# Withanolides from *Salpichroa origanifolia*[1]

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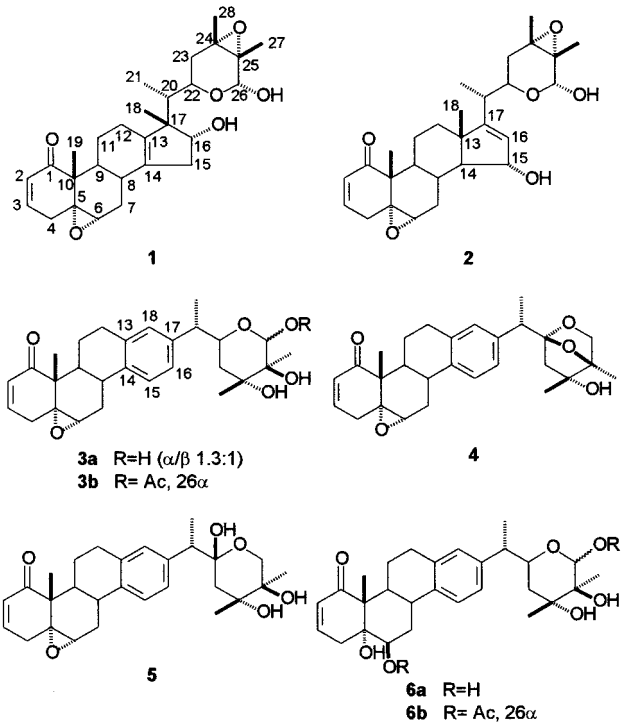
Five new withanolides, 5α,6α:22,26:24,25-triepoxy-16α,26-dihydroxy-18(13→17)-*abeo*-ergosta-2,13-dien-1-one (salpichrolide N, **1**), 5α,6α:22,26:24,25-triepoxi-15α,26-dihydroxyergosta-2,16-dien-1-one (salpichrolide L, **2**), 5α,6α:22,26-diepoxi-24,25,26-trihydroxy-17(13→18)-*abeo*-ergosta-2,13,15,17-tetraen-1-one (salpichrolide M, **3a**), 5α,6α:22,25:22,26-triepoxy-24-hydroxy-17(13→18)-*abeo*-ergosta-2,13,15,17-tetraen-1-one (salpichrolide J, **4**), and 5α,6α:22,26-diepoxy-22,24,25-trihydroxy-17(13→18)-*abeo*-ergosta-2,13,15,17-tetraen-1-one (salpichrolide K, **5**), were isolated from the leaves of *Salpichroa origanifolia* and characterized by a combination of spectroscopic (1D and 2D NMR, MS) and chemical methods.

Withanolides are a group of naturally occurring steroids built on an ergostane skeleton, in which C-22 and C-26 are appropriately oxidized in order to form a δ-lactone ring. Biogenetic transformations, however, can produce highly modified compounds, both at the steroid nucleus and at the side chain. Such compounds have been described as withasteroids.[2] As part of a program aimed at the discovery of novel withanolides from species of the Solanaceae family growing in Argentina, we have previously reported the isolation of nine withanolides and two ergostane derivatives closely related to withanolides from *Salpichroa origanifolia* (Lam.) Thell growing in the provinces of Cordoba and Buenos Aires (central and east Argentina, respectively).[3−6] Several of these showed antifeedant activity on neonatae larvae of *Musca domestica*.[7]

We now report the isolation of five new withanolides from *S. origanifolia* collected in the province of Salta (northwest of the country), salpichrolides N (**1**), L (**2**), M (**3a**), J (**4**), and K (**5**), and their structure elucidation by spectroscopic and chemical methods with the aid of molecular modeling.




## Results and Discussion

The HREIMS of salpichrolide N (**1**) showed a molecular ion corresponding to the molecular formula $C_{28}H_{38}O_6$. The $^1H$ and $^{13}C$ NMR data of rings A and B and the side chain (see Experimental Section) were closely related to those of salpichrolide A;[3] however no signals from an aromatic D ring were observed. The $^{13}C$ NMR, as well as the DEPT experiments, which sorted 28 carbons into five methyls, six methylenes, nine methines, and eight nonprotonated carbons, were in accordance with the molecular formula. The presence in the $^{13}C$ NMR spectrum (Table 2) of two nonprotonated carbons at δ 134.6 and δ 138.1 and a methine at δ 82.5, which correlated to the proton at δ 4.04 in the HMQC spectrum, suggested the presence of a tetrasubstituted double bond and a hydroxylated carbon in ring D. The singlet at δ 1.11 was assigned to the 18-methyl and correlated to the carbon signal at δ 27.4 in the HMQC; however the HMBC data indicated that this methyl was not in its normal position. The HMBC experiment displayed correlations between H-18 (δ 1.11) and carbons at δ 138.1 (C-13), 82.5 (C-16), and 41.4 (C-20), which were in agreement with the presence of a $\Delta^{13}$-16-hydroxy arrangement in ring D with the angular methyl shifted to C-17. The other correlations shown in the COSY 45, HMQC, and HMBC spectra confirmed the assignments made and the structure proposed for compound **1**. A similar rearranged structure has been found in a compound related to withanolides isolated from *Tubocapsicum anomalum*.[8]

The stereochemistry at C-16 and C-17 was deduced from the 500 MHz NOESY spectrum. Thus, the correlation for the pair H-18 (δ 1.113)/H-16 (δ 4.045) indicated that the 16-hydroxyl and the methyl group (H-18) were on opposite sides of ring D. Furthermore, NOE correlations of H-15β (δ 2.395) with H-7β, H-8β, and H-16 indicated that the latter proton was on the β-face of the steroid and allowed unambiguous assignment of the α-stereochemistry to the 16-hydroxyl and in consequence the β-orientation for the methyl group at C-17 (Figure 1, Supporting Information).

The structure of salpichrolide N (**1**) is particularly interesting regarding the biosynthesis of withanolides with

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**Table 1.** $^1$H NMR Spectral Data for Relevant Protons of Compounds **2**−**6** (chemical shifts ($\delta$) downfield from TMS, *J* couplings (in parentheses) in Hz)

| H | **2**[b] | **3a**[b,e] | **3b**[b] | **4**[a] | **5**[b] | **6a**[b,e] |
|---|---|---|---|---|---|---|
| 2 | 5.95 dd (10.1, 2.3) | 6.00 dd (10.0, 2.6) | 6.00 dd (10.0, 2.5) | 6.00 dd (10.2, 2.2) | 6.00 dd (10.1,2.2) | 5.86 dd (10.0, 2.4) |
| 3 | 6.70 ddd (10.1, 5.0, 2.3) | 6.76 ddd (10.0, 5.0, 2.6) | 6.75 ddd (10.0, 5.0, 2.5) | 6.76 ddd (10.2, 5.0, 2.2) | 6.75 ddd (10.1, 5.0, 2.2) | 6.69 ddd (10.0, 5.2, 2.4) |
| 4α | 1.85 dd (19.5, 5.0) | 1.90 dd (19.2, 5.0) | 1.90 dd (19.2, 5.0) | 1.90 dd (19.6, 5.0) | 1.90 dd (19.6, 5.0) | 2.13 dd (20.0, 5.2) |
| 4*β* | 3.12 dt (19.5, 2.3) | 3.13 dt (19.2, 2.6) | 3.13 dt (19.2, 2.5) | 3.14 dt (19.6, 2.2) | 3.15 dt (19.6, 2.2) | 3.25 dt (20.0, 2.4) |
| 6 | 3.08 d (5.0) | 3.22 d (4.6) | 3.23 d (4.8) | 3.22 d (4.8) | 3.23 d (4.0) | 3.78 br s |
| 7α | 1.70 m | 1.90 m | | 1.84 m | 1.84 m | |
| 7*β* | 2.15 m | 2.70 m | | 2.65 m | 2.65 m | |
| 14 | 1.20 m | | | | | |
| 15 | 4.51 d (8.5) | 7.11 d (8.0) | 7.10 d (8.0) | 7.10 br s | 7.12 br s | 7.20 d (8.0) |
| 16 | 5.39 br s | 6.98 dd (8.0, 1.0) | 6.99 dd (8.0, 1.0) | 7.10 br s | 7.12 br s | 7.00 dd (8.0, 1.0) |
| 18 | 0.82 s | 6.91 d (1.0) | 6.94 d (1.0) | 6.99 br s | 7.00 br s | 6.98 d (1.0) |
| 19 | 1.40 s | 1.38 s | 1.38 s | 1.37 s | 1.38 s | 1.38 s |
| 20 | 2.10 m | 2.78 m [2.88 m] | | 3.05 q (7.0) | 2.81 q (7.0) | |
| 21 | 1.01 d (7.1) | 1.23 d (7.0) | 1.25 d (6.9) | 1.36 d (7.0) | 1.35 d (7.0) | 1.23 d (7.0) |
| 22 | 3.70 ddd (10.6, 6.9, 3.7) | 4.13 m [3.67 m] | 3.80 m | | | 4.13 m [3.65 m] |
| 23α | 1.85 m | 1.55 m | | 1.73 *d*[c] (12.9) | 1.54 bs | |
| 23*β* | 1.55 m | 1.73 m [1.55 m] | | 1.70 *d*[c] (12.9) | 1.54 bs | |
| 26α | 4.96 d (9.0) | 4.92 br s [4.60 br s] | 5.57 s | 4.38 d (6.7) | 4.12 d (12.1) | 4.89 br s [4.61 br s] |
| 26*β* | | | | 3.31 d (6.7) | 3.28 d (12.1) | |
| 27 | 1.41 s | 1.43 s [1.35 s] | 1.24 s | 1.35 s[d] | 1.19 s[d] | 1.45 s [1.36 s] |
| 28 | 1.39 s | 1.38 s [1.21 s] | 1.38 s | 1.25 s[d] | 1.12 s[d] | 1.38 s [1.21 s] |
| 26-OH | 3.45 d (9.0) | | | | | |
| AcO | | | 2.14 s | | | |

[a] 500.13 MHz. [b] 200.13 MHz. [c] Asigned by $^1$H selective decoupling in the $^{13}$C NMR spectra; assignments may be interchanged. [d] Assignments are based on COSY LR correlations. [e] 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 square brackets.

**Table 2.** $^{13}$C NMR Spectral Data of Compounds **1** ($CDCl_3$, 100.61 MHz) and **2**−**6** ($CDCl_3$, 50.32 MHz)

| C | **1** | **2** | **3a**[a] | **3b** | **4** | **5** | **6a**[a] |
|---|---|---|---|---|---|---|---|
| 1 | 203.4 | 202.5 | 202.7 | 202.1 | 202.4 | 203.0 | 203.4 |
| 2 | 128.8 | 129.0 | 128.9 | 128.9[d] | 128.9 | 128.9 | 128.1[e] |
| 3 | 142.6 | 142.1 | 142.5 | 142.4 | 142.5 | 142.5 | 142.3 |
| 4 | 33.7 | 34.2 | 33.6 | 33.6 | 33.6 | 33.6 | 35.0 |
| 5 | 65.2 | 64.9 | 64.6 | 64.7 | 64.7 | 64.7 | 76.2 |
| 6 | 59.5 | 58.7 | 59.0 | 59.0 | 59.1 | 59.0 | 74.1 |
| 7 | 27.5 | 29.7 | 30.3 | 30.6 | 30.6 | 30.6[b] | 33.5 |
| 8 | 32.5 | 36.2 | 33.2 | 33.2 | 33.3 | 33.3 | 32.3 |
| 9 | 36.6 | 38.4 | 36.4 | 36.4 | 36.4 | 36.4 | 38.0 |
| 10 | 48.4 | 48.7 | 48.7 | 48.8 | 48.8 | 48.8 | 52.1 |
| 11 | 25.0 | 22.1 | 25.4 | 25.4 | 25.4 | 25.4 | 25.7 |
| 12 | 25.8 | 33.7 | 30.6 | 30.4 | 30.4 | 30.4[b] | 30.5 |
| 13 | 138.1 | 47.2 | 137.7 [137.9] | 137.9 | 138.0 | 137.3 | 138.3 |
| 14 | 134.6 | 65.2 | 137.1 | 137.1 | 137.0 | 138.0 | 137.2 |
| 15 | 41.1 | 78.2 | 125.4 [125.7] | 125.7 | 126.1 | 126.6 | 125.2 [125.0] |
| 16 | 82.5 | 127.8 | 126.5 | 126.4 | 126.4 | 126.9 | 125.3 |
| 17 | 53.8 | 157.6 | 141.3 [140.8] | 139.8 | 139.4 | 139.4 | 140.6 |
| 18 | 27.4 | 18.9 | 128.2 [128.3] | 128.7[d] | 128.8 | 130.0 | 127.9[e] |
| 19 | 15.0 | 15.7 | 14.8 | 14.9 | 14.9 | 14.9 | 14.0 |
| 20 | 41.4 | 28.9 | 43.9 [43.6] | 43.1 | 42.9 | 42.9 | 43.5 |
| 21 | 13.2 | 17.0 | 18.0 [17.1] | 16.7 | 15.7 | 15.6 | 17.5 [16.8] |
| 22 | 66.4 | 66.3 | 71.7 [75.9] | 76.1 | 111.9 | 99.1 | 71.1 [75.5] |
| 23 | 35.8 | 34.7 | 40.2 [39.8] | 39.2 | 51.2 | 53.4 | 40.2 [40.0] |
| 24 | 63.7 | 64.7 | 73.7 [74.2] | 74.0 | 76.3 | 72.5[c] | 73.5 [73.7] |
| 25 | 65.0 | 63.8 | 76.5 [73.0] | 75.6 | 86.3 | 71.5[c] | 76.2 [72.7] |
| 26 | 91.5 | 91.6 | 97.2 [96.7] | 94.9 | 70.6 | 66.0 | 97.0 [96.2] |
| 27 | 16.4 | 16.5 | 20.9 [14.8] | 16.0 | 12.1 | 17.1 | 20.5 [14.4] |
| 28 | 18.9 | 19.1 | 24.2 [22.7] | 22.6 | 26.5 | 22.9 | 23.6 [22.0] |
| $CH_3CO$ | | | | 21.1 | | | |
| $CH_3CO$ | | | | 170.0 | | | |

[a] Chemical shift data correspond to the major epimer (26*S**). Distinct resonances for the 26*R** (minor) epimer observed in the spectrum of the epimeric mixture are shown in square brackets. [b−f] Assignments may be interchanged.

an aromatic D ring. Whiting[9] has proposed as a possible pathway to ring D aromatization the oxidation of C-18 followed by a 1,2-shift of C-17 to form a new six-membered ring via a cyclopropyl fused intermediate; this would lead to salpichrolide A and related compounds upon cleavage of the C-13−C-17 bond (Figure 2, pathway a, Supporting Information). The cleavage of the C-13−C-18 bond of the cyclopropyl intermediate would result in migration of the angular methyl (C-18) to C-17 via a 13,15-diene intermediate to yield salpichrolide N (**1**) (Figure 2, pathway b, Supporting Information). Salpichrolide L (**2**) described below may be the precursor of a putative 14,16-diene intermediate.

The $^1$H and $^{13}$C NMR spectra of salpichrolide L (**2**) showed the same pattern as those of rings A and B and the side chain moiety of salpichrolide N (Tables 1 and 2). Absence of the characteristic signals of an aromatic D ring and the presence of a singlet at $\delta$ 0.82 assigned to $CH_3$-18

were indicative of a nonrearranged ergostane skeleton. The doublet at $\delta$ 4.51 ($J$ = 8.5 Hz) and the broad singlet at $\delta$ 5.39 in the $^1$H NMR spectrum in conjunction with the $^{13}$C NMR data of ring D showed a close similarity with the 15-hydroxylated withanolides nicaphysalin B and C, isolated from *Nicandra physaloides*.[10] The $^{13}$C resonances at $\delta$ 78.2, 127.8, and 157.6, assigned to C-15, C-16, and C-17, respectively, and the correlation peaks for the pairs H-15($\delta$ 4.51)/H-16($\delta$ 5.39) and H-14($\delta$ 1.20)/H-15($\delta$ 4.51) observed in the COSY 45 spectrum, supported the existence of a $\Delta^{16}$-15-hydroxy functionality. The α-stereochemistry for the 15-hydroxy group was assigned by comparison with the NMR data of nicaphysalins B and C. FABMS (thioglycerol, $K_2CO_3$) showed a $[M + K]^+$ quasimolecular ion at $m/z$ 509 (100), in accordance with the proposed structure.

Salpichrolide M (**3a**) was isolated as a nonresolvable 1.3:1 epimeric mixture at C-26 as determined from the $^1$H NMR spectrum upon integration of the H-26 signals at $\delta$ 4.92 and 4.60 (Table 2). The HREIMS showed a molecular ion corresponding to the molecular formula $C_{28}H_{36}O_6$. The $^1$H and $^{13}$C NMR chemical shifts for rings A−D of compound **3a** (Tables 1 and 2) were almost identical for both stereoisomers. The presence of signals at $\delta$ 73.7 (C-24), 76.5 (C-25), and 97.2 (C-26) in the $^{13}$C NMR spectrum corresponding to the major isomer of compound **3a** indicated a 24,25-dihydroxylactol functionality closely related to salpichrolide H, previously isolated also as an epimeric mixture at C-26, from plants of *Salpichroa origanifolia* growing in Buenos Aires.[6] However, the differences observed in the $^1$H NMR data of compound **3a** and salpichrolide H for hydrogens at positions 22, 26, and 28 suggested a different stereochemistry at the hydroxylated carbons. The chemical shifts corresponding to the side chain of **3a** were almost identical to those observed in the $^1$H and $^{13}$C NMR spectra of the synthetic derivative **6a** (Tables 1 and 2), a stereoisomer of salpichrolide H at C-24 and C-25 obtained by treatment of salpichrolide A with aqueous sulfuric acid in THF. We have previously described[6] that acetylation of **6a** ($Ac_2O$, pyridine, 25 °C) gave exclusively the (26*R*)-acetate (**6b**), which was assigned the stereochemistry 24*R*,25*S*,26*R* on the basis of NOESY spectral data and molecular modeling calculations; acetylation of the epimeric mixture of salpichrolide M (**3a**) also gave a single product, **3b** (Tables 1 and 2), almost identical in the side chain to compound **6b**;[6] thus **3a** was assigned the 24*R**,25*S** stereochemistry. Withanolides isolated as 26*R*/26S mixtures have been reported only from *Salpichroa origanifolia*[6] and *Physalis pubescens*.[11]

The $^1$H and $^{13}$C NMR data (Tables 1 and 2) of salpichrolide J (**4**) indicated that this compound differed from salpichrolide A in the substitution pattern of the side chain.[3] There were no signals corresponding to lactol or lactone, characteristic of most withanolides. The $^{13}$C NMR complemented by DEPT spectra (Table 2) showed the presence of three methyls, two methylenes, one methine, and three nonprotonated carbons in the side chain. Signals observed at $\delta$ 76.3 (C), 86.3 (C), and 70.6 ($CH_2$) corresponded to three oxygenated carbons and were assigned to C-24, C-25, and C-26, respectively, while the nonprotonated carbon resonance at $\delta$ 111.9 was assigned to C-22; the high chemical shift value of the latter carbon suggested the presence of a ketal functionality. In the $^1$H NMR spectrum (Table 1) the two mutually coupled doublets at $\delta$ 4.38 and 3.31 ($J$ = 6.7 Hz) were attributed to $CH_2$-26. Singlets at $\delta$ 1.37, 1.35, and 1.25 were assigned to methyls 19, 27, and 28, respectively, based on the correlations peaks observed in the long-range $^1$H−$^1$H COSY spectrum for the pairs H-27($\delta$ 1.35)/H-26($\delta$ 4.38) and H-28($\delta$ 1.25)/H-23($\delta$ 1.70−1.73). The doublet at $\delta$ 1.36 ($J$ = 7.0 Hz) assigned to $CH_3$-21 correlated in the COSY 45 spectrum with the quartet at $\delta$ 3.05 attributed to H-20. In the $^{13}$C NMR the assignment of C-23 at $\delta$ 51.2 ($CH_2$) was confirmed by selective irradiation of the $^1$H AB quartet centered at $\delta$ 1.72. The HREIMS of **4** showed the $[M]^+$ ion corresponding to the molecular formula $C_{28}H_{34}O_5$, and the EIMS spectrum showed peaks at $m/z$ 307 (9%) and 143 (5%) due to cleavage of the C-20−C-22 bond with loss of the cyclic ketal ring, which confirmed the proposed structure.

Molecular modeling calculations and the NOESY spectrum of compound **4** showed that the stereoisomers 22*S**,24*S**,25*R** and 22*S**,24*R**,25*R** could only be distinguished by the NOE correlation between H-26 and H-28. The NOESY spectrum showed correlations between the pairs H-23/H-28, H-27/H-28, and H-26/H-27, but no correlation was observed for the pair H-26/H-28, which indicated that compound **4** would have the stereochemistry 22*S**,24*R**,25*R** (Figure 3a, Supporting Information). It should be noted that the configuration at C-24 and C-25 is coincident with that of **3a**.

Salpichrolide K (**5**) had $^1$H and $^{13}$C NMR similarly related to those of compound **4**, the main difference being the upfield shift of C-22 and C-26 to $\delta$ 99.1 and 66.0, respectively, in the $^{13}$C NMR spectrum. This was attributed to the presence of a 26→22 cyclic hemiketal ring, which was in agreement with the fact that compound **5** slowly cyclized to **4** in solution. In the $^1$H NMR, the mutually coupled doublets at $\delta$ 4.12 and 3.28 ($J$ = 12.1 Hz) were assigned to $CH_2$-26, and the singlets at $\delta$ 1.19 and 1.12 were attributed to $CH_3$-27 and $CH_3$-28, respectively, based on the cross-peak observed at $\delta$ 1.19 and 3.28 (H-26) in the long-range $^1$H−$^1$H COSY spectrum. The COSY 45 spectrum also showed a correlation peak for signals at $\delta$ 1.35 and 2.81 assigned to $CH_3$-21 and CH-20, respectively. Mass measurements were in accordance with the structural assignments: the EIMS showed a small molecular ion peak at $m/z$ 468 (1%) and peaks at $m/z$ 307 (25%) and 143 (5%), which corresponded to cleavage between C-20 and C-22 and the loss of the hemiketal ring; the HREIMS showed a molecular ion corresponding to $C_{28}H_{38}O_6$.

Considering the relation between **5** and **4**, it was evident that both compounds should have the same stereochemistry at C-24 and C-25. The NOESY spectrum of **5** showed correlations between the pairs H-26eq/H-27 and H-26ax/H-28, these correlations being possible only for configuration 22*R**,24*R**,25*R** (Figure 3b, Supporting Information), in agreement with the stereochemistry assigned to compound **4**.

A common feature of most withanolides is the oxidation levels of C-22 and C-26, C-26 being oxidized in most instances to the carboxylic acid level, thus allowing the formation of a 22,26-lactone. In some withanolides (e.g., most salpichrolides) it is at the aldehyde level, allowing the formation of a 22,26-lactol. Salpichrolides J (**4**) and K (**5**) are, to our knowledge, the first withanolides to be reported with a side chain in which oxidation levels at C-22 and C-26 are reversed.

## Experimental Section

**General Experimental Procedures.** $^1$H and $^{13}$C NMR spectra were recorded on a Bruker AC-200 NMR spectrometer at 200.13 and 50.32 MHz, a Bruker AM-500 at 500.13 and 125.77 MHz, or a Bruker DPX-400 at 400.13 and 100.61 MHz. Multiplicity determinations (DEPT) and 2D spectra (COSY 45, COSY LR, NOESY, HMQC, and HMBC) were obtained using standard Bruker software. Chemical shifts are given in ppm

(δ) 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 Hyperchem 5.1. Melting points were taken on a Fisher-Johns apparatus and are uncorrected. Vacuum liquid chromatography (VLC) and column chromatography were carried out on Kieselgel 60-G (Merck) and Kieselgel S 0.040−0.063 mm, respectively. TLC analysis was performed on silica gel 60 F254 (0.2 mm thick).

**Plant Material.** Aerial parts of *S. origanifolia* were collected in the summer (1996) in Salta, Argentina. A voucher specimen is deposited at the Museo Botánico, Universidad de Córdoba [CORD].

**Extraction and Isolation.** Fresh leaves and stems (1500 g) were triturated and extracted successively with ether and EtOH at room temperature. The residue obtained after evaporation of the combined extracts was chromatographed on Kieselgel 60-G. Elution with hexane−EtOAc mixtures of increasing polarity (100:0−0:100) afforded three fractions containing withanolides. These fractions were further fractionated using a combination of chromatographic techniques including flash chromatography and preparative TLC. This led to the isolation of the known withanolides salpichrolide A[3] (800 mg) and C[4] (40 mg) and of salpichrolide N (**1**) (4 mg), salpichrolide L (**2**) (12 mg), salpichrolide M (**3**) (10 mg), salpichrolide J (**4**) (8 mg), and salpichrolide K (**5**) (3 mg). Known compounds were identified by comparison ($^1$H and $^{13}$C NMR spectra, TLC) with authentic standards.

**Salpichrolide N (1):** white crystals (EtOAc−hexane), mp 157−159 °C; $[\alpha]^{25}_D$ −6.0° (c 0.05, MeOH); UV (MeOH) $\lambda_{max}$ 228 nm; IR (dry film) $\nu_{max}$ 3394, 2925, 1687, 1072, 1043 cm$^{-1}$; $^1$H NMR data (500.13 MHz) δ 6.737 (1H, ddd, *J* = 10.1, 5.0, 2.3 Hz, H-3), 5.976 (1H, dd, *J* = 10.1, 2.3 Hz, H-2), 4.996 (1H, br s, H-26), 4.045 (1H, br t, *J* = 8.1 Hz, H-16), 3.626 (1H, ddd, *J* = 11.1, 8.1, 2.7 Hz, H-22), 3.130 (1H, d, *J* = 5.0 Hz, H-6), 3.078 (1H, dt, *J* = 19.4, 2.3 Hz, H-4β), 2.48 (1H, m, H-15α), 2.445 (1H, dt, *J* = 11.0, 2.8 Hz, H-12β), 2.395 (1H, dd, *J* = 15.1, 8.1 Hz, H-15β), 2.098 (1H, dt, *J* = 14.8, 5.0, H-7β), 2.042 (1H, dd, *J* = 14.3, 2.7 Hz, H-23α), 1.992 (2H, m, H-11), 1.978 (1H, m, H-8), 1.883 (1H, dt, *J* = 2.0, 12.3 Hz, H-9), 1.853 (1H, dd, *J* = 19.5, 5.0 Hz, H-4α), 1.832 (1H, br quintet, *J* = 7.3 Hz, H-20), 1.769 (1H, dd, *J* = 14.8, 11.9 Hz, H-7α), 1.633 (1H, dd, *J* = 14.3, 11.1 Hz, H-23β), 1.395 (3H, s, H-27), 1.387 (3H, s, H-28), 1.299 (3H, s, H-19), 1.113 (3H, s, H-18), 1.041 (1H, td, *J* = 11.0, 3.0 Hz, H-12α), 0.92 (3H, d, *J* = 7.3 Hz, H-21); $^{13}$C NMR data (100.61 MHz), Table 2; EIMS *m/z* 470 (0.6) $[M]^+$, 452 (1, M − $H_2O$), 434 (1), 282 (10), 171 (7), 143 (7), 109 (10); FABMS (glycerol, $K_2CO_3$) *m/z* 509 (100) $[M + K]^+$; HREIMS *m/z* 470.2662 (calcd for $C_{28}H_{38}O_6$, 470.2668).

**Salpichrolide L (2):** white crystals (EtOAc−hexane), mp 159−161 °C; $[\alpha]^{25}_D$ +36.0° (*c* 0.05, MeOH); UV (MeOH) $\lambda_{max}$ 220 nm; IR (dry film) $\nu_{max}$ 3422, 2930, 1690, 1034 cm$^{-1}$; $^1$H NMR data (200.13 MHz), Table 1; $^{13}$C NMR data (50.32 MHz), Table 2; EIMS *m/z* 452 (1, M − $H_2O$), 387 (1), 310 (3), 322 (1), 171 (3), 143 (3); FABMS (thioglycerol, $K_2CO_3$) *m/z* 509 (100) $[M + K]^+$; HREIMS *m/z* 452.2570 (calcd for $C_{28}H_{36}O_5$, 452.2563).

**Salpichrolide M (3a):** amorphous solid; $[\alpha]^{25}_D$ −50.8° (*c* 0.05, MeOH); UV (MeOH) $\lambda_{max}$ 222, 268, 276 nm; IR (dry film) $\nu_{max}$ 3429, 2937, 1690, 1070, 742 cm$^{-1}$; $^1$H NMR data (200.13 MHz), Table 1; $^{13}$C NMR data (50.32 MHz), Table 2; EIMS *m/z* 468 (0.6) $[M]^+$, 450 (1, M − $H_2O$), 307 (26), 263 (2), 193 (2), 171 (6), 143 (6), 109 (5); HREIMS *m/z* 468.2519 (calcd for $C_{28}H_{36}O_6$, 468.2512).

**Acetylation of Salpichrolide M (3a).** Salpichrolide N (**3a**) (4 mg) was dissolved in $Ac_2O$−pyridine (1:1, 0.1 mL) and left for 4 h at 25 °C. Dilution with EtOH and evaporation under a stream of nitrogen afforded acetate **3b** as an amorphous solid: $^1$H NMR data (200.13 MHz), Table 1; $^{13}$C NMR data (50.32 MHz), Table 2.

**Preparation of 6a and 6b.** Salpichrolide A (50 mg) was dissolved in THF (3 mL), and 1.5 N $H_2SO_4$ (0.2 mL) was added; the reaction mixture was stirred for 6 h at 25 °C. Neutralization with aqueous $KHCO_3$ and extractive workup afforded compound **6a**: $^1$H NMR data (200.13 MHz), Table 1; $^{13}$C NMR data (50.32 MHz), Table 2. Acetylation with $Ac_2O$−pyridine as previously described[6] afforded, after purification by PTLC, compound **6b** as an amorphous solid.

**Salpichrolide J (4):** white crystals (EtOAc−hexane), mp 172−173 °C; $[\alpha]^{25}_D$ −25.0° (*c* 0.04, MeOH); UV (MeOH) $\lambda_{max}$ 218, 276 nm; IR (dry film) $\nu_{max}$ 3427, 2930, 1690, 1084, 742 cm$^{-1}$; $^1$H NMR data (500.13 MHz), Table 1; $^{13}$C NMR data (50.32 MHz), Table 2; EIMS *m/z* 450 (3) $[M]^+$, 432 (2), 402 (6), 387 (12), 307 (9), 262 (1), 109 (6), 171 (5), 143 (5); FABMS (*m*-nitrobenzyl alcohol) *m/z* 451 (100) $[M + 1]^+$; HREIMS *m/z* 450.2396 (calcd for $C_{28}H_{34}O_5$, 450.2406).

**Salpichrolide K (5):** amorphous solid; $[\alpha]^{25}_D$ −28.0° (*c* 0.03, MeOH); UV (MeOH) $\lambda_{max}$ 222, 268, 276 nm; IR (dry film) $\nu_{max}$ 3420, 2930, 1690, 1082, 744 cm$^{-1}$; $^1$H NMR data (200.13 MHz), Table 1; $^{13}$C NMR data (50.32 MHz), Table 2; EIMS *m/z* 468 (1) $[M]^+$, 450 (1), 432 (2), 387 (12), 307 (25), 262 (1), 143 (5), 109 (7); HREIMS *m/z* 468.2518 (calcd for $C_{28}H_{36}O_6$, 468.2512).

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**Supporting Information Available:** AM1 calculated structures for compounds **1**, **4**, and **5** indicating relevant NOEs observed. Hypothetical biogenetic pathways leading to ring D aromatic withanolides and **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

## References and Notes

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