New Hydroxylated Withanolides from *Salpichroa origanifolia*

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From the leaves of *Salpichroa origanifolia* three new withanolides, (20*S*,22*R*,24*S*,25*S*,26*R*)-5α,6α:22,26:24,25-triepoxy-15,26-dihydroxy-17(13→18)-*abeo*-ergosta-2,13,15,17-tetraen-1-one (salpichrolide G, **1**), (20*S*,22*R*,24*S*,25*R*)-5α,6α:22,26-diepoxy-24,25,26-trihydroxy-17(13→18)-*abeo*-ergosta-2,13,15,17-tetraen-1-one (salpichrolide H, **2**), and (20*S*,22*R*,25*S*)-5α,6α:22,26-diepoxy-25,26-dihydroxy-17(13→18)-*abeo*-ergosta-2,13,15,17,23-pentaen-1-one (salpichrolide I, **3**), were isolated and characterized by spectroscopic methods and with the aid of molecular modeling. The latter two compounds were obtained as an epimeric mixture at C-26.

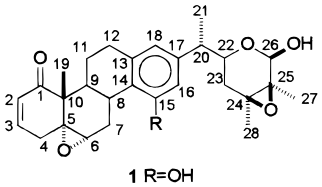
The withanolides are a group of naturally occurring C-28 steroidal lactones and lactols built on an intact or rearranged ergostane framework, isolated from several genera of the Solanaceae.[1] Many of them exhibit biological activity as insecticides, feedant deterrants, and ecdysteroid antagonists, being related to chemical defense mechanisms.[1−3]

Previous studies on extracts of plants of *Salpichroa origanifolia* (Lam.) Thell growing in Argentina showed the presence of four withanolides and two ergostane derivatives closely related to withanolides (salpichrolides A−F).[4−6] The most remarkable structural feature of five of these compounds was an aromatic ring D carrying a side chain displaced from its customary site adjacent the C/D junction of typical steroids. Although a few natural steroids containing a six-membered aromatic ring were known in the withanolide family, they had only been found in the peruvian "shoofly" plant *Nicandra physaloides*, and until these findings, it was thought that they were restricted to this plant.[7,8] Preliminary studies have shown that several salpichrolides have antifeedant and insecticidal properties.

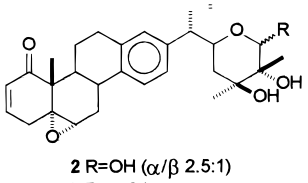
Continuing our investigations on the withanolides of *S. origanifolia*, we now report the isolation of three new withanolides, salpichrolides G (**1**), H (**2**), and I (**3**), structurally related to the compounds previously isolated from this plant. Salpichrolide G was isolated from plants collected in spring, while salpichrolides H and I were obtained from plants collected in winter. The structures of the new compounds were elucidated by spectroscopic methods and with the aid of molecular modeling.

Results and Discussion

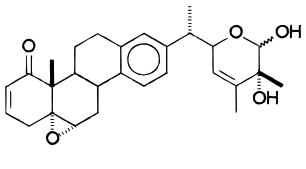
The molecular formula of Salpichrolide G (**1**) was determined by HREIMS as $C_{28}H_{34}O_6$. The EIMS of **1** showed a small molecular ion peak at *m*/*z* 466 (1%) and a peak at 171 (7%) that represented the δ-epoxy lactol side chain.



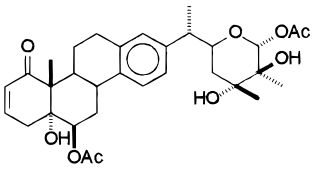
1 R=OH
4 R=H



2 R=OH (α/β 2.5:1)
5 R=α-OAc



3 (α/β 1:3.5)



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The ^{1}H and ^{13}C NMR data of rings A−C and the side chain of **1** (Tables 1 and 2) were closely related to those of salpichrolide A (**4**), indicating that they differed in the substitution pattern of ring D.[4] Its ^{1}H NMR spectrum exhibited only two aromatic hydrogens as broad singlets at δ 6.40 and 6.49, suggesting the presence of a hydroxyl group at C-15. The signal for a nonprotonated carbon at 154.9 ppm in the ^{13}C NMR spectrum assigned to C-15 and the resonances between 112.0 and 142.0 ppm for the other aromatic carbons were in agreement with this structure. Spectral assignments were confirmed by DEPT and COSY-45 spectra.

Salpichrolide H (**2**) was isolated as a 2.5:1 epimeric mixture at C-26 as determined from the ^{1}H NMR (Table 1, see below); the major stereoisomer could be separated by preparative TLC, but it slowly reverted to the epimeric mixture. Compound **2**, $C_{28}H_{36}O_6$, did not show a molecular ion in its mass spectrum, but a peak at *m*/*z* 450 (1) corresponding to the $[M - H_2O]^+$ ion was observed. Another significant peak was at *m*/*z* 307 (40), which represented the loss of ring E from the side chain. The FABMS (thioglycerol, KCl) showed a $[M + K]^+$ ion at 507 (9) that was consistent with the proposed formula. The ^{1}H and ^{13}C NMR chemical shifts for rings

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Table 1. ^{1}H NMR Spectral Data for the Relevant Protons of Compounds **1**−**3**, **5**, and **6** (in $CDCl_3$, δ from TMS, Couplings in Parentheses in Hz)

H	**1**	**2**[a]	**3**[b]	**5**	**6**
2	5.99 dd (10.2, 2.0)	6.00 dd (10.2, 2.2)	6.01 dd (10.1, 2.2)	6.00 dd (10.1, 2.2)	5.94 dd (10.0, 2.5)
3	6.75 ddd (10.2, 5.0, 2.0)	6.75 ddd (10.2, 5.0, 2.2)	6.76 ddd (10.1, 5.0, 2.2)	6.75 ddd (10.1, 5.0, 2.2)	6.65 ddd (10.0, 5.0, 2.5)
4α	1.95 dd (19.4, 5.0)	1.95 dd (19.6, 5.0)	1.95 dd (19.6, 5.0)	1.95 dd (19.6, 5.0)	2.15 dd (19.0, 5.0)
4*β*	3.12 dt (19.4, 2.0)	3.13 dt (19.6, 2.2)	3.14 dt (19.6, 2.2)	3.15 dt (19.6, 2.2)	2.95 dt (19.0, 2.5)
6	3.23 d (5.0)	3.22 d (4.8)	3.23 d (5.0)	3.23 d (4.8)	4.98 br s
7α	1.55 m	1.85 m	1.90 m	1.80 m	1.95 m
7*β*	2.83 m	2.75 m	2.70 m	2.73 m	2.35 m
15		7.10 d (8.0)	7.12 d (8.0)	7.10 d (8.0)	7.12 d (8.0)
16	6.40 br s	6.99 dd (8.0, 1.0)	6.99 dd (8.0, 1.0)	6.99 dd (8.0, 1.0)	6.99 dd (8.0, 1.0)
18	6.49 br s	6.94 d (1.0)	6.94 d (1.0)	6.94 d (1.0)	6.95 d (1.0)
19	1.38 s	1.38 s	1.38 s	1.38 s	1.39 s
20	2.69 m	2.80 m	2.95 m	2.80 m	2.85 m
21	1.20 d (7.2)	1.25 d (7.0)	1.26 d (7.2)	1.26 d (7.0)	1.29 d (7.0)
22	3.83 ddd (14.5, 2.5)	4.23 ddd [4.05 m] (10.6, 6.4, 3.5)	4.38 m	4.18 m	3.80 dt (12.5, 7.0, 3.0)
23α	1.85 dd (14.5, 2.5)	1.60 m	5.37 br s [5.25 br s]	1.60 m	1.44 dd (12.5, 3.0)
23*β*	1.60 t (14.5)	1.60 m		1.60 m	1.58 t (12.5)
26	5.00 br s	4.82 d [4.94 br s] (7.4)	4.97 d (5.2) [4.75 d (5.0)]	5.92 s	5.60 s
27	1.37 s	1.15 s [1.13 s]	1.19 s [1.22 s]	1.16 s	1.24 s
28	1.39 s	1.19 s [1.20 s]	1.75 br s	1.20 s	1.36 s
26-OH	3.25 br s	4.35 d (7.4)	3.05 d (5.2)		
Ac				2.13 s	2.13 s

[a] Chemical shift data correspond to the major epimer (26*S*). Distinct resonances for the 26*R* epimer observed in the spectrum of the epimeric mixture are shown in brackets. [b] Chemical shift data correspond to the major epimer (26*R*). Distinct resonances for the 26*S* epimer observed in the spectrum of the epimeric mixture are shown in brackets.

A−D of compound **2** (Tables 1 and 2) were identical for both stereoisomers and closely related to those of salpichrolide A (**4**). However, differences were observed in the side-chain atoms. For the major stereoisomer, two nonprotonated carbons at 75.0 and 71.3 ppm and a methine at 99.5 ppm in the ^{13}C NMR spectrum suggested the presence of a 24,25-dihydroxy lactol moiety in the side chain probably arising from the hydrolytic cleavage of a 24,25-epoxide. This agreed with the downfield shift of the H-22 resonance from δ 3.84 in salpichrolide A (**4**) to δ 4.23 in the ^{1}H NMR spectrum. Singlets at δ 1.15 and 1.19 were assigned to H-27 and H-28, with the corresponding carbons appearing at 21.5 and 23.9 ppm in the ^{13}C NMR. The COSY-45 spectrum showed correlations between signals at δ 4.82 and 4.35, which were assigned to H-26 and the 26-OH, respectively. The minor stereoisomer of **2** could not be isolated, and its spectral data were obtained from the epimeric mixture. The most remarkable difference in the ^{1}H NMR spectrum was the downfield shift of H-26 to δ 4.94 (Table 1); this chemical shift is almost identical to that found in the other salpichrolides and related epoxy lactols with a 26*R* configuration.[4,5] The chemical shift difference observed for H-26 between both C-26 epimers of **2** is coincident with that found for another pair of C-26 stereoisomeric lactols (physapubescin) isolated from *Physalis pubescens*, suggesting that in this case the major isomer (with the higher chemical shift for H-26) has the 26*S* configuration.[9] The proportion of each epimer in the mixture could be determined from the relative integration of the respective resonances of H-26. A shift of the C-26 resonance was also observed in the ^{13}C NMR spectra of the C-26 epimers (Table 2); in this case, the 26*R* epimer had C-26 at 95.9 ppm, 3.6 ppm upfield from the 26*S* resonance. Differences were also observed for the other carbons of the side chain.

Table 3 shows the relevant correlations observed in the NOESY spectrum of the major stereoisomer of **2** together with the distances between the corresponding hydrogens in the most stable conformer of the proposed structure (AM1, AMPAC 5.0) (Figure 1a). The strong correlation observed for the pair H-26/H-22 is only possible in a 26*S* stereoisomer in which these hydrogens have a 1,3-diaxial relationship.[10] The strong NOE observed between the methyl groups at positions 24 and 25 indicated a diaxial or axial/equatorial diol; however, no correlations were observed for the pairs H-22/H-28 and H-23*β*/H-27. AM1 calculations predicted these distances to be larger than 4.4 Å only in the 24*S*,25*R*,-26*S* stereoisomer (Figure 1a).

DEPT spectral data and the ^{1}H−^{1}H correlations (COSY-45 and NOESY) confirmed the assignments made and the proposed structure for salpichrolide H (**2**). Acetylation of the epimeric mixture of salpichrolide H (Ac_2O/pyridine, 25 °C) gave exclusively the (26*R*)-acetate (**5**) derived from the 26*S* lactol, as determined from the NOESY spectrum, which showed a strong correlation for the H-26/H-22 pair.[11]

Table 2. ^{13}C NMR Spectral Data of Compounds **1**−**3**, **5**, and **6**

C	1	2[a]	3[b]	5	6
1	203.3	202.6	202.6	202.6	202.6
2	128.5	128.9	128.9	128.9	128.9
3	142.8	142.5	142.4	142.5	140.4
4	33.3	33.6	33.6	33.6	35.2
5	64.5	64.7	64.7	64.7	75.6
6	60.6	59.0	59.0	59.0	76.2
7	29.2	30.4	30.4	30.4[c]	30.6
8	32.2	33.2	33.2	33.2	33.0
9	38.3	36.9	36.4	36.4	38.0
10	49.1	48.8	48.8	48.8	52.3
11	24.2	25.4	25.4	25.4	25.9
12	31.5	30.6	30.6	30.6[c]	30.5
13	141.3	137.8	138.0[c]	137.7	137.4
14	124.0	137.2	137.0[c]	137.0	137.3
15	154.9	125.5	125.6	125.7	125.5
16	112.3	126.5	126.4	126.4	125.5
17	140.0	141.1	140.3	141.0	140.0
18	120.8	128.4	128.4	128.6	128.9
19	14.8	14.9	14.9	14.9	14.6
20	42.6	43.6	43.0	42.8	43.2
21	17.0	17.6	16.8	17.0	16.8
22	67.5	68.2 [73.6]	73.6 [78.6]	74.0	75.6
23	33.6	39.0 [38.7]	121.3 [121.8]	38.2	39.3
24	64.8	71.3 [74.7[c]]	136.5[c]	74.2[d]	74.0[c]
25	63.4	75.0 [75.3[c]]	69.5 [71.6]	75.6[d]	76.1[c]
26	91.6	99.5 [95.9]	96.1 [97.7]	95.2	94.5
27	16.5	21.5 [15.5]	15.8 [15.2]	16.1	15.6
28	18.5	23.9 [24.1]	23.7	24.0	22.4
CH_3CO				21.3	21.3, 21.4
CH_3CO				170.3	169.9

[a] Chemical shift data corresponds to the major epimer (26*S*). Distinct resonances for the 26*R* epimer observed in the spectrum of the epimeric mixture are shown in brackets. [b] Chemical shift data corresponds to the major epimer (26*R*). Distinct resonances for the 26*S* epimer observed in the spectrum of the epimeric mixture are shown in brackets. [c,d] Assignments may be interchanged.

To give further support to the above structures, salpichrolide A (**4**) was treated with aqueous sulfuric acid in THF and acetylated to give, after preparative TLC, diacetate **6**. The ^{13}C NMR spectrum of **6** (Table 2) had signals for C-20 to C-28 closely related to those of compound **5**, suggesting a similar substitution pattern; however, the differences observed in the ^{1}H NMR spectrum for hydrogens at positions 23, 26, 27, and 28 (Table 1) suggested a different stereochemistry at the hydroxylated carbons. The NOESY spectrum of **6** showed a strong correlation for the pair H-26/H-22, indicating a 26*R* stereochemistry as in **5** (Table 3). However, correlations were now observed for the pairs H-22/H-28 and H-23β/H-27 while no correlation was observed between the methyl groups (H-27/H-28), indicating a diequatorial 24,25-diol.[12] Thus, **6** was assigned the isomeric stereochemistry 24*R*,25*S*,26*R* (Figure 1b).

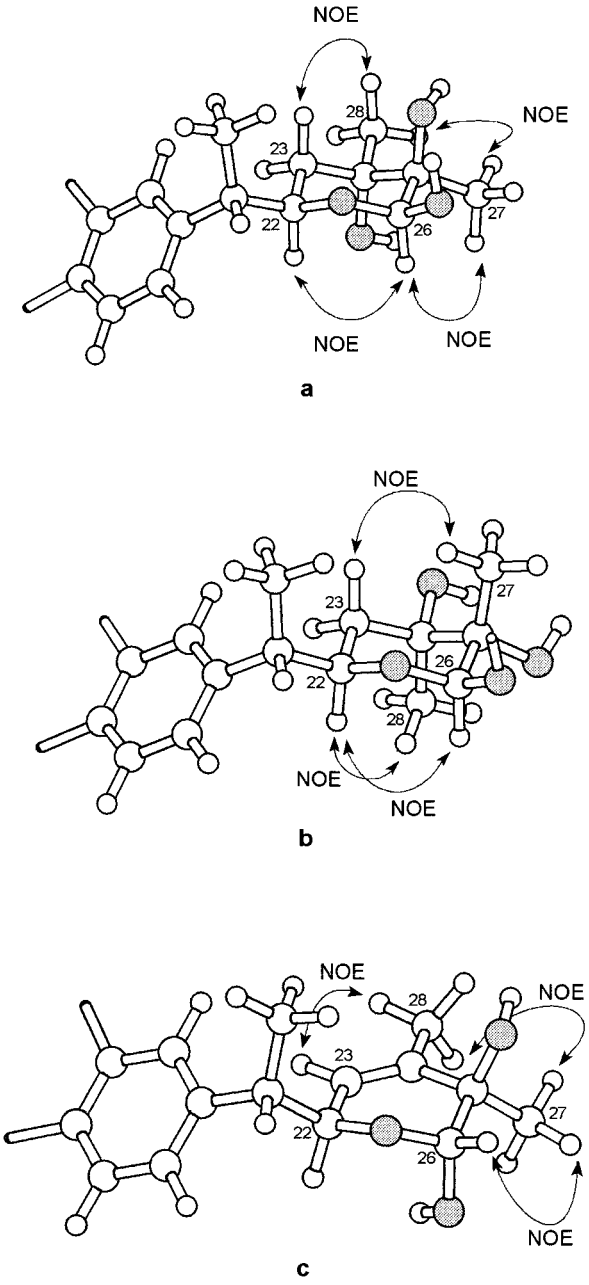

Figure 1. Most stable conformers for ring E of (a) **2** (major epimer, 26*S*), (b) **6** (26-acetate not shown for simplicity), and (c) **3** (major epimer, 26*R*) from AM1 calculations (AMPAC 5.0). Observed NOEs correspond to those shown in Table 3.

Salpichrolide I (**3**) was isolated as a ca. 3.5:1 epimeric mixture at C-26, as determined from the ^{1}H NMR (Table 1, see below). As in the case of salpichrolide H (**2**), this mixture could not be separated. Compound **3** did not

Table 3. Relevant Correlations Displayed by Compounds **2**, **3**, and **6** in the NOESY NMR Spectrum[a]

	2 (26*S*)[b]		**3** (26*R*)[b]		**6**	
H	δ	correlates with δ	δ	correlates with δ	δ	correlates with δ
22	4.23	4.82 (H-26, 2.5)	4.38	5.37 (H-23, 2.6)	3.80	5.60 (H-26, 2.5) 1.36 (H-28, 2.1)
23β	1.60	1.19 (H-28, 2.4)	5.37	1.75 (H-28, 2.4)	1.58	1.24 (H-27, 2.3)
26	4.82	1.15 (H-27, 2.4)	4.97	1.19 (H-27, 2.7)	5.60	1.24 (H-27, 2.6[c])
27	1.15	1.19 (H-28, 2.1)	1.19	1.75 (H-28, 2.4)		

[a] Distances (Å) between interacting hydrogens, from AM1 calculations, are given in parentheses. When more than one hydrogen is present at a given position, the shortest distance is indicated. Interactions between vicinal and geminal hydrogens are not included. [b] Data for **2** and **3** correspond to the major epimer as indicated.[10] [c] This distance measured in the ring E boat conformer of **6** which is 0.8 kcal/mol less stable than the chair conformer (AM1).[12]

show a molecular ion in its mass spectrum but presented a peak at *m/z* 307 (54) corresponding to the loss of ring E and a peak at *m/z* 171 (9) representing the side chain. The FABMS (thioglycerol) showed a $[M - H_2O + 1]^+$ ion at *m/z* 433 (100), in accordance with the proposed structure.

Comparison of the ^1H and ^{13}C NMR spectra of the major epimer of **3** with those of compound **2** (Tables 1 and 2) showed almost identical signals for all nuclei, except those corresponding to the side chain. The presence of a singlet at δ 5.37 in the ^1H NMR spectrum and the signals at δ 121.3, 138.0, and 73.6, which corresponded, respectively, to a methine and two nonprotonated carbons, were in agreement with a Δ^{23}-25-hydroxy functionality in the side chain. Singlets at δ 1.19 and 1.75 were assigned to Me-27 and Me-28, respectively, and H-22 was observed as a broad double doublet at δ 4.38.

The COSY-45, DEPT, and NOESY spectra of **3** confirmed the proposed structure. The NOESY spectrum and molecular modeling calculations (Table 3, Figure 1c) were in accordance with a 26*R* configuration for the major stereoisomer of **3** (no correlation between H-26 and H-22), which is further supported by the H-26 resonance at δ 4.97, identical to that previously reported for the compounds isolated from this plant with 26*R* stereochemistry.[4,5] The minor epimer of **3** (26*S*) had H-26 shifted upfield to δ 4.75 and showed the expected correlation with H-22 in the NOESY NMR spectrum. The spectroscopic data did not allow the unequivocal assignment of the stereochemistry at C-25 of salpichrolide I, as molecular modeling predicted the same NOE correlations for both diastereomers. A 25*R* configuration may be assumed by analogy with the stereochemistry of salpichrolide H (**2**). Thus, **3** may arise in the plant, from elimination of the axial 24-hydroxyl in **2**. Compounds **2** and **3** are, with the exception of physapubescin,[9] the only withanolide lactols isolated as a 26*R*/26*S* mixture.

Experimental Section

General Experimental Procedures. ^1H and ^{13}C NMR spectra were recorded on a Bruker AC-200 NMR spectrometer at 200.13 and 50.32 MHz, respectively. Multiplicity determinations (DEPT) and 2D spectra (COSY-45, HETCOR, NOESY) were obtained using standard Bruker software. Chemical shifts are given in ppm downfield from TMS as internal standard. EIMS were collected on a VG Trio-2 mass spectrometer at 70 eV by direct inlet; FABMS and HREIMS (70 eV) were measured on a VG ZAB-BEQQ mass spectrometer. IR and UV spectra were measured on a Nicolet Magna 550 FT IR and a Hewlett-Packard 8451A spectrophotometer, respectively. AM1 calculations were performed with AMPAC 5.0 (Semichem). Melting points were taken on a Fisher-Johns apparatus and are uncorrected. Column chromatography was performed on Kieselgel 60-G (Merck) and on Kieselgel S 0.032−0.063 mm. TLC analysis was performed on Si gel 60 F254 (0.2 mm thick).

Plant Material. Aerial parts of *S. origanifolia* were collected in spring (November 1995) and in winter (August 1996) in the surroundings of the University campus in Buenos Aires, Argentina and processed separately. A voucher specimen is deposited at the Museo Botánico, Universidad de Córdoba under no. CORD 254.

Extraction and Isolation. Fresh leaves and stems (500 g) were triturated and extracted succesively with ether and ethanol at room temperature. The residue obtained after evaporation of the combined extracts was chromatographed on Kieselgel 60-G. Elution with hexanes−EtOAc mixtures of increasing polarity (100:0−0:100) afforded six fractions containing withanolides. The fractions eluted with hexanes−EtOAc from 40:60 to 0:100 were further fractionated by flash chromatography on silica gel yielding salpichrolide A (**4**) as the major component. After purification of the minor fractions by preparative TLC, salpichrolide G (**1**) (10 mg) was isolated from the extract of the plants collected in spring and salpichrolide H (**2**) (8 mg) and salpichrolide I (**3**) (7 mg) were isolated from the extract of the plants collected in winter.

Salpichrolide G (1): white crystals (EtOAc−hexane); mp 155−156 °C; UV (MeOH) λ_{max} 216, 362 nm; IR (dry film) ν_{max} 3423, 1685, 732 cm^{-1}; ^1H NMR ($CDCl_3$) see Table 1; ^{13}C NMR ($CDCl_3$) see Table 2; EIMS *m/z* $[M]^+$ 466 (1), 448 (2), 324 (7), 306 (7), 171 (7), 123 (10); HREIMS *m/z* found $[M]^+$ 466.2355 ($C_{28}H_{34}O_6$ requires 466.2355); FABMS (*m*-nitrobenzyl alcohol, KCl) *m/z* $[M + K]^+$ 505 (28).

Salpichrolide H (2): amorphous solid; UV (MeOH) λ_{max} 216, 276, 362 nm; IR (dry film) ν_{max} 3412, 1692, 917, 720 cm^{-1}; ^1H NMR ($CDCl_3$) see Table 1; ^{13}C NMR ($CDCl_3$) see Table 2; EIMS *m/z* $[M - H_2O]^+$ 450 (1), 432 (1), 308 (75), 307 (40), 290 (31), 171 (25), 157 (30); FABMS (*m*-nitrobenzyl alcohol, KCl) *m/z* $[M + K]^+$ 507 (9).

Acetylation of Salpichrolide H (2). Salpichrolide H (**2**) (4 mg) was dissolved in Ac_2O/pyridine (1:1, 0.1 mL) and left for 4 h at 25 °C. Dilution with ethanol and evaporation under a stream of nitrogen afforded acetate **5** as a white amorphous solid: ^1H NMR ($CDCl_3$) see Table 1; ^{13}C NMR ($CDCl_3$) see Table 2.

Preparation of Compound 6 from 4. Salpichrolide A (**4**) (50 mg) was dissolved in THF (3 mL), 1.5 N H_2SO_4 (0.2 mL) was added, and the reaction mixture stirred for 6 h at 25 °C. Neutralization with aqueous $KHCO_3$ and extractive workup, followed by acetylation with Ac_2O/pyridine (1:1, 1 mL) and purification by preparative TLC, afforded compound **6**: ^1H NMR ($CDCl_3$) see Table 1; ^{13}C NMR ($CDCl_3$) see Table 2.

Salpichrolide I (3): amorphous solid; UV (MeOH) λ_{max} 225, 268, 364 nm; IR (dry film) ν_{max} 3405, 2922, 1692, 1051, 734 cm^{-1}; ^1H NMR ($CDCl_3$) see Table 1; ^{13}C NMR ($CDCl_3$) see Table 2; EIMS *m/z* $[M - \text{ring E}]^+$ 307 (54) 290 (8), 171 (9), 143 (20); FABMS (glycerol) $[M - H_2O + 1]^+$ 433 (100).

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(10) The NOESY spectrum of the epimeric mixture of **2** did not show a correlation peak for the H-26/H-22 pair of the minor stereoisomer (26*R*).
(11) Acetate **5** showed in its NOESY spectrum the same correlations as the major epimer of **2** (26*S*). AM1 calculations did not show significant differences between the most stable conformers of both compounds.
(12) A correlation was also observed for the pair H-26/H-27 of **6**. Although these hydrogens are not expected to show NOE in the structure shown in Figure 1b, they are only 2.5 Å apart in the boat conformer of ring E, which is only 0.8 kcal/mol less stable than the chair (AM1). In **2**, the boat conformer is ca. 3 kcal/mol less stable than the chair and is not expected to contribute significantly.

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