

The α,β-unsaturated ketones are good substrates for ene-reductases, giving dihydro ketones.[36−38] Thus, reduction of the double bond of an α,β-unsaturated system in substrate **1** catalyzed by an ene-reductase in *C. elegans* would lead to a 2,3-dihydro-1-oxo derivative that would later be reduced by an alcohol dehydrogenase from *C. elegans* to give metabolite **13** (Figure 6). The reduction of the double bond can compete with reduction of the carbonyl group, giving, in this case, the *S*-configured allyl alcohol at C-1. Enzymatic epoxidation of this alcohol and its subsequent opening would give trihydroxy derivative **15** (Figure 6). Epoxidation and formation of dihydroxy derivatives from the double bonds of diterpene substrates have been observed in some biotransformations with *C. elegans* and other filamentous fungi.[27,39−42]

The results obtained showed that the application of biotransformation through fungi is an efficient tool to obtain structural diversity on relatively poorly reactive metabolites such as withanolides.[3] The derivatives obtained are particularly valuable not only for their biological potential but for the fact that functionalities such as hydroxy and carbonyl groups have the ability to access a greater number and larger variety of further derivatives.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO P-1010 polarimeter. UV spectra were obtained using a Shimadzu-260 spectrophotometer, and IR spectra were produced using a Nicolet 5-SXC spectrophotometer. NMR spectra were recorded on a Bruker AVANCE II AV-400 NMR spectrometer operating at 400.13 MHz for ^{1}H and 100.63 MHz for ^{13}C or a Varian direct drive (499.79 and 600.24 MHz for ^{1}H and 125.68 and 150.95 MHz Inova operating at 500.13 MHz for ^{1}H and 125.03 MHz for ^{13}C) NMR spectrometer, while 2D spectra (COSY, HSQC, HMBC, and NOESY) were obtained using standard software. Chemical shifts are given in ppm (δ) downfield from the tetramethylsilane (TMS) internal standard. HRESIQTOFMS were determined on a Micro TOF II Bruker Daltonics or a Waters model LCT Premier XE instrument. Conformational searches were performed using the MMFF94 force field in Macromodel with a 5 kcal/mol energy window, a chloroform solvent model, and a dielectric constant of 1.0, taking the electrical charges from the parameters of the force field, and using the mixed torsional/low-mode sampling method. The chromatographic separa-

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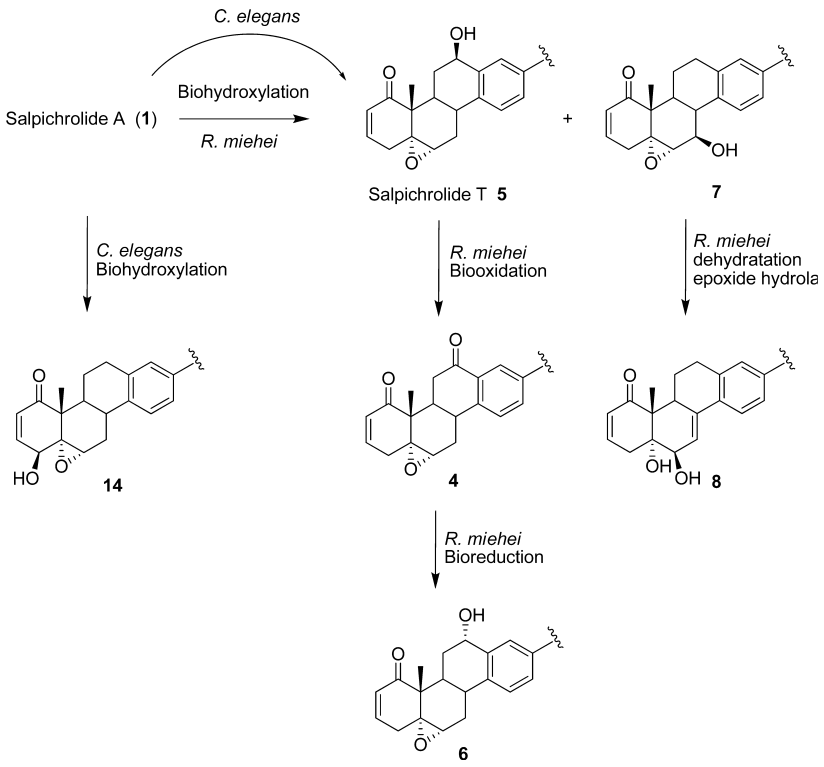

Figure 4. Possible microbial pathway to obtain metabolites **4**–**8** and **14** from salpichrolide A (**1**).

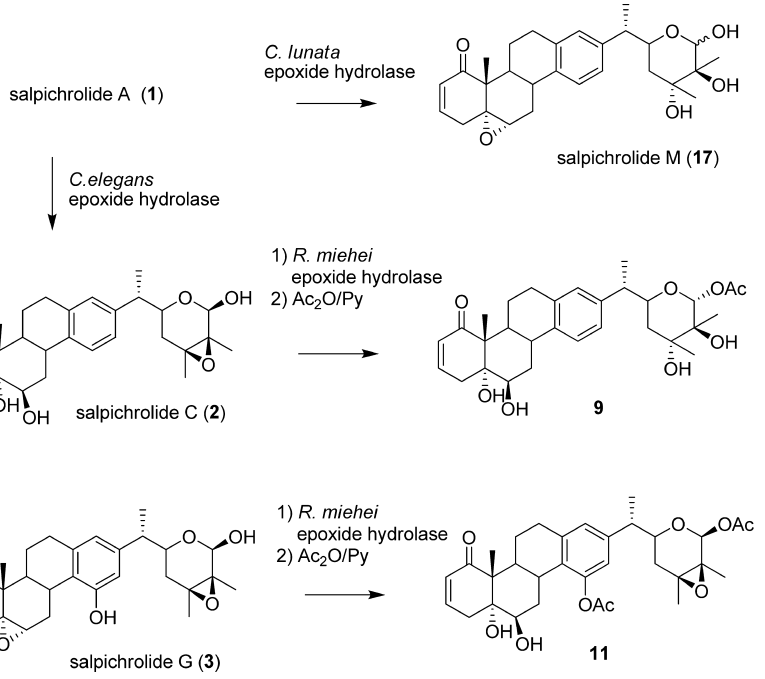

Figure 5. Possible microbial pathway to obtain metabolites **2**, **9**, **11**, and **17**.

tions were performed by column chromatography on silica gel 60 (0.063–0.200 mm) and Sephadex LH-20, and preparative TLC was carried out using silica gel 60 F_{245} (0.2 nm thick) plates.

Plant Material. *Salpichroa origanifolia* (Lam.) Baillon was collected in Córdoba, Argentina, in June 2013. A voucher specimen has been deposited at the Museo Botánico Córdoba, Universidad Nacional de Córdoba, under CORD 52365. The plant material was identified by Prof. Gloria E. Barboza (IMBIV-CONICET, Córdoba, Argentina).

Extraction and Isolation. The fresh plants of *S. origanifolia* (ca. 5.0 kg) were triturated with EtOH at room temperature immediately after collection. The residue obtained after solvent evaporation was partitioned with hexane–EtOH–H_2O (10:3:1), and the aqueous EtOH phase was concentrated, with the resulting aqueous phase extracted with CH_2Cl_2. The CH_2Cl_2 extract was dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness at reduced pressure. The residue obtained (5.3 g) was fractionated initially by vacuum-liquid chromatography. Elution with hexane–EtOAc mixtures of increasing polarity (80:20 to 0:100) afforded four fractions containing withanolides. These fractions were pooled (3.8 g) and further chromatographed on Si gel. Elution with CH_2Cl_2–MeOH (100:0 to 90:10)

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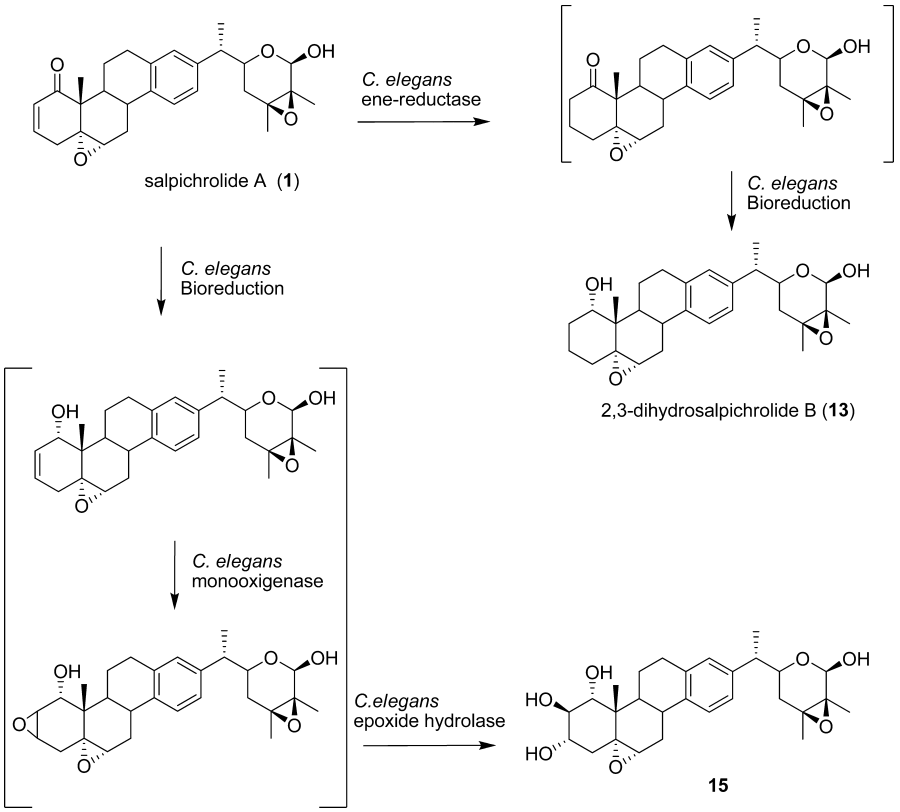

Figure 6. Possible microbial pathway to obtain metabolites **13** and **15** from salpichrolide A (**1**).

afforded (in order of elution) salpichrolide A (**1**, 1.8 g), salpichrolide G (**3**, 0.3 g), and salpichrolide C (**2**, 0.8 g). The physical and spectoscopic data were in agreement with those reported for these compounds.[4,5,7]

Microorganisms and Culture Medium. *Rhizomucor miehei* (CECT 2749), *Cunninghamella elegans* (CECT 2113), and *Curvularia lunata* (CECT 2131) were obtained from the Spanish Type Culture Collection (CECT), Departamento de Microbiología, Universidad de Valencia, Spain. In all microbial transformation experiments, a medium of potato dextrose broth (Scharlau 02-483) was used for microorganism proliferation. Erlenmeyer flasks (250 mL) containing 90 mL of sterilized medium were inoculated with 1 mL of microorganism suspended in saline solution (9%). Incubations were maintained at 28 °C with gyratory shaking (150 rpm) for 6 days, after which substrates (5−10%) in EtOH were added.

Recovery and Purification of Metabolites. Cultures were filtered and pooled, cells were washed thoroughly with water, and the liquid was saturated with NaCl and extracted twice with CH_2Cl_2. Dried fungal cells were washed repeatedly with CH_2Cl_2. Both extracts were pooled, dried with dry Na_2SO_4, and evaporated under reduced pressure. The resulting mixture of compounds was chromatographed on a silica gel column and further purified by preparative TLC.

Biotransformation of Salpichrolide A (1) by *Rhizomucor miehei*. Salpichrolide A (**1**, 400 mg) was dissolved in EtOH (8 mL), distributed among eight Erlenmeyer-flask cultures (*R. miehei*), and incubated for 7 days in a shaker at room temperature, after which the cultures were processed as indicated above to obtain starting material **1** (18.5 mg, 4.6%), **4** (1.3 mg, 0.3%), salpichrolide T[9] (**5**, 16.6 mg 4%), **6** (8.1 mg, 2%), **7** (3.4 mg 0.8%), and **8** (4.9 mg, 1.2%).

[(20S,22R,24S,25S,26R)-5α,6α:22,26:24,25-Triepoxy-26-hydroxy-17(13→18)abeo-5α-ergosta-2,13,15,17-tetraene-1,12-dione] ***(4):*** white, amorphous solid; $[\alpha]^{21}_D$ +24.3 (*c* 0.11, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 212 (3.58) nm, 252 (3.11) nm; IR (dried film) ν_{max} 2927, 1686, 1031, 675 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESITOFMS *m*/*z* [M + Na]$^+$ 487.2103 (calcd for $C_{28}H_{32}O_6Na$ 487.2091).

[(20S,22R,24S,25S,26R)-5α,6α:22,26:24,25-Triepoxy-12α,26-dihydroxy-17(13→18)abeo-5α-ergosta-2,13,15,17-tetraen-1-one] ***(6):*** white, amorphous solid; $[\alpha]^{21}_D$ +19.5 (*c* 0.62, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 218 (3.88) nm; IR (dried film) ν_{max} 3443, 2927, 1686, 1031, 738 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESITOFMS *m*/*z* [M + Na]$^+$ 489.2255 (calcd for $C_{28}H_{34}O_6Na$ 489.2248).

[(20S,22R,24S,25S,26R)-5α,6α:22,26:24,25-Triepoxy-7β-26-dihydroxy-17(13→18)abeo-5α-ergosta-2,13,15,17-tetraen-1-one] ***(7):*** white, amorphous solid; $[\alpha]^{21}_D$ +7.7 (*c* 0.51, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 218 (3.65) nm; IR (dried film) ν_{max} 3439, 2927, 1681, 1031, 733 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESITOFMS *m*/*z* [M + Na]$^+$ 489.2248 (calcd for $C_{28}H_{34}O_6Na$ 489.2248).

[(20S,22R,24S,25S,26R)-22,26:24,25-Diepoxy-5α,6β,26-trihydroxy-17(13→18)abeo-5α-ergosta-2,7,13,15,17-pentaen-1-one] ***(8):*** white, amorphous solid; $[\alpha]^{21}_D$ −23.4 (*c* 0.31, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 217 (4.00) nm, 262 (3.81) nm; IR (dried film) ν_{max} 3430, 2932, 1681, 1031, 742 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESITOFMS *m*/*z* [M + Na]$^+$ 489.2266 (calcd for $C_{28}H_{34}O_6Na$ 489.2248).

Biotransformation of Salpichrolide C (2) by *Rhizomucor miehei*. Salpichrolide C (**2**, 250 mg) was dissolved in EtOH (5 mL), distributed among three Erlenmeyer-flask cultures (*R. miehei*), and incubated for 7 days in a shaker at room temperature, after which the cultures were processed as indicated above to obtain salpichrolide C (**2**, 81.4 mg, 32%) and a mixture of polar metabolites, which was acetylated with pyridine (3 mL) and Ac_2O (1.5 mL) for 12 h under darkness at 25 °C to give **9** (25.0 mg, 8%)[7] and **10** (4.4 mg, 1.4%).

[(20S,22R,24R,25S,26R)-6β,26-Diacetoxy-22,26-epoxy-5α,24,25-trihydroxy-17(13→18)abeo-5α-ergosta-2,13,15,17-tetraen-1-one] ***(9):*** white, amorphous solid; $[\alpha]^{21}_D$ −8.4 (*c* 1.44, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 219 (3.90) nm; IR (dried film) ν_{max} 3466, 2932, 1739, 1681, 1378, 1054, 738 cm^{-1}; HRESITOFMS *m*/*z* [M + Na]$^+$ 591.2565 (calcd for $C_{32}H_{40}O_9Na$ 591.2565).

[(20S,22R,24R,25S,26R)-6β,25,26-Diacetoxy-22,26-epoxy-5α,24-dihydroxy-17(13→18)abeo-5α-ergosta-2,13,15,17-tetraen-1-one]

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(10): white, amorphous solid; $[\alpha]^{21}_{D}$ +8.1 (*c* 0.57, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 220 (4.04) nm; IR (dried film) ν_{max} 3439, 2932, 1739, 1686, 1383, 1241, 1058, 742 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; HRESITOFMS *m*/*z* $[M + Na]^+$ 633.2673 (calcd for $C_{34}H_{42}O_{10}Na$ 633.2670).

Biotransformation of Salpichrolide G (3) by *Rhizomucor miehei*. Salpichrolide G (**3**, 150 mg) was dissolved in EtOH (3 mL), distributed among three Erlenmeyer-flask cultures (*R. miehei*), and incubated for 7 days in a shaker at room temperature, after which the cultures were processed as indicated above to obtain salpichrolide G (**3**, 23.0 mg, 15%) and mixture of polar metabolites, which was acetylated with pyridine (3 mL) and Ac_2O (1.5 mL) for 12 h under darkness at 25 °C to give **11** (5.6 mg, 3%) and **12** (5.2 mg, 2.7%).

[(20S,22R,24S,25S,26R)-15,26-Diacetoxy,22,26:24,25-diepoxy-5α,6β-dihydroxy-17(13→18)abeo-5α-ergosta-2,7,13,15,17-pentaen-1-one] (11): white, amorphous solid; $[\alpha]^{21}_{D}$ −45.1 (*c* 0.3, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 217 (3.90) nm; IR (dried film) ν_{max} 3501, 2932, 1743, 1681, 1378, 1218, 733 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; HRESITOFMS *m*/*z* $[M + Na]^+$ 591.2573 (calcd for $C_{32}H_{40}O_9Na$ 591.2565).

[(20S,22R,24S,25S,26R)-6,15,26-Triacetoxy,22,26:24,25-diepoxy-5α-hydroxy-17(13→18)abeo-5α-ergosta-2,7,13,15,17-pentaen-1-one] (12): white, amorphous solid; $[\alpha]^{21}_{D}$ −52.3 (*c* 0.3, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 217 (3.95) nm; IR (dried film) ν_{max} 3488, 2927, 1743, 1681, 1370, 1249, 738 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; HRESITOFMS *m*/*z* $[M + Na]^+$ 633.2691 (calcd for $C_{34}H_{42}O_{10}Na$ 633.2670).

Biotransformation of Salpichrolide A (1) by *Cunninghamella elegans*. Salpichrolide A (**1**, 300 mg) was dissolved in EtOH (6 mL), distributed among six Erlenmeyer-flask cultures (*C. elegans*), and incubated for 7 days in a shaker at room temperature, after which the cultures were processed as indicated above to obtain starting material **1** (34.7 mg, 11.6%), salpichrolide C[5] (**2**, 5.0 mg), salpichrolide T[9] (**5**, 12.1 mg), 2,3-dihydrosalpichrolide B[12] (**13**, 7.4 mg), **14** (17.3 mg), and **15** (9.7 mg).

[(20S,22R,24S,25S,26R)-5α,6α:22,26:24,25-Triepoxy-4β,26-dihydroxy-17(13→18)abeo-5α-ergosta-2,13,15,17-tetraen-1-one] (14): white, amorphous solid; $[\alpha]^{21}_{D}$ +9.5 (*c* 1.19, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 214 (3.94) nm; IR (dried film) ν_{max} 3506, 2932, 1694, 1272, 1036, 742 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; HRESITOFMS *m*/*z* $[M + Na]^+$ 489.2245 (calcd for $C_{28}H_{34}O_6Na$ 489.2253).

[(20S,22R,24S,25S,26R)-5α,6α:22,26:24,25-Triepoxy-1α,2α,3β,26-tetrahydroxy-17(13→18)abeo-5α-ergosta-13,15,17-triene] (15): white, amorphous solid; $[\alpha]^{21}_{D}$ −59.4 (*c* 0.73, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 214 (3.74) nm; IR (dried film) ν_{max} 3415, 2932, 1681, 1031, 762 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; HRESITOFMS *m*/*z* $[M + Na]^+$ 509.2507 (calcd for $C_{28}H_{38}O_7Na$ 509.2515).

Acetylation of 15. Metabolite **15** (9.7 mg) was dissolved in pyridine (1 mL) and Ac_2O (0.5 mL). The reaction was maintained for 12 h under darkness at 25 °C and extracted in the usual way. The solvent was evaporated to give a mixture of compounds, which was chromatographed by preparative TLC to afford **16** (5.2 mg).

[(20S,22R,24S,25S,26R)-2α,3β,26-Triacetoxy-5α,6α:22,26:24,25-triepoxy-1α-hydroxy-17(13→18)abeo-5α-ergosta-13,15,17-triene] (16): white, amorphous solid; $[\alpha]^{21}_{D}$ −52.6 (*c* 0.51; CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 216 (3.78) nm; IR (dried film) ν_{max} 2936, 1748, 1378, 1227, 1120, 1045, 760 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; HRESITOFMS *m*/*z* $[M + Na]^+$ 635.2831 (calcd for $C_{34}H_{44}O_{10}Na$ 635.2832).

Biotransformation of Salpichrolide A (1) by *Curvularia lunata*. Salpichrolide A (**1**, 300 mg) was dissolved in EtOH (6 mL), distributed among six Erlenmeyer-flask cultures (*C. lunata*), and incubated for 7 days in a shaker at room temperature, after which the cultures were processed as indicated above to obtain starting material **1** (72 mg, 24%), salpichrolide C (**2**, 20 mg, 6.4%), and salpichrolide M[8] (**17**, 4 mg, 1.3%).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.6b00310.

^1H and ^{13}C NMR spectra of new compounds, relevant HMBC correlations of compounds **4**, **6**, **10**, **14**, and **16**, relevant NOE correlations of compounds **10** and **16**, and most stable conformers for rings A−D of 1α-hydroxy-2β,3α-diacetoxy and 1α-hydroxy-2α,3β-diacetoxy epimers (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Ray, A. B.; Gupta, M. In *Progress in the Chemistry of Organic Natural Products*; Herz, W.; Kirby, G. W.; Moore, R. E.; Steglich, W.; Tamm, Ch., Eds.; Springer-Verlag: Vienna, 1994; Vol. *63*, pp 1−106.

(2) Chen, L.; He, H.; Qiu, F. *Nat. Prod. Rep.* **2011**, *28*, 705−740.

(3) Misico, R. I.; Nicotra, V.; Oberti, J. C.; Barboza, G.; Gil, R. R.; Burton, G. In *Progress in the Chemistry of Organic Natural Products*; Kinghorn, A. D.; Falk, H.; Kobayashi, J., Eds.; Springer-Verlag: Vienna, 2011; Vol. *94*, pp 127−229.

(4) Veleiro, A. S.; Oberti, J. C.; Burton, G. *Phytochemistry* **1992**, *31*, 935−937.

(5) Veleiro, A. S.; Burton, G.; Bonetto, G. M.; Gil, R. R.; Oberti, J. C. *J. Nat. Prod.* **1994**, *57*, 1741−1745.

(6) Tettamanzi, M. C.; Veleiro, A. S.; Oberti, J. C.; Burton, G. *Phytochemistry* **1996**, *43*, 461−463.

(7) Tettamanzi, M. C.; Veleiro, A. S.; Oberti, J. C.; Burton, G. *J. Nat. Prod.* **1998**, *61*, 338−342.

(8) Tettamanzi, M. C.; Veleiro, A. S.; de la Fuente, J. R.; Burton, G. *J. Nat. Prod.* **2001**, *64*, 783−786.

(9) Nicotra, V. E.; Basso, A. V.; Ramacciotti, N. S.; Misico, R. I. *J. Nat. Prod.* **2013**, *76*, 2219−2225.

(10) Mareggiani, G.; Picollo, M. I.; Zerba, E.; Burton, G.; Tettamanzi, M. C.; Benedetti-Doctorovich, M. O. V.; Veleiro, A. S. *J. Nat. Prod.* **2000**, *63*, 1113−1116.

(11) Mareggiani, G.; Picollo, M. I.; Veleiro, A. S.; Tettamanzi, M. C.; Benedetti-Doctorovich, M. O. V.; Burton, G.; Zerba, E. *J. Agric. Food Chem.* **2002**, *50*, 104−107.

(12) Bado, S.; Mareggiani, G.; Amiano, N.; Burton, G.; Veleiro, A. S. *J. Agric. Food Chem.* **2004**, *52*, 2875−2878.

(13) Misico, R. I.; Song, L. L.; Veleiro, A. S.; Cirigliano, A. M.; Tettamanzi, M. C.; Burton, G.; Bonetto, G. M.; Nicotra, V. E.; Silva, G. L.; Gil, R. R.; Oberti, J. C.; Kinghorn, A. D.; Pezzuto, J. M. *J. Nat. Prod.* **2002**, *65*, 677−680.

(14) Machin, R. P.; Veleiro, A. S.; Nicotra, V. E.; Oberti, J. C.; Padrón, J. M. *J. Nat. Prod.* **2010**, *73*, 966−968.

(15) Nighat, S.; Zafar Saeed, S. *J. Enzyme Inhib. Med. Chem.* **2013**, *28*, 1113−1128.

DOI: 10.1021/acs.jnatprod.6b00310
J. Nat. Prod. XXXX, XXX, XXX−XXX

(16) Donova, M. V.; Egorova, O. V. *Appl. Microbiol. Biotechnol.* **2012**, *94*, 1423−1447.
(17) Parra, A.; Rivas, F.; García-Granados, A.; Martínez, A. *Mini-Rev. Org. Chem.* **2009**, *6*, 307−320.
(18) Simeo, Y.; Sinisterra, J. V. *Mini-Rev. Org. Chem.* **2009**, *6*, 128−134.
(19) García-Granados, A.; Martínez, A.; Parra, A.; Rivas, F. *Curr. Org. Chem.* **2007**, *11*, 679−692.
(20) Fernandes, P.; Cruz, A.; Angelova, B.; Pinheiro, H. M.; Cabral, J. M. S. *Enzyme Microb. Technol.* **2003**, *32*, 688−705.
(21) Gustafson, M. E.; Nicholas, A. W.; Rosazza, J. P. *J. Chromatogr.* **1977**, *137*, 465−457.
(22) Fuska, J.; Prousek, J.; Rosazza, J.; Budesinsky, M. *Steroids* **1982**, *40*, 157−169.
(23) Wijeratne, E. M. K.; Xu, Y.; Marron, M. T.; Whitesell, L.; Gunatilaka, A. A. L. *42nd Western Regional Meeting of the American Chemical Society*; Las Vegas 2008; p WRM-221.
(24) Damu, A. G.; Kuo, P.-C.; Su, C.-R.; Kuo, T.-H.; Chen, T.-H.; Bastow, K. F.; Lee, K.-H.; Wu, T.-S. *J. Nat. Prod.* **2007**, *70*, 1146−1152.
(25) Martínez, A.; Perojil, A.; Rivas, F.; Parra, A.; García-Granados, A.; Fernández-Vivas, A. *Phytochemistry* **2015**, *117*, 500−508.
(26) Martínez, A.; Rivas, F.; Perojil, A.; Parra, A.; García-Granados, A.; Fernández-Vivas, A. *Phytochemistry* **2013**, *94*, 229−237.
(27) García-Granados, A.; Liñán, E.; Martínez, A.; Onorato, M. E.; Arias, J. M. *J. Nat. Prod.* **1995**, *58*, 1695−1701.
(28) García-Burgos, C.; Carballeira, J. D.; Sinisterra, J. V. *J. Mol. Catal. B: Enzym.* **2006**, *41*, 16−20.
(29) Cano, A.; Ramírez-Apan, M. T.; Delgado, G. *J. Braz. Chem. Soc.* **2011**, *22*, 1177−1182.
(30) García-Granados, A.; Fernandez, A.; Gutiérrez, A.; Martínez, A.; Quirós, R.; Rivas, F.; Arias, J. M. *Phytochemistry* **2004**, *65*, 107−115.
(31) García-Granados, A.; Liñán, E.; Martínez, A.; Rivas, F.; Arias, J. M. *Phytochemistry* **1995**, *38*, 1237−1244.
(32) Prelog, V. *Pure Appl. Chem.* **1964**, *9*, 119−130.
(33) García-Granados, A.; Jiménez, M. B.; Martínez, A.; Parra, A.; Rivas, F.; Arias, J. M. *Phytochemistry* **1994**, *37*, 741−747.
(34) García-Granados, A.; Martínez, A.; Quirós, R.; Extremera, A. *Tetrahedron* **1999**, *55*, 8567−8578.
(35) Fraga, M.; González, P.; Hernández, M. G.; Suárez, S. *Tetrahedron* **1999**, *55*, 1781−1792.
(36) Romagnolo, A.; Spina, F.; Brenna, E.; Crotti, M.; Parmeggiani, F.; Varese, G. C. *Fungal Biol.* **2015**, *119*, 487−493.
(37) Toogood, H. S.; Knaus, T.; Scrutton, N. S. *ChemCatChem* **2014**, *6*, 951−954.
(38) Winkler, C. K.; Tasnádi, G.; Clay, D.; Hall, M.; Faber, K. *J. Biotechnol.* **2012**, *162*, 381−389.
(39) García-Granados, A.; Liñán, E.; Martínez, A.; Rivas, F.; Arias, J. M. *Phytochemistry* **1995**, *38*, 1237−1244.
(40) García-Granados, A.; Martínez, A.; Ortiz, A.; Onorato, M. E.; Arias, J. M. *J. Nat. Prod.* **1990**, *53*, 441−450.
(41) García-Granados, A.; Martínez, A.; Onorato, M. E.; Ruiz, M. L.; Sanchez, J. M.; Arias, J. M. *Phytochemistry* **1990**, *29*, 121−126.
(42) García-Granados, A.; Martínez, A.; Onorato, M. E.; Arias, J. M. *J. Nat. Prod.* **1986**, *49*, 126−132.

DOI: 10.1021/acs.jnatprod.6b00310
J. Nat. Prod. XXXX, XXX, XXX−XXX