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Withanolides with Phytotoxic Activity from Two Species of the Genus *Salpichroa*: *S. origanifolia* and *S. tristis* var. *lehmannii*

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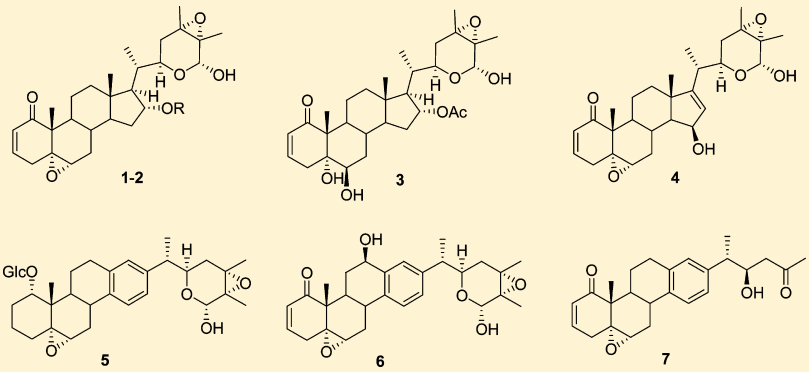
Supporting Information



ABSTRACT: Seven new withanolides, salpichrolides O−U (**1**−**7**), the known 2,3-dihydrosalpichrolide B (**9**), a substance not previously isolated from a natural source, and three known compounds, salpichrolide D (**8**), salpichrolide A (**10**), and salpichrolide C (**11**), were isolated and characterized from the aerial parts of *Salpichroa origanifolia* and *S. tristis* var. *lehmannii*. Compounds **1**−**4** and **8** have an oxygenated D ring, while compounds **5**−**7** and **9**−**11** possess a six-membered aromatic D ring. The structures of the isolated compounds were identified by analysis of their spectroscopic data including NMR and MS. Withanolides **1**, **3**, **8**, **10**, and **11** exhibited selective radicle growth inhibition toward *Lactuca sativa* (lettuce) at 150 and 400 ppm.

Withanolides constitute a group of natural C-28 steroids based on an ergostane skeleton, with oxidation at C-1, C-22, and C-26 being a common feature. 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.[1−4]

The genus *Salpichroa* Miers is a small group of about 16 South American species. *Salpichroa origanifolia* (Lam.) Baillon is indigenous to low-altitude areas (0−2000 m) of southeastern Bolivia, Paraguay, southern Brazil, Uruguay, and Argentina, while *Salpichroa tristis* var. *lehmannii* (Dammer) Keel is widespread along the Andes from Venezuela to northestern Argentina.[5] A family of aromatic D-ring withanolides and related ergostane derivatives has been isolated from *S. origanifolia*,[6] and many of these compounds exhibit interesting biological activities such as antifeedant,[7] cancer chemopreventive,[8] and antiproliferative effects.[9]

Continuing our investigations into the withanolides of the genus *Salpichroa*, we reported the isolation of four new withanolides from *S. origanifolia* (**1**−**4**) and three new withanolides from *S. tristis* var. *lehmannii* (**5**−**7**). Additionally, the known salpichrolide D (**8**) was isolated from *S. origanifolia* and *S. tristis* var. *lehmannii*, while 2,3-dihydrosalpichrolide B (**9**), salpichrolide A (**10**), and salpichrolide C (**11**) were purified from *S. tristis* var. *lehmannii*. Selective phytotoxicity has been reported for several withanolide types including normal skeleton, norbornane, sativolide, C-22 spiranoid, and trechonolide derivatives.[10] In order to evaluate such activity in compounds with a different withanolide skeleton, the phytotoxic activity of the major compounds obtained in the present investigation were evaluated against *Lactuca sativa* (lettuce) and *Avena sativa* (oats).

RESULTS AND DISCUSSION

The dichloromethane extracts of the aerial parts of two *Salpichroa* species were subjected to chromatographic purification to give seven new withanolides (**1**−**7**). All known salpichrolides obtained from these species were isolated previously from *S. origanifolia* collected in different regions and at different times of the year,[6] except for the semisynthetic compound 2,3-dihydrosalpichrolide B (**9**), obtained by hydrogenation of salpichrolide B,[7c] which is reported here for the first time as a natural product.

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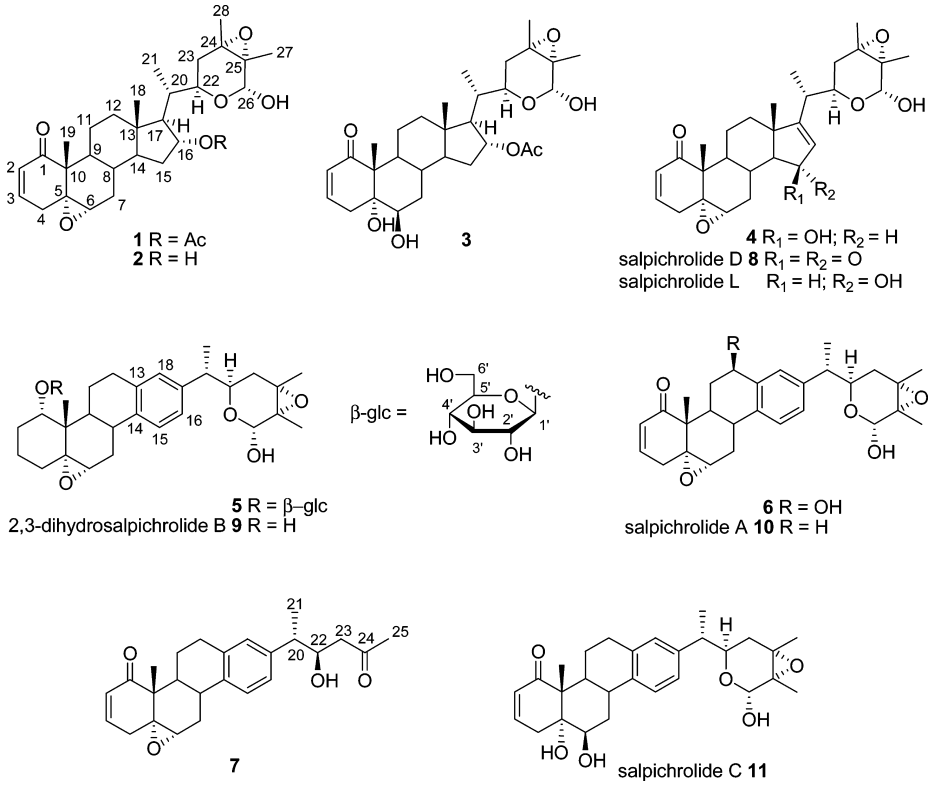
Chart 1



S. origanifolia collected in Entre Ríos, east of Argentina, gave the new compounds **1**–**4** and the known salpichrolide D (**8**);[6b] all of these withanolides have the ring D oxygenated substituents. The HRFABMS of salpichrolide O (**1**) showed a quasimolecular ion $[M + Na]^+$ at m/z 537.2802, corresponding to an elemental formula of $C_{30}H_{42}O_7Na$. The IR spectrum revealed the presence of hydroxy (3414 cm^{-1}), ester (1729 cm^{-1}), and keto (1682 cm^{-1}) groups. Inspection of the 1D and 2D NMR data revealed that compound **1** possesses the rings A and B and the side chain closely related to those of salpichrolide A (**10**);[6] however no signals from an aromatic ring D were observed. Compound **1** was found to contain the following moieties in common with **10**: an α,β-unsaturated ketone in ring A [^{13}C: δ 202.7 (C-1), 128.9 (C-2), 142.2 (C-3); ^{1}H: δ 5.96 (dd, J = 10.0 and 2.2 Hz, H-2), δ 6.72 (ddd, J = 10.0, 4.9, and 2.2 Hz, H-3)]; a 5α,6α-epoxy group in ring B [^{13}C: δ 64.7 (C-5), 58.5 (C-6); ^{1}H: δ 3.05 (d, J = 4.9 Hz, H-6)]; and a side chain with an epoxy-δ-lactol group [^{13}C: δ 65.0 (C-22), 64.7 (C-24), 63.8 (C-25), 91.8 (C-26), 16.5 (C-27), 18.9 (C-28); ^{1}H: δ 3.52 (1H, dt, J = 10.9, 2.9 Hz, H-22), 4.97 (1H, d, J = 11.0 Hz, H-26), 1.407 (3H, s, H_3-27), 1.402 (3H, H_3-28)]), as supported by ^{1}H–^{1}H COSY and HMBC experiments. The NMR data corresponding to rings C/D and the side ring were closely related to those of virginol A isolated from *Physalis virginiana*.[11] The presence of an acetoxy group in **1** was evidenced by the NMR spectra [^{13}C: δ 170.9 (CH_3CO–), 21.2 (CH_3CO–); ^{1}H: δ 2.07 (3H, s, CH_3CO–)]. This group was located at C-16 by the HMBC correlations of H-16 (δ 4.99) with CH_3CO– (δ 21.2), C-14 (δ 54.0), and C-20 (δ 37.9), while C-16 (δ 78.7) correlated with H-15α (δ 1.42) and H-17 (δ 1.35). The H-16 signal (δ 4.99) was a broad triplet with a J = 7.1 Hz. This coupling constant agrees with an α-orientation of the C-16-acetoxy group, like that of virginol A, whose structure was unambiguously established.[11] This assumption was supported by the NOESY experiment, which showed correlation of H-16 with CH_3-18 (δ 0.70). Accordingly, the structure of **1** was elucidated as (17*R*,20*S*,22*R*,24*S*,25*S*,26*R*)-16α-acetoxy-5α,6α:22,26:24,25-triepoxy-26-hydroxyergost-2-en-1-one.

Salpichrolide P (**2**) revealed a molecular formula of $C_{28}H_{40}O_6$ by HRESIMS and NMR experiments. Compound **2** exhibited IR absorptions for hydroxyl (3414 cm^{-1}) and an α,β-unsaturated ketone (1682 cm^{-1}). The ^{1}H and ^{13}C NMR data were closely comparable to those of salpichrolide O (**1**). The obvious differences between **2** and **1** were the absence of the signals corresponding to an acetoxy group in **2**, suggesting that **2** is a 16-hydroxy derivative of **1**. This observation was supported by the low-frequency shift of H-16 (δ 4.23 in **2** and δ 4.96 in **1**) in the ^{1}H NMR spectrum, the low-frequency shift of C-16 (δ 75.1 in **2** and 78.7 in **1**), and the high-frequency shift of C-17 (δ 64.4 in **2** and δ 57.9 in **1**) in the ^{13}C NMR spectrum. The NOESY experiment showed a cross-correlation peak between H-16 and CH_3-18, thus establishing an α-orientation of the hydroxy group at C-16 in **2**, as shown. The structure of **2** was elucidated as (17*R*,20*S*,22*R*,24*S*,25*S*,26*R*)-5α,6α:22,26:24,25-triepoxy-16α,26-dihydroxyergost-2-en-1-one and subsequently named salpichrolide P.

Salpichrolide Q (**3**) revealed a molecular formula of $C_{30}H_{44}O_8$ by HRFABMS. The ^{1}H and ^{13}C NMR spectra of compound **3** were closely related to those of **1**. The almost identical ^{13}C NMR data for rings C and D and the side chain of compound **1** indicated that structural differences between these withanolides were restricted to substituents in rings A and B. The ^{1}H and ^{13}C NMR data of **3** were consistent with a 5α,6β-diol pattern.[12] The small coupling in the signal at δ 3.66 (brs, H-6) confirmed the β-axial orientation of the OH-6 group. The ^{13}C NMR spectrum showed the expected chemical shifts for

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signals of carbons C-5 and C-6 at δ 77.4 and 74.1, respectively. The orientation of the acetoxy group at C-16 was assigned as α on the basis of the NOE observed between H-16 (δ 4.98) and CH_3-18 (δ 0.77). Accordingly, the structure of compound **3** was determined as (17*R*,20*S*,22*R*,24*S*,25*S*,26*R*)-16α-acetoxy-22,26:24,25-diepoxy-5α,6β,26-trihydroxyergost-2-en-1-one and named salpichrolide Q.

The HREIMS of salpichrolide R (**4**) did not show a molecular ion, but revealed an M − H_2O fragment corresponding to $C_{28}H_{36}O_5$. Its NMR data exhibited a close resemblance to those of salpichrolide L,[6e] possessing the same nine-carbon side chain with an δ-epoxylactol, identical rings A and B, and a 15-hydroxy-16(17)-ene system in ring D. The obvious differences between **4** and salpichrolide L were the signals of H-15 [δ 4.43 (dd, J = 4.8, 2.8 Hz) in **4**, δ 4.51 (d, J = 8.5 Hz) in salpichrolide L] and H-16 [δ 5.62 (d, J = 2.6 Hz) in **4**, δ 5.39 (brs) in salpichrolide L] in the ^{1}H NMR spectra. Analysis of the ^{13}C NMR showed chemical shifts for the C-14 to C-18 in ring D (δ 65.2, 78.2, 127.8, 157.3, and 18.9 in salpichrolide L and δ 59.6, 73.6, 124.0, 162.9, and 22.8 in compound **4**). This evidence suggested a 15β-hydroxy-16(17)-ene system in ring D. This assumption was confirmed by comparison of the ^{1}H and ^{13}C NMR data of salpichrolide L and compound **4** with those of nicaphysalins B and C, respectively. These withanolides isolated from *Nicandra physalodes*[13] have the same structural arrangement in ring D and the side chain, nicaphysalin B has a hydroxyl group at C-15 with α-orientation, while nicaphysalin C has a hydroxy group at C-15 with β-orientation. Salpichrolide L and nicaphysalin B showed almost identical chemical shift signals for C-15 to C-18 carbons, the differences being smaller than 1 ppm. On the other hand, not only the chemical shift for H-15 but also the multiplicity and coupling constant value are almost identical. The same similarity was observed between compound **4** and nicaphysalin C in their NMR spectra data. Thus, the structure of compound **4** was established as (20*S*,22*R*,24*S*,25*S*,26*R*)-5α,6α:22,26:24,25-triepoxy-15β,26-dihydroxyergost-2,16-dien-1-one. All withanolides isolated from *S. origanifolia* in the present investigation (**1**−**4** and **8**) were found to have a five-membered D ring with different degrees of oxidation. All the previous studies of *S. origanifolia* showed salpicholide A (**10**) as a major component with the exception of the population analyzed in this paper. This species has a cosmopolitan distribution, and probably the absence of **10** is due to the influence of the environment conditions. However, although salpichrolide A was not found, all the other withanolides isolated have the ring D oxygenated, and these compounds may be involved in the biosynthetic pathway leading to ring D expansion. A pathway for this conversion in which the dehydrogenation steps precede ring expansion has been postulated by Whiting in *Nicandra physalodes*.[14]

From *S. tristis* var. *lehmannii*, three new withanolides were isolated (**5**−**7**) along with four known compounds, salpichrolide D (**8**),[6b] 2,3-dihydrosalpichrolide B (**9**),[7c] salpichrolide A (**10**),[6a] and salpichrolide C (**11**).[6b] The molecular formula of salpichrolide S (**5**) was determined by HRAPCI/ESIMS as $C_{34}H_{48}O_{10}$. The ^{1}H and ^{13}C NMR spectra of compound **5** exhibited signals for a monoglycosylated withanolide, for which the aglycone is 2,3-dihydrosalpichrolide B (**9**), previously obtained from the reduction of 2,3-dihydrosalpichrolide A.[7c] The main difference observed between compounds **5** and **9** was the shift of the C-1 signal (δ 80.8 in **5** and δ 72.2 in **9**), consistent with the presence of a glycosidic functionality at C-1. This was confirmed by the HMBC correlations of H_3-19/C-1 (1.06/80.8) and H-1′/C-1 (4.30/80.8). The identification of the sugar was performed by analyses of COSY, HSQC, and HMBC experiments. The chemical shifts corresponding to the sugar moiety were in good agreement with the presence of D-glucose. The anomeric signals at δ_H 4.30 (d, J = 7.6 Hz)/δ_C 105.0 were assigned to a β-glucopyranosyl unit.[15] In order to determine the absolute configuration of the glucose, compound **5** was hydrolyzed with 3 M hydrochloric acid to yield D-glucose, as indicated by its identical R_f value compared to a D-glucose standard by TLC analysis and its positive optical rotation. The structure of compound **5** was determined as (20*S*,22*R*,24*S*,25*S*,26*R*)-5α,6α:22,26:24,25-triepoxy-1α-O-D-glucosyl-26-hydroxy-17(13→18)*abeo*-ergosta-13,15,17-triene.

The molecular formula of salpichrolide T (**6**) was determined by HREIMS as $C_{28}H_{34}O_6$. The ^{1}H and ^{13}C NMR data of rings A, B, and D and the side chain were closely related to those of salpichrolide A, indicating that they differed in the substitution pattern of ring C. The ^{1}H NMR spectrum of **6** exhibited a signal at δ 4.93 assigned to the proton geminal to a β-hydroxy group at C-12. The spin−spin coupling pattern of H-12 (dd, J = 10.8, 5.0 Hz) indicated its axial orientation. The substitution pattern in ring C was confirmed by HMBC correlations between H-12/C-14 (4.93/137.2) and H-11α/C-12 (2.88/69.8). Other differences apparent in the ^{1}H and ^{13}C NMR data were the high-frequency shift of the H-18 signal from δ 6.90 for salpichrolide A (**10**) to δ 7.43 for **6** and the C-11 resonance from δ 25.4 to 36.1, respectively. The structure of **6** was determined as shown.

Salpichrolide U (**7**) revealed a molecular formula of $C_{25}H_{30}O_4$ by HREIMS. The IR spectrum revealed the presence of hydroxy (3459 cm^{-1}) and two keto (1708 and 1684 cm^{-1}) groups. Compound **7** exhibited ^{1}H and ^{13}C NMR spectra closely related to those of the A−D rings of salpichrolide A (**10**). The main differences between these substances resulted from signals corresponding to the side chain. Regarding the attachment to C-17, an opened chain substituent was evident in **7**. The characteristic ^{1}H NMR signals for this side chain were (i) a signal at δ 4.17 (ddd, J = 12.6, 6.4, and 3.1 Hz) assigned to H-22, (ii) a doublet for the C-21 methyl group at δ 1.27 (J = 7.1 Hz), indicating the absence of a hydroxy group at C-20, and (iii) a singlet at δ 2.16, typical of a methyl bonded to a carbonyl. The ^{13}C NMR spectrum of **7** was in agreement with the structure proposed for the signals at δ 71.8, 209.5, and 30.9 assigned to C-22, C-24, and C-25, respectively. Salpichrolide A may precede compound **7** in terms of its biosynthetic formation; thus, if the 24,25-epoxy function in salpichrolide A (**10**) is opened, e.g., hydrolyzed to a diol, retro-aldol cleavage can proceed, giving rise to **7**.[16] The configurations at C-20 and C-22 may be assigned as the same as in salpichrolide A (20*S*,22*R*), based on the above-mentioned biosynthetic considerations. Withanolides with the same biogenetic relationship between **7** and salpichrolide A (e.g., Nic-17 and Nic-1, respectively) have been isolated from *Nicandra physalodes*.[16,17] Accordingly, the structure of **7** was elucidated as (5α,6α-epoxy-22-hydroxy-26,27-dinor-17(13→18)*abeo*-5α-cholesta-2,13,15,17-tetraene-1,24-dione on the basis of biogenetic grounds and subsequently named salpichrolide U.

Several withanolides isolated from species of the genera *Iochroma* and *Jaborosa* have shown selective phytotoxic activity toward dicotyledonous species.[10] To evaluate for this type of bioactivity, the effect of compounds **1**, **3**, **5**, **8**, **9**, **10**, and **11** on seed germination and radicle length of a dicotyledon [*L. sativa*

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Table 1. Effect of Withanolides at Different Concentrations on Radicle Length of *Lactuca sativa* and *Avena sativa*

	effect on radicle length (%)[a]					
	Lactuca sativa			*Avena sativa*		
compound	15 ppm	150 ppm	400 ppm	15 ppm	150 ppm	400 ppm
1	2.0 ± 0.1	−30.2 ± 3.6	−42.4 ± 4.5	−12.0 ± 5.7	−6.4 ± 2.7	−26.9 ± 3.6
3	−9.6 ± 3.1	−10.4 ± 3.0	−62.4 ± 25.0	9.9 ± 3.5	1.6 ± 0.6	−14.2 ± 6.0
5	−10.7 ± 3.8	−14.9 ± 5.3	−4.7 ± 1.2	22.0 ± 7.1	11.3 ± 3.8	9.5 ± 2.9
8	−29.2 ± 10.4	−50.7 ± 15.9	−61.9 ± 23.2	8.8 ± 3.8	0.8 ± 0.3	−10.4 ± 4.8
9	−0.14 ± 0.04	−29.0 ± 8.5	−68.8 ± 24.2	−1.7 ± 0.5	−36.7 ± 13.7	−65.0 ± 32.0
10	1.8 ± 0.1	−50.0 ± 7.0	−86.0 ± 14.0	−5.6 ± 0.7	−23.0 ± 2.0	−31.0 ± 4.0
11	−4.4 ± 1.2	−33.8 ± 8.4	−78.3 ± 16.3	−11.5 ± 5.5	−18.5 ± 3.8	−42.4 ± 9.9

[a]The data are presented as percentage differences from the control (zero value); negative values represent inhibition, and positive values represent stimulation. The percentages were calculated from 3 × 20 replicates. No effect was observed on germination for any of the test compounds at the doses used. Germination and root length values of treated and control experiments were analyzed by the Student's *t* test ($p < 0.05$).

(lettuce)] and of a monocotyledon [*A. sativa* (oat)] as standard target species has been studied in the concentration range 15−400 ppm. The assays were performed according to the procedures previously described.[10a] The results are reported as percentage differences from the control (Table 1). The effect produced by the test compounds on germination was not significant against the species assayed. On the other hand, all evaluated compounds, with the exception of **5**, caused strong inhibition of radicle elongation in *L. sativa* at 150 and 400 ppm, and a dose-dependent effect was observed. The inhibition values observed at 400 ppm were between 86.0% for **10** and 42.4% for **1**. The responses on *A. sativa* radicle elongation were different. Compound **9** showed similar inhibition with respect to *L. sativa*, while compounds **1**, **10**, and **11** showed lesser inhibitory effects, and compounds **3**, **5**, and **8** were inactive. These show selective phytotoxic properties of withanolides **1**, **3**, **8**, **10**, and **11** against lettuce and oat. Thus, these compounds may act as a selective phytogrowth controller against weed species. A similar selective inhibition effect on dicotyledon species was reported for withanolides with different arrangements at the side chain: withanolides type-normal skeleton (4,7,20-trihydroxy withanolides from *Iochroma australe*),[10a] -norbornane (jaborosalactol 18 from *Jaborosa bergii*),[10b] -C22 espiranoid (jaborosalactones 29 and 43 from *J. rotacea* and *J. kurtzii*, respectively),[10c,d] -sativolides (jaborosalactone 38 from *J. caulescens* var. *caulescens*),[10e] and -trechonolides (12-*O*-ethyljaborosalactone 42 from *J. caulescens* var. *bipinnatifida*),[10e] and for withanolides with different substitution patterns in rings A/B. However, there is not enough information available yet to make an analysis of structure−activity relationship.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were measured on a mercury thermometer apparatus and were uncorrected. Optical rotations were measured on a JASCO P-1010 polarimeter. UV spectra were obtained using a Shimadzu-260 spectrophotometer. IR spectra were obtained in a Nicolet 5-SXC spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC-200 NMR spectrometer at 200.13 (^{1}H) and 50.32 (^{13}C) MHz or a Bruker AVANCE II AV-400 operating at 400.13 MHz for ^{1}H and 100.63 MHz for ^{13}C. Multiplicity determinations (DEPT) and 2D spectra (COSY, HSQC, HMBC, and NOESY) were obtained using standard Bruker software. Chemical shifts are given in ppm (δ) downfield from the TMS internal standard. FABMS and HRFABMS were measured on an NBA−sodium matrix using a VG-ZAB mass spectrometer. EIMS and HREIMS were determined at 70 eV on a VG Auto Speccon mass spectrometer, HRESIMS was recorded on a LCT premier XE Micromass spectrometer, and HRAPCI/ESIMS was recorded on an Agilent LCTOF mass spectrometer. Chromatographic separations were performed by vacuum-liquid chromatography. Column chromatography was performed on silica gel 60 (0.063−0.200 mm) or silica gel 100 C_{18} reversed-phase, radial chromatography with a radial Chromatotron model 7924 T on silica gel 60 PF_{254} Merck (1 mm thick), and preparative TLC on silica gel 60 F_{254} (0.2 mm thick) plates.

Plant Material. *Salpichroa origanifolia* (Lam.) Baillon was collected in Paraná, Entre Ríos, Argentina, in December 2002. A voucher specimen has been deposited at Museo Botánico Córdoba, Universidad Nacional de Córdoba, under CORD 895. *Salpichroa tristis* var. *lehmannii* (Dammer) Keel was collected in Yavi Chico, Departamento Yavi, Jujuy, Argentina, in March 2006. A voucher specimen has been deposited at Museo Botánico Córdoba, Universidad Nacional de Córdoba, under Barboza et al. 1734. The plant material was identified by Prof. Gloria E. Barboza (IMBIV-CONICET, Córdoba, Argentina).

Extraction and Isolation. The fresh plants of *S. origanifolia* (ca. 4.0 kg) were triturated with EtOH at room temperature immediately after collection. The residue obtained after evaporation of the solvent was partitioned with hexane−EtOH−H_2O (10:3:1), the aqueous EtOH phase was concentrated, and the resulting aqueous phase was 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 (3.4 g) was fractionated initially by vacuum-liquid chromatography. Elution with hexane−EtOAc mixtures of increasing polarity (80:20 to 0:100) and EtOAc−MeOH (100:0 to 50:50) afforded four fractions containing withanolides: fractions I−IV [*n*-hexane−EtOAc (10:90) to EtOAc−MeOH (90:10)]. These fractions were pooled (240 mg) and further processed using the radial chromatographic technique. Elution with CH_2Cl_2−MeOH (100:0 to 0:100) afforded (in order of elution) compounds **1** (73.0 mg), **8** (12.6 mg), **2** (36.5 mg), **4** (21.0 mg), and **3** (84.4 mg).

The fresh plants of *S. tristis* var. *lehmannii* (700 g) were triturated with EtOH at room temperature immediately after collection, and the EtOH extract was concentrated under reduced pressure. The residue was defatted by partition in hexane−EtOH−H_2O (10:3:1), the EtOH−H_2O phase was washed with hexane (3 × 300 mL), and the EtOH was evaporated at reduced pressure. The residue was diluted with H_2O and extracted with CH_2Cl_2 (3 × 200 mL). The CH_2Cl_2 extract was dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness at reduced pressure. The residue (1.8 g) was subjected to column chromatography with *n*-hexane−EtOAc and EtOAc−MeOH mixtures of increasing polarity to give six fractions containing withanolides (I−V). Fraction I [*n*-hexane−EtOAc (60:40), 330 mg] was chromatographed over silica gel, eluting with mixtures of increasing polarity of *n*-hexane−EtOAc and EtOAc−MeOH to yield compounds **10** (150.1 mg) and **9** (60.3 mg). Column chromatography of fraction II [*n*-hexane−EtOAc (40:60), 140 mg] with *n*-hexane−EtOAc mixtures of increasing polarity gave **11** (50.2 mg). Fraction III [*n*-hexane−EtOAc (10:90), 120 mg] was separated by column chromatography with *n*-hexane−EtOAc and EtOAc−MeOH mixtures of increasing polarity to give two main fractions containing

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withanolides: A (4.0 mg) and B (34.0 mg). Fraction A was fractionated by preparative TLC with CH_2Cl_2–MeOH (94:06) yielding **7** (2.0 mg), while fraction B was chromatographed with *n*-hexane–EtOAc (30:70) to give **6** (4.0 mg). Fraction IV [EtOAc–MeOH (98:2), 130 mg] was chromatographed on C_{18} silica gel using MeOH–H_2O (55:45) as solvent, yielding **5** (41.0 mg). Column chromatography of fraction V [EtOAc–MeOH (92:8), 65.0 mg] with EtOAc–MeOH mixtures of increasing polarity gave **8** (7.0 mg).

*Salpichrolide O [(17R,20S,22R,24S,25S,26R)-16α-acetoxy-5α,6α:22,26:24,25-triepoxy-26-hydroxyergost-2-en-1-one] (**1**):* colorless crystals (*n*-hexane–EtOAc); mp 208 °C (dec); $[\alpha]^{21}_D$ +3.7 (*c* 1.7, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 220 (3.87) nm; IR (dried film) ν_{max} 3414, 2930, 1729, 1682, 1378, 1246, 1031 cm^{-1}; ^1H NMR ($CDCl_3$–D_2O (95:05), 400.13 MHz) δ 6.72 (1H, ddd, *J* = 10.0, 4.9, 2.2 Hz, H-3), 5.96 (1H, dd, *J* = 10.0, 2.2 Hz, H-2), 4.97 (1H, sa, *J* = 11.0 Hz, H-26), 4.96 (1H, t, *J* = 7.1 Hz, H-16), 3.52 (1H, dt, *J* = 10.9, 2.9 Hz, H-22), 3.10 (1H, dt, *J* = 19.6, 2.5 Hz, H-4β), 3.05 (1H, d, *J* = 4.9 Hz, H-6), 2.31 (1H, ddd, *J* = 13.6, 7.0, 3.2 Hz, H-11α), 2.07 (3H, s, CH_3CO-16), 1.92 (1H, m, H-12β), 1.91 (2H, m, H-7β and, H-23α), 1.87 (1H, m, H-20), 1.84 (1H, dd, *J* = 19.6, 5.0 Hz, H-4α), 1.74 (1H, m, H-9), 1.69 (1H, m, H-15β), 1.63 (1H, dd, *J* = 14.1, 11.8 Hz, H-23β), 1.44 (1H, m, H-7α), 1.42 (1H, m, H-15α), 1.407 (3H, s, H_3-27), 1.402 (3H, H_3-28), 1.40 (1H, m, H-12α), 1.35 (1H, m, H-17), 1.33 (1H, m, H-8), 1.33 (3H, s, H-19), 1.31 (1H, m, H-14), 1.30 (1H, m, H-11β), 0.91 (3H, d, *J* = 6.8 Hz, H-21), 0.70 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 50.03 MHz) δ 202.7 (C, C-1), 170.9 (C, CH_3CO-16), 142.2 (CH, C-3), 128.9 (CH, C-2), 91.8 (CH, C-26), 78.7 (CH, C-16), 65.0 (CH, C-22), 64.7 (C, C-5, C-24), 63.8 (C, C-25), 58.5 (CH, C-6), 57.9 (CH, C-17), 54.0 (CH, C-14), 48.3 (C, C-10), 43.2 (C, C-13), 39.5 (CH_2, C-12), 38.0 (CH, C-9), 37.6 (CH, C-20), 34.4 (CH_2, C-15), 34.0 (CH_2, C-4), 30.1 (CH, C-8), 29.5 (CH_2, C-23), 28.6 (CH_2, C-7), 22.3 (CH_2, C-11), 21.2 (CH_3, CH_3CO-16), 18.9 (CH_3, C-28), 16.5 (CH_3, C-27), 15.5 (CH_3, C-19), 13.2 (CH_3, C-18), 12.8 (CH_3, C-21); FABMS *m*/*z* 537 $[M + Na]^+$ (36), 497 $[M + H - H_2O]^+$ (10), 468 (9), 342 (22), 341 (84), 313 (37), 193 (35), 155 (41), 123 (64), 111 (52); HRFABMS *m*/*z* $[M + Na]^+$ 537.2802 (calcd for $C_{30}H_{42}O_7Na$, 537.2828).

*Salpichrolide P [(17R,20S,22R,24S,25S,26R)-5α,6α:22,26:24,25-triepoxy-16α,26-dihydroxyergost-2-en-1-one] (**2**):* white, amorphous powder; $[\alpha]^{21}_D$ +35.9 (*c* 0.4, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 222 (3.51) nm; IR (dried film) ν_{max} 3414, 2925, 1682, 1382, 1087, 1038, 864, 736 cm^{-1}; ^1H NMR ($CDCl_3$, 400.13 MHz) δ 6.71 (1H, ddd, *J* = 10.1, 5.2, 2.2 Hz, H-3), 5.95 (1H, dd, *J* = 10.1, 1.9 Hz, H-2), 5.02 (1H, brs, H-26), 4.23 (1H, brt, *J* = 6.7 Hz, H-16), 3.80 (1H, ddd, *J* = 11.2, 5.7, 2.4 Hz, H-22), 3.09 (1H, dt, *J* = 19.5, 2.5 Hz, H-4β), 3.05 (1H, d, *J* = 4.9 Hz, H-6), 2.28 (1H, ddd, *J* = 13.6, 7.2, 3.5 Hz, H-11α), 2.08 (1H, dd, *J* = 14.3, 2.5 Hz, H-23α), 1.93 (1H, m, H-7β), 1.88 (1H, m, H-12β), 1.83 (1H, dd, *J* = 19.5, 5.2 Hz, H-4α), 1.74 (1H, m, H-20), 1.72 (1H, m, H-9), 1.60 (1H, m, H-23β), 1.57 (2H, m, H_2-15), 1.45 (1H, m, H-7α), 1.42 (3H, s, H_3-27), 1.41 (3H, s, H_3-28), 1.39 (1H, m, H-14), 1.38 (1H, m, H-12α), 1.32 (3H, s, H_3-19), 1.29 (1H, m, H-8), 1.25 (1H, m, H-11β), 1.13 (1H, dd, *J* = 10.8, 5.6 Hz, H-17), 0.91 (3H, d, *J* = 6.8 Hz, H_3-21), 0.67 (3H, s, H_3-18); ^{13}C NMR ($CDCl_3$, 50.03 MHz) δ 202.9 (C, C-1), 142.2 (CH, C-3), 129.0 (CH, C-2), 91.8 (CH, C-26), 75.1 (CH, C-16), 67.5 (CH, C-22), 65.2 (C, C-5), 64.7 (C, C-24), 64.4 (CH, C-17), 63.4 (C, C-25), 58.7 (CH, C-6), 53.4 (CH, C-14), 48.3 (C, C-10), 44.4 (C, C-13), 39.9 (CH_2, C-12), 38.3 (CH, C-9), 38.0 (CH, C-20), 36.4 (CH_2, C-15), 34.0 (CH_2, C-4), 32.4 (CH_2, C-23), 30.2 (CH, C-8), 28.7 (CH_2, C-7), 22.4 (CH_2, C-11), 18.9 (CH_3, C-28), 16.4 (CH_3, C-27), 15.5 (CH_3, C-19), 14.5 (CH_3, C-21), 13.5 (CH_3, C-18); EIMS *m*/*z* 424 (10), 406 (10), 472 (3), 312 (4), 283 (25), 163 (21), 123 (100), 109 (43), 97 (17), 55 (24); HRESIMS *m*/*z* $[M + Na]^+$ 495.2729 (calcd for $C_{28}H_{40}O_6Na$, 495.2723).

*Salpichrolide Q [(17R,20S,22R,24S,25S,26R)-16α-acetoxy-22,26:24,25-diepoxy-5α,6β,26-trihydroxyergost-2-en-1-one] (**3**):* white, amorphous powder; $[\alpha]^{21}_D$ +16.7 (*c* 1.8, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 222 (3.78) nm; IR (dry film) ν_{max} 3440, 2935, 1714, 1671, 1380, 1248, 1029 cm^{-1}; ^1H NMR ($CDCl_3$, 400.13 MHz) δ 6.61 (1H, ddd, *J* = 10.0, 4.7, 2.0 Hz, H-3), 5.90 (1H, dd, *J* = 10.2, 2.5 Hz, H-2), 4.99 (1H, s, H-26), 4.98 (1H, t, *J* = 7.1 Hz, H-16), 3.66 (1H, brs, H-6), 3.58 (1H, d, *J* = 11.6 Hz, OH-26), 3.53 (1H, dt, *J* = 11.8, 2.8 Hz, H-22), 3.33 (1H, dt, *J* = 19.6, 2.5 Hz, H-4β), 2.27 (1H, ddd, *J* = 13.6, 6.8, 3.2 Hz, H-11α), 2.07 (3H, s, CH_3CO-16), 2.06 (1H, m, H-4α), 1.96 (1H, dt, *J* = 12.8, 3.3 Hz, H-12β), 1.92 (1H, m, H-20), 1.89 (1H, m, H-23α), 1.81 (1H, m, H-9), 1.75 (1H, m, H-15β), 1.74 (1H, m, H-8), 1.70 (1H, m, H-7β), 1.64 (1H, dd, *J* = 14.3, 11.5 Hz, H-23β), 1.51 (1H, t, *J* = 15.8 Hz, H-14), 1.49 (1H, m, H-7α), 1.48 (1H, m, H-12α), 1.47 (1H, m, H-15α), 1.41 (6H, s, H_3-27, H_3-28), 1.39 (1H, m, H-17), 1.33 (1H, m, H-11β), 1.32 (3H, s, H_3-19), 0.93 (3H, d, *J* = 6.5 Hz, H_3-21), 0.77 (3H, s, H_3-18); ^{13}C NMR ($CDCl_3$, 50.03 MHz) δ 204.7 (C, C-1), 171.3 (C, CH_3CO-16), 141.5 (CH, C-3), 128.6 (CH, C-2), 91.8 (CH, C-26), 79.1 (CH, C-16), 77.4 (C, C-5), 74.1 (CH, C-6), 65.2 (CH, C-22), 64.8 (C, C-24), 63.7 (C, C-25), 58.2 (CH, C-17), 53.1 (CH, C-14), 51.9 (C, C-10), 43.7 (C, C-13), 41.3 (CH, C-9), 40.0 (CH_2, C-12), 37.6 (CH, C-20), 35.8 (CH_2, C-4), 34.3 (CH_2, C-15), 33.4 (CH_2, C-23), 29.5 (CH_2, C-7), 29.4 (CH, C-8), 22.8 (CH_2, C-11), 21.2 (CH_3, CH_3CO-16), 18.8 (CH_3, C-28), 16.4 (CH_3, C-27), 15.6 (CH_3, C-19), 13.5 (CH_3, C-18), 12.8 (CH_3, C-21); FABMS *m*/*z* 555 $[M + Na]^+$ (17), 429 (5), 381 (12), 341 (94), 313 (69), 155 (33), 136 (72), 123 (41), 111 (32); HRFABMS *m*/*z* $[M + Na]^+$ 555.2926 (calcd for $C_{30}H_{44}O_8Na$, 555.2934).

*Salpichrolide R [(20S,22R,24S,25S,26R)-5α,6α:22,26:24,25-triepoxy-15β,26-dihydroxyergost-2,16-dien-1-one] (**4**):* white, amorphous powder; $[\alpha]^{21}_D$ +9.8 (*c* 0.13, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 222 (3.69) nm; IR (dry film) ν_{max} 3414, 2930, 1729, 1682, 1378, 1246, 1031 cm^{-1}; ^1H NMR ($CDCl_3$, 400.13 MHz) δ 6.72 (1H, ddd, *J* = 10.0, 5.0, 2.2 Hz, H-3), 5.96 (1H, dd, *J* = 10.0, 2.2 Hz, H-2), 5.62 (1H, d, *J* = 2.6 Hz, H-16), 5.00 (1H, s, H-26), 4.43 (1H, dd, *J* = 4.8, 2.8 Hz, H-15), 3.77 (1H, ddd, *J* = 10.5, 7.5, 2.3 Hz, H-22), 3.12 (1H, dt, *J* = 19.6, 2.8 Hz, H-4β), 3.12 (1H, d, *J* = 5.1 Hz, H-6), 2.46 (1H, m, H-11α), 2.32 (1H, dt, *J* = 14.8, 5.5, Hz, H-7β), 2.19 (1H, m, H-20), 2.00 (1H, dd, *J* = 14.3, 2.5 Hz, H-23α), 1.85 (1H, dd, *J* = 19.6, 5.0 Hz, H-4α), 1.81 (1H, m, H-9), 1.75 (1H, m, H-8), 1.63 (1H, m, H-23β), 1.62 (1H, m, H-7α), 1.45 (1H, m, H-11β), 1.424 (3H, s, H_3-19), 1.415 (6H, s, H_3-27, H_3-28), 1.23 (1H, dd, *J* = 10.3, 4.3 Hz, H-14), 1.07 (3H, s, H-18), 0.96 (3H, d, *J* = 6.9 Hz, H-21); ^{13}C NMR ($CDCl_3$, 50.03 MHz) δ 202.9 (C, C-1), 162.9 (C, C-17), 142.2 (CH, C-3), 129.0 (CH, C-2), 124.0 (CH, C-16), 91.6 (CH, C-26), 73.6 (CH, C-15), 66.6 (CH, C-22), 65.0 (C, C-5), 64.6 (C, C-24), 63.7 (C, C-25), 59.6 (CH, C-14), 58.9 (CH, C-6), 48.8 (C, C-10), 47.0 (C, C-13), 39.0 (CH, C-9), 36.4 (CH, C-20), 35.0 (CH_2, C-23), 34.3 (CH_2, C-4), 34.2 (CH_2, C-12), 27.9 (CH_2, C-7), 26.9 (CH, C-8), 22.8 (CH_3, C-18), 22.0 (CH_2, C-11), 18.8 (CH_3, C-28), 17.4 (CH_3, C-21), 16.6 (CH_3, C-27), 15.6 (CH_3, C-19); EIMS *m*/*z* 452 $[M - H_2O]^+$ (3), 434 $[M - 2H_2O]^+$ (4), 422 (8), 404 (30), 389 (20), 328 (8), 310 (24), 309 (27), 171 (24), 123 (100), 109 (28), 91 (18), 55 (22); HREIMS *m*/*z* $[M - H_2O]^+$ 452.2567 (calcd for $C_{28}H_{36}O_5$, 452.2563).

*Salpichrolide S [(20S,22R,24S,25S,26R)-5α,6α:22,26:24,25-triepoxy-1α-O-D-glucosyl-26-hydroxy-17(13→18)abeo-ergosta-13,15,17-triene] (**5**):* colorless crystals (hexane–EtOAc), mp 153–155 °C; $[\alpha]^{21}_D$ −53.3 (*c* 1.2, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 215 (3.96) nm; IR (dry film) ν_{max} 3416, 2934, 1505, 1081, 1034, 754 cm^{-1}; ^1H NMR ($CDCl_3$–D_2O (95:05), 400.13 MHz) δ 7.06 (1H, d, *J* = 8.1 Hz, H-15), 6.95 (1H, dd, *J* = 8.1, 1.5 Hz, H-16), 6.89 (1H, brs, H-18), 4.99 (1H, s, H-26), 4.30 (1H, d, *J* = 7.6 Hz, H-1′), 3.97 (1H, brs, H-1), 3.88 (1H, ddd, *J* = 10.9, 6.5, 2.1 Hz, H-22), 3.84 (1H, dd, *J* = 11.9, 3.2 Hz, H-6′a), 3.77 (1H, dd, *J* = 11.9, 4.7 Hz, H-6′b), 3.52 (1H, t, *J* = 8.9 Hz, H-4′), 3.49 (1H, t, *J* = 8.8 Hz, H-3′), 3.42 (1H, t, *J* = 7.9 Hz, H-2′), 3.33 (1H, dt, *J* = 8.1, 4.3 Hz, H-5′), 2.92 (1H, d, *J* = 4.7 Hz, H-6), 2.81 (1H, m, H-8), 2.80 (1H, m, H-12a), 2.70 (1H, m, H-12b), 2.67 (1H, q, *J* = 6.8 Hz, H-20), 2.62 (1H, ddd, *J* = 15.2, 7.8, 4.7 Hz, H-7β), 2.24 (1H, brt, *J* = 11.9 Hz, H-9), 2.16 (1H, td, *J* = 13.3, 4.2 Hz, H-2a), 2.06 (1H, brd, *J* = 14.1 Hz, H-3a), 1.89 (1H, dd, *J* = 14.2, 2.4 Hz, H-23α), 1.85 (2H, m, H-2b, H-7α), 1.79 (1H, m, H-11α), 1.57 (1H, dd, *J* = 14.2, 11.4 Hz, H-23β), 1.38 (3H, s, H_3-27), 1.37 (3H, s, H_3-28), 1.28 (1H, m, H-11β), 1.21 (3H, d, *J* = 7.1 Hz, H_3-21), 1.06 (3H, s, H_3-19), 1.01 (1H, brd, *J* = 14.1 Hz, H-3b); ^{13}C NMR ($CDCl_3$, 100.63 MHz) δ 140.6 (C, C-17), 138.6 (C, C-13), 136.5 (C, C-14), 128.0 (CH, C-18), 126.4 (CH, C-16), 125.8 (CH, C-15), 105.0 (CH, C-1′), 91.6 (CH, C-

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26), 80.7 (CH, C-1), 76.4 (CH, C-2′), 75.4 (CH, C-5′), 73.5 (CH, C-3′), 70.0 (CH, C-4′), 67.6 (CH, C-22), 64.8 (C, C-5), 64.1[a] (C, C-24), 63.3[a] (C, C-25), 62.0 (CH_2, C-6′), 57.6 (CH, C-6), 43.2 (CH, C-20), 40.5 (C, C-10), 34.5 (CH, C-9), 34.2 (CH_2, C-23), 32.1 (CH, C-8), 30.0 (CH_2, C-3, C-4, C-7, C-12), 28.0 (CH_2, C-2), 22.2 (CH_2, C-11), 18.6 (CH_3, C-28), 17.7 (CH_3, C-21), 16.6 (CH_3, C-27), 15.8 (CH_3, C-19) ([a]assignments may be interchanged); EIMS m/z 568 (47), 553 (38), 436 $[M - Glc]^+$ (4), 406 (55), 388 (47), 373 (28), 294 (78), 157 (25), 123 (100), 113 (49), 55 (20); HRAPCI/ESIMS m/z $[M + NH_4]^+$ 634.3587 (calcd for $C_{34}H_{52}NO_{10}$, 634.3585).

Hydrolysis and Identification of the Sugar Moiety in Compound 5. Compound **5** was dissolved in MeOH (5 mL) and refluxed with 0.5 N HCl (3 mL) for 4 h. The reaction mixture was diluted with H_2O and extracted with $CHCl_3$ (3 × 10 mL). The aqueous layer was then neutralized by Ag_2CO_3, and the precipitate filtered to give a monosaccharide, which was dissolved in 1 mL of H_2O and kept overnight before TLC analysis and determination of optical rotations. The monosaccharide from compounds **5** gave an R_f value of 0.22 ($CHCl_3$–MeOH–H_2O, 13:7:2 (lower phase)) and a specific rotation ($[\alpha]^{22}_D$ +34 (c 0.1, H_2O) consistent with D-glucose.

*Salpichrolide T [(20S,22R,24S,25S,26R)-5α,6α:22,26:24,25-trieepoxy-12β,26-dihydroxy-17(13→18)abeo-ergosta-2,13,15,17-tetraen-1-one] (**6**):* white, amorphous powder; $[\alpha]^{21}_D$ −4.8 (c 0.2, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 216 (4.00) nm; IR (dried film) ν_{max} 3428, 2930, 1684, 1377, 1022, 750 cm^{-1}; ^1H NMR ($CDCl_3$, 400.13 MHz) δ 7.43 (1H, brs, H-18), 7.12 (1H, d, J = 8.3 Hz, H-15), 7.09 (1H, dd, J = 8.3, 1.5 Hz, H-16), 6.78 (1H, ddd, J = 10.3, 5.0, 2.3 Hz, H-3), 6.02 (1H, dd, J = 10.3, 3.0 Hz, H-2), 4.98 (1H, d, J = 9.0 Hz, H-26), 4.93 (1H, dd, J = 10.8, 5.0 Hz, H-12), 3.87 (1H, ddd, J = 11.3, 5.8, 2.5 Hz, H-22), 3.43 (1H, d, J = 9.0 Hz, OH-26), 3.23 (1H, d, J = 5.0 Hz, H-6), 3.14 (1H, dt, J = 19.6, 2.8 Hz, H-4β), 2.88 (1H, ddd, J = 11.4, 5.0, 1.8 Hz, H-11α), 2.80 (1H, m, H-8), 2.79 (1H, m, H-20), 2.68 (1H, m, H-7β), 2.21 (1H, td, J = 12.1, 1.8 Hz, H-9), 1.92 (1H, dd, J = 19.6, 5.0 Hz, H-4α), 1.85 (1H, m, H-23α), 1.83 (1H, m, H-7α), 1.58 (1H, m, H-23β), 1.40 (3H, s, H_3-19), 1.38 (3H, s, H_3-27), 1.37 (3H, s, H_3-28), 1.32 (1H, m, H-11β), 1.24 (3H, d, J = 7.0 Hz, H_3-21); ^{13}C NMR ($CDCl_3$, 100.63 MHz) δ 202.2 (C, C-1), 142.6 (CH, C-3), 141.2 (C, C-17), 139.7 (C, C-13), 137.2 (C, C-14), 128.8 (CH, C-2), 127.2 (CH, C-16), 126.1 (CH, C-15), 125.9 (CH, C-18), 91.7 (CH, C-26), 69.8 (CH, C-12), 67.4 (CH, C-22), 64.9 (C, C-24), 64.5 (C, C-5), 63.7 (C, C-25), 58.8 (CH, C-6), 48.3 (C, C-10), 43.2 (CH, C-20), 36.1 (CH_2, C-11), 34.2 (CH_2, C-4), 33.7[a] (CH, C-9), 33.5[a] (CH, C-8), 33.4[a] (CH_2, C-23), 30.2 (CH_2, C-7), 18.8 (CH_3, C-28), 17.3 (CH_3, C-21), 16.5 (CH_3, C-27), 14.2 (CH_3, C-19) ([a]assignments may be interchanged); EIMS m/z 466 $[M]^+$ (1), 448 $[M - H_2O]^+$ (6), 432 (7), 420 (30), 418 (49), 405 (69), 403 (99), 326 (47), 306 (100), 263 (35), 123 (93), 109 (65), 97 (40), 57 (24), 55 (42); HREIMS m/z $[M]^+$ 466.2370 (calcd for $C_{28}H_{34}O_6$, 466.2355).

*Salpichrolide U [(5α,6α-epoxy-22-hydroxy-26,27-dinor-17(13→18)abeo-5α-cholesta-2,13,15,17-tetraene-1,24-dione] (**7**):* white, amorphous powder; $[\alpha]^{21}_D$ −14.9 (c 0.09, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 204 (4.09) nm; IR (dried film) ν_{max} 3459, 2918, 1708, 1684, 1377, 1268 cm^{-1}; ^1H NMR ($CDCl_3$, 400.13 MHz) δ 7.15 (1H, d, J = 8.0 Hz, H-15), 7.02 (1H, dd, J = 8.0, 1.6 Hz, H-16), 6.95 (1H, brs, H-18), 6.77 (1H, ddd, J = 10.1, 5.0, 2.2 Hz, H-3), 6.01 (1H, ddd, J = 10.1, 2.9, 1.0 Hz, H-2), 4.17 (1H, ddd, J = 12.6, 6.4, 3.1 Hz, H-22), 3.65 (1H, s, OH-22), 3.24 (1H, d, J = 4.8 Hz, H-6), 3.14 (1H, dt, J = 19.5, 2.6 Hz, H-4β), 2.93 (1H, ddd, J = 16.5, 12.2, 4.4 Hz, H-12a), 2.76 (2H, m, H-8, H-20), 2.75 (1H, m, H-12b), 2.71 (1H, m, H-7β), 2.61 (1H, m, H-23a), 2.59 (1H, m, H-11α), 2.49 (1H, dd, J = 17.1, 9.5 Hz, H-23b), 2.16 (3H, s, H_3-25), 2.09 (1H, td, J = 11.7, 2.3 Hz, H-9), 1.91 (1H, ddd, J = 19.5, 5.0, 1.0 Hz, H-4α), 1.84 (1H, td, J = 13.8, 2.9 Hz, H-7α), 1.38 (3H, s, H_3-19), 1.29 (1H, td, J = 12.0, 4.3 Hz, H-11β), 1.27 (3H, d, J = 7.1 Hz, H_3-21); ^{13}C NMR ($CDCl_3$, 100.63 MHz) δ 209.5 (C, C-24), 202.6 (C, C-1), 142.5 (CH, C-3), 140.2 (C, C-17), 138.2 (C, C-13), 137.5 (C, C-14), 128.9 (CH, C-2), 128.5 (CH, C-18), 126.8 (CH, C-15), 125.6 (CH, C-16), 71.8 (CH, C-22), 64.6 (C, C-5), 59.0 (CH, C-6), 48.7 (C, C-10), 47.4 (CH_2, C-23), 44.6 (CH, C-20), 36.4 (CH, C-9), 33.6 (CH, C-8), 33.2 (CH_2, C-4), 30.9 (CH_3, C-25), 30.6 (CH_2, C-12), 30.4 (CH_2, C-7), 25.3 (CH_2, C-11), 17.1 (CH_3, C-21), 14.8 (CH_3, C-19); EIMS m/z 394 $[M]^+$ (1), 376 $[M - H_2O]^+$ (19), 336 (21), 308 (49), 307 (100), 290 (9), 171 (10), 117 (12), 91 (9), 55 (6); HREIMS m/z $[M]^+$ 394.2125 (calcd for $C_{25}H_{30}O_4$, 394.2144).

Seed Germination Bioassays. Seeds of *Lactuca sativa* (lettuce) and *Avena sativa* (oats) were obtained from Laboratorio de Semillas (Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Argentina). All undersized and damaged seeds were discarded, and the assay seeds were selected for uniformity. Bioassays were carried out as reported previously.[10a] To a filter paper (Whatman No. 1) in a Petri dish (60 mm diameter) was added a known amount of the test compound in 100% EtOH. Control consisted of EtOH in place of the test solutions. The solvent was evaporated in a desiccator under reduced pressure. There were three replicates for the weeds of each treatment, and parallel controls. After adding 3 mL of H_2O to the dish, 20 plant seeds were sown on the paper. They were incubated at 25 ± 2 °C. After 5 days for *A. sativa* and 7 days for *L. sativa*, inhibition of seed germination was judged by comparing the treated plant with that of the control experiment. The radicle protrusion was taken as a parameter for the test. The growth inhibition test, in which the elongation of the roots and a total plant elongation were measured, was a length of 5 days in *A. sativa* and 7 days in *L. sativa*. Data are reported as percentage differences from control in the table. Thus, zero represents the control, positive values represent stimulation of the parameter studied, and negative values represent inhibition.

Germination and root length values of treated and control experiments were analyzed by the Student's t test ($p < 0.05$).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^1H and ^{13}C NMR spectra of compounds **1**–**7**. This information is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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