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## NEW WITHANOLIDES FROM *SALPICHROA ORIGANIFOLIA*

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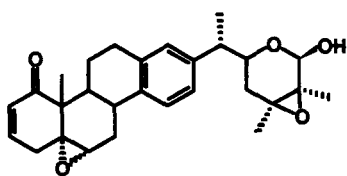
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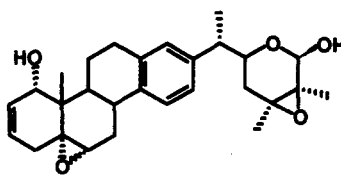
ABSTRACT.—From whole plants of *Salpichroa organifolia*, collected before the flowering stage, three new withanolides were isolated and their structures established by spectroscopic and chemical methods. Two of them, salpichrolides B [2] and C [3], contain a six-membered aromatic D ring, while the third one, salpichrolide D [4], has a five-membered D ring with a  $\Delta^{16-15}$  ketone system.

*Salpichroa organifolia* (Lam.) Thell. (Solanaceae) is the most widespread species of the genus *Salpichroa* Miers (tribe Jaborosea), occurring in North and South America, Europe, Africa, and Australia (1). Our earlier investigation on the withanolides of *S. organifolia* was conducted on plants collected in the flowering stage in which only a single withanolide,

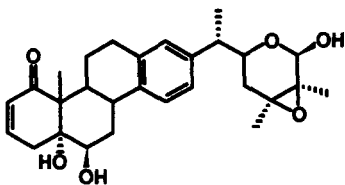
salpichrolide A [1], was present (2). This compound contained two unusual features, an aromatic ring D and a  $5\alpha,6\alpha$ -epoxide. A few withanolides containing a six-membered aromatic D ring and possessing insect repellent properties have been found previously in the Peruvian "shoofly" plant *Nicandra physaloides* (3,4). However, no withanolides with a 5,6-



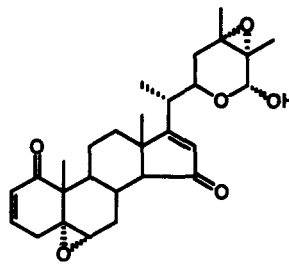
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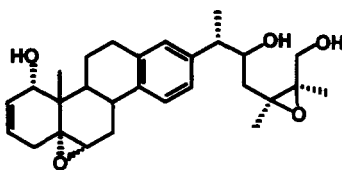
2



3



4



5

epoxide with  $\alpha$  stereochemistry were known previously.

This paper describes the investigation of *S. organifolia* plants collected before the flowering stage; in this case we isolated, besides **1**, three new withanolides. Two of these, salpichrolides B [**2**] and C [**3**], are closely related structurally to **1**, while the third one, salpichrolide D [**4**], has a five-membered D ring with a  $\Delta^{16}$ -15-ketone. The structures were assigned based on chemical and spectroscopic evidence.

The  $^1\text{H}$ -nmr spectrum of salpichrolide B [**2**] exhibited two olefinic protons at  $\delta$  6.03 and 5.81, which differed from those in the typical 2-en-1-one system in ring A of **1** (**2**). These data, in conjunction with the doublet at  $\delta$  3.96 (H-1), were consistent with the presence of a hydroxy group at C-1; the C-19 methyl singlet appeared at  $\delta$  1.05. Application of Garbisch equations (5) to the minimum energy conformers of the  $1\alpha$ - and  $1\beta$ -hydroxy isomers obtained from AM1 calculations (Hyperchem 2.0) gave the coupling constants shown in Table 1 for H-1/H-2 and H-1/H-3, the experimental data being in accordance with the  $\alpha$ -stereochemistry at C-1. Evidence for the presence of a  $5\alpha,6\alpha$ -epoxy group was provided by the doublet observed at  $\delta$  3.08 ( $J=5.0$  Hz). Analysis of the  $^{13}\text{C}$ -nmr and DEPT spectra of compound **2** showed the absence of a ketone carbonyl and the presence of an additional methine at  $\delta$  70.86 assigned to C-1; when compared to salpichrolide A [**1**] C-3 was shifted upfield to 129.15 ppm as expected. All other values for both the  $^1\text{H}$ - and  $^{13}\text{C}$ -nmr data were closely related to those of salpichrolide A, indicating an

aromatic D ring and a  $\delta$ -lactol side-chain with the same stereochemistry. The COSY 45 spectrum of **1** confirmed the  $^1\text{H}$ - $^1\text{H}$  nmr connectivities. Mass measurements were in accordance with the structural assignment; the eims showed a small molecular ion peak at  $m/z$  452 (3.4%) and a peak at  $m/z$  143 (5.2%) corresponding to the  $\delta$ -lactol ring in the side-chain. The cleavage between C-20 and C-22 rendered an ion at  $m/z$  309 (10.5%), while this cleavage followed by the loss of  $\text{H}_2\text{O}$  gave rise to the base peak ( $m/z$  292). Reduction of salpichrolide A [**1**] with  $\text{NaBH}_4$  in EtOH afforded compound **5**, which gave  $^1\text{H}$ - and  $^{13}\text{C}$ -nmr chemical shifts for rings A–D that are similar to those obtained for **2**, thus confirming this portion of the proposed structure. Compound **2** is the first withanolide structure reported that contains a 2-en-1-hydroxy system in ring A.

Salpichrolide C [**3**] also had  $^1\text{H}$ - and  $^{13}\text{C}$ -nmr spectra closely related to those of salpichrolide A [**1**], the main difference being the downfield shift of H-6 to  $\delta$  3.77 and the presence of  $^{13}\text{C}$ -nmr signals at  $\delta$  72.23 and 74.89 assigned to C-5 and C-6. These data indicated the presence of a second hydroxyl group at the  $6\beta$ -position in a  $5\alpha,6\beta$ -dihydroxysteroid. The nmr spectral assignments were confirmed by DEPT and HETCOR spectra (Table 2). The molecular ion was absent in the mass spectrum of **3**, but a peak at  $m/z$  450 (2.5%) corresponding to the ion  $[\text{M}-\text{H}_2\text{O}]^+$  was observed. Another significant peak was at  $m/z$  325 (65.8%) due to the scission of the C-20–C-22 bond and was accompanied by a peak at  $m/z$  143 (38.2%) which represented the  $\delta$ -lactol side-chain. The fabms (thioglycerol,

TABLE 1. AM1 Data (H(1)-C-C-H(2) and H(1)-C(1)...C(3)-H(3) Angles) and Calculated Coupling Constants for the Minimum Energy Conformers of **2** and Its  $1\beta$  Isomer.\*

|                                 | H(1)-C-C-H(2) | H(1)-C(1)...C(3)-H(3) | $^3J_{1,2}$ | $^4J_{1,3}$ |
|---------------------------------|---------------|-----------------------|-------------|-------------|
| <b>2</b> .....                  | 46.00°        | 53.50°                | 4.5 Hz      | 1.2 Hz      |
| $1\beta$ -OH-( <b>2</b> ) ..... | 74.00°        | 80.10°                | 2.8 Hz      | 2.5 Hz      |

\*Experimental coupling constants for **2** are:  $^3J_{1,2}=4.5$  Hz;  $^4J_{1,3}<1$  Hz.

TABLE 2.  $^{13}\text{C}$ - and  $^1\text{H}$ -(HETCOR) Nmr Spectral Data of Compound 3.

| Carbon | $\delta_{\text{C}}$ | H- $\alpha$ | $\delta_{\text{H}}$ | H- $\beta$ | $J_{\text{gem}}$ (Hz) |
|--------|---------------------|-------------|---------------------|------------|-----------------------|
| 1      | 204.07              |             | —                   |            |                       |
| 2      | 128.88              |             | 5.94                |            |                       |
| 3      | 141.46              |             | 6.65                |            |                       |
| 4      | 35.53               | 2.10        |                     | 3.32       | 19.6                  |
| 5      | 72.23               |             | —                   |            |                       |
| 6      | 74.89               |             | 3.77                |            |                       |
| 7      | 34.25               | 1.59        |                     | 1.84       | 19.1                  |
| 8      | 32.39               |             | 3.00                |            |                       |
| 9      | 38.35               |             | 2.13                |            |                       |
| 10     | 52.38               |             | —                   |            |                       |
| 11     | 25.94               | 2.45        |                     | 1.36       | 15.0                  |
| 12     | 29.77               |             |                     |            |                       |
| 13     | 137.59              |             | —                   |            |                       |
| 14     | 138.07              |             | —                   |            |                       |
| 15     | 125.62              |             | 7.20                |            |                       |
| 16     | 125.42              |             | 6.99                |            |                       |
| 17     | 140.55              |             | —                   |            |                       |
| 18     | 128.76              |             | 6.95                |            |                       |
| 19     | 14.93               |             | 1.30                |            |                       |
| 20     | 43.14               |             | 2.71                |            |                       |
| 21     | 17.37               |             | 1.24                |            |                       |
| 22     | 67.59               |             | 3.87                |            |                       |
| 23     | 33.83               | 1.88        |                     | 2.16       | 16.0                  |
| 24     | 64.87               |             | —                   |            |                       |
| 25     | 63.65               |             | —                   |            |                       |
| 26     | 91.75               |             | 4.98                |            |                       |
| 27     | 16.64               |             | 1.39                |            |                       |
| 28     | 18.83               |             | 1.30                |            |                       |

\*Not observed.

KCl) showed a  $[\text{M} + \text{K}]^+$  ion at  $m/z$  507 which was consistent with the formula  $\text{C}_{28}\text{H}_{36}\text{O}_6$  for compound 3. Treatment of salpichrolide A [1] with 1.5 N  $\text{H}_2\text{SO}_4$  in THF afforded a product identical to 3, thus confirming the structure proposed for this compound.

The  $^1\text{H}$ -nmr spectrum of salpichrolide D [4] exhibited two olefinic protons at  $\delta$  5.96 and 6.72, typical of a 2-en-1-one system in ring A. The H-19 methyl singlet at  $\delta$  1.39 and the doublet at  $\delta$  3.10 ( $J=5.5$  Hz) confirmed the presence of a 1-oxo- $\Delta^2$ -5 $\alpha$ ,6 $\alpha$ -epoxy withanolide. The absence of the signals characteristic of an aromatic D ring and the presence of a singlet at  $\delta$  0.98 assigned to the H-18 methyl, indicated the presence of a five-membered D ring. The  $^{13}\text{C}$ - and  $^1\text{H}$ -nmr chemical shifts of C-22 through C-28 correspond to an epoxy lactol side-chain with the same stereochemistry as in the previous compounds.

An unusual substitution pattern was evident in ring D of 4: the presence of signals at  $\delta$  125.70, 184.43, and 207.30 in the  $^{13}\text{C}$ -nmr spectrum indicated an  $\alpha,\beta$ -unsaturated ketone in this ring. The  $^1\text{H}$ -nmr spectrum showed an olefinic proton at  $\delta$  5.79 which was correlated by selective decoupling to C-16 ( $\delta$  125.70) and by long-range  $^1\text{H}$ - $^1\text{H}$  COSY nmr to H-20 supporting the  $\Delta^{16}$ -15-keto functionality. The nmr spectral assignments were confirmed by heteronuclear selective decoupling, DEPT, and DQF-COSY spectra. The fabms ( $m$ -nitrobenzylalcohol) showed a  $[\text{M} + 1]^+$  ion at  $m/z$  469, consistent with the formula  $\text{C}_{28}\text{H}_{36}\text{O}_6$  for this compound. Although withanolides containing a 16,17-double bond (6,7) have been reported, this is the first one that con-

tains an unsaturated ketone system in ring D. This fact, in conjunction with the presence of ring-D aromatic withanolides in *Salpicbroa origanifolia*, suggests that salpichrolide D or a related compound (e.g., a  $\Delta^{16}$ -15-hydroxy withanolide or its dehydration product) 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 physaloides* (8,9).

## EXPERIMENTAL

**GENERAL EXPERIMENTAL PROCEDURES.**—<sup>1</sup>H- and <sup>13</sup>C-nmr spectra were recorded on a Bruker AC-200 spectrometer at 200.13 and 50.23 MHz, respectively. Multiplicity determinations (DEPT) and 2D nmr spectra (COSY, HETCOR) 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 instrument; fabms were collected on a VG ZAB-BEQQ spectrometer. AM1 calculations were performed with Hyperchem 2.0 (Autodesk Inc.). Mps were taken on a Fisher-Johns apparatus and are uncorrected. Cc was performed on Kieselgel S 0.032–0.063 mm; tlc was performed on Si gel 60 F<sub>254</sub>, 0.2 mm thick.

**PLANT MATERIAL.**—Whole *Salpicbroa origanifolia* plants were collected just before the flowering stage, in the surroundings of the University campus in Córdoba, Córdoba Province, Argentina. A voucher specimen is deposited at the Museo Botánico, Universidad Nacional de Córdoba under No. CORD.89.

**EXTRACTION AND ISOLATION.**—Fresh plants (2.2 kg) were triturated with EtOH at room temperature immediately after collection. The residue obtained after evaporation of the solvent was partitioned with hexane-MeOH-H<sub>2</sub>O (10:3:1), the aqueous-MeOH phase concentrated *in vacuo*, and the resulting aqueous phase extracted with CHCl<sub>3</sub>. The CHCl<sub>3</sub> extract was dried (Na<sub>2</sub>SO<sub>4</sub>), evaporated, and the residue (2.1 g) was chromatographed on Si gel. Elution with hexane-EtOAc mixtures of increasing polarity (9:1 to 7:3) afforded salpichrolide A [1] (250 mg) and fractions containing a partially resolved mixture of three other withanolides. The latter fractions were pooled and further fractionated by flash chromatography to yield two main fractions containing withanolides 2 (5 mg) and 3 (7 mg). Prep. tlc of the pooled intermediate fractions afforded compound 4 (1.2 mg).

*Salpichrolide B* [2].—White solid; mp 164–

165° (EtOAc/hexane); uv  $\lambda$  max (MeOH) (log  $\epsilon$ ) 214 (3.90), 268 (3.05), 276 (2.86) nm; <sup>1</sup>H nmr (CDCl<sub>3</sub>)  $\delta$  7.13 (1H, d,  $J$ =8.0 Hz, H-15), 6.98 (1H, dd,  $J$ =8.0 and 1.5 Hz, H-16), 6.89 (1H, d,  $J$ =1.5 Hz, H-18), 6.03 (1H, ddd,  $J$ =10.2, 4.5, and 1.5 Hz, H-2, confirmed by irradiation at  $\delta$  3.96), 5.81 (1H, ddd,  $J$ =10.2, 5.0, and 2.0 Hz, H-3), 5.00 (1H, d,  $J$ =9.5 Hz, H-26), 3.96 (1H, br s, H-1; d,  $J$ =4.5 Hz after exchange with D<sub>2</sub>O), 3.65 (1H, ddd,  $J$ =12.0, 6.0, and 2.5 Hz, H-22), 3.34 (1H, d,  $J$ =9.5 Hz, OH-26), 3.08 (1H, d,  $J$ =5.0 Hz, H-6), 1.38 (3H, s, H<sub>3</sub>-27), 1.35 (3H, s, H<sub>3</sub>-28), 1.23 (3H, d,  $J$ =7.0 Hz, H<sub>3</sub>-21), 1.05 (3H, s, H<sub>3</sub>-19); <sup>13</sup>C nmr (CDCl<sub>3</sub>)  $\delta$  140.29 (C-17), 138.10 (C-13), 136.50 (C-14), 129.15 (C-3), 128.78 (C-18), 126.68 (C-16), 125.78 (C-2), 125.70 (C-15), 91.71 (C-26), 70.86 (C-1), 67.43 (C-22), 64.90 (C-24), 64.90 (C-5), 63.66 (C-25), 56.39 (C-6), 42.99 (C-20), 39.40 (C-10), 35.19 (C-9), 33.67 (C-23), 32.39 (C-4), 32.32 (C-8), 31.08 (C-7), 30.55 (C-12), 22.05 (C-11), 18.76 (C-28), 17.17 (C-21), 16.55 (C-27), 15.07 (C-19); eims  $m/z$  452 [M]<sup>+</sup> (3.4), 310 (20.5), 309 (10.5), 292 (100), 171 (7.3), 143 (5.2).

*Salpichrolide C* [3].—White solid; mp 179–180° (EtOAc/hexane); uv  $\lambda$  max (MeOH) (log  $\epsilon$ ) 228 (4.20), 268 (3.04), 276 (3.02) nm; <sup>1</sup>H nmr (CDCl<sub>3</sub>)  $\delta$  7.20 (1H, d,  $J$ =8.1 Hz, H-15), 6.99 (1H, dd,  $J$ =8.1 and 1.5 Hz, H-16), 6.95 (1H, d,  $J$ =1.5 Hz, H-18), 6.65 (1H, ddd,  $J$ =10.1, 5.1, and 2.5 Hz, H-3), 5.94 (1H, dd,  $J$ =10.1 and 2.8 Hz, H-2), 4.98 (1H, br s, H-26), 3.87 (1H, ddd,  $J$ =12.0, 6.0, and 2.5 Hz, H-22), 3.77 (1H, d,  $J$ =2.5 Hz, H-6), 3.37 (1H, br s, OH-26), 3.32 (1H, ddd,  $J$ =19.6, 2.8, and 2.5 Hz, H-4 $\beta$ ), 1.39 (3H, s, H<sub>3</sub>-27), 1.30 (6H, s, H<sub>3</sub>-28 and H<sub>3</sub>-19), 1.24 (3H, d,  $J$ =6.5 Hz, H<sub>3</sub>-21); <sup>13</sup>C-nmr data, see Table 2; eims  $m/z$  450 [M-H<sub>2</sub>O]<sup>+</sup> (2.5), 327 (13.6), 326 (63.4), 325 (65.8), 171 (21.4), 143 (38.2); fabms (thioglycerol, KCl)  $m/z$  507 [M+K]<sup>+</sup> (13.3).

*Salpichrolide D* [4].—Amorphous white solid; mp 154–155° (EtOAc/hexane); uv  $\lambda$  max (MeOH) (log  $\epsilon$ ) 226 (4.18) nm; <sup>1</sup>H nmr (CDCl<sub>3</sub>)  $\delta$  6.72 (1H, ddd,  $J$ =10.0, 5.1, and 2.2 Hz, H-3), 5.96 (1H, dd,  $J$ =10.0 and 2.8 Hz, H-2), 5.79 (1H, s, H-16), 4.98 (1H, d,  $J$ =9.5 Hz, H-26), 3.82 (1H, ddd,  $J$ =11.7, 7.0, and 2.9 Hz, H-22), 3.39 (1H, d,  $J$ =9.5 Hz, OH-26), 3.12 (1H, ddd,  $J$ =21.0, 2.8, and 2.2 Hz, H-4 $\beta$ ), 3.10 (1H, d,  $J$ =5.5 Hz, H-6), 2.88 (1H, dd,  $J$ =11.8 and 5.5 Hz, H-7 $\beta$ ), 2.42 (1H, quin,  $J$ =7.0 Hz, H-20), 1.94 (1H, dd,  $J$ =14.4 and 2.9 Hz, H-23 $\alpha$ ), 1.86 (1H, dd,  $J$ =21.0 and 5.1 Hz, H-4 $\alpha$ ), 1.75 (1H, dd,  $J$ =14.0 and 11.8 Hz, H-7 $\alpha$ ), 1.62 (1H, dd,  $J$ =14.4 and 11.7 Hz, H-23 $\beta$ ), 1.41 (3H, s, H<sub>3</sub>-27), 1.40 (3H, s, H<sub>3</sub>-28), 1.39 (3H, s, H<sub>3</sub>-19), 1.10 (3H, d,  $J$ =7.0 Hz, H<sub>3</sub>-21), 0.98 (3H, s, H<sub>3</sub>-18); <sup>13</sup>C nmr (CDCl<sub>3</sub>)  $\delta$  207.30 (C-15), 202.55 (C-1), 184.43 (C-17), 142.46 (C-3), 128.81 (C-2), 125.70 (C-16), 91.71

(C-26), 66.37 (C-22), 65.71 (C-25), 64.66 (C-24 and C-14), 64.42 (C-5), 58.57 (C-6), 48.51 (C-10), 46.47 (C-13), 38.63 (C-9), 37.50 (C-8), 34.43 (C-23), 34.05 (C-4), 32.23 (C-12), 29.70 (C-7), 27.57 (C-20), 23.11 (C-18), 21.69 (C-11), 18.76 (C-28), 17.19 (C-21), 16.54 (C-27), 15.56 (C-19); *fabms* (matrix) *m/z* (*m*-nitrobenzylalcohol) 469  $[M+1]^+$  (24.1), (glycerol) 469  $[M+1]^+$  (5.2), (glycerol + Na<sub>2</sub>CO<sub>3</sub>) 491  $[M+Na]^+$  (43.9), (glycerol + K<sub>2</sub>CO<sub>3</sub>) 507  $[M+K]^+$  (100).

**PREPARATION OF 3 FROM 1.**—Compound **1** (6 mg) in THF (4 ml) was treated with 1.5 N H<sub>2</sub>SO<sub>4</sub> (0.03 ml) at room temperature for 18 h. The solution was diluted with H<sub>2</sub>O, neutralized with aqueous NaHCO<sub>3</sub>, and extracted with CH<sub>2</sub>Cl<sub>2</sub>. The product obtained by evaporation of the solvent (6 mg) was identical (tlc, <sup>1</sup>H nmr) to compound **3**.

**PREPARATION OF 5 FROM 1.**—NaBH<sub>4</sub> (10 mg) was added to a solution of compound **1** (10 mg), in EtOH (4 ml). After 1 h at 50° the solution was neutralized with 1 N HCl and the solvent was removed; dilution with H<sub>2</sub>O and extractive workup afforded compound **5** (6 mg); <sup>1</sup>H nmr (CDCl<sub>3</sub>; chemical shifts in square brackets were measured in C<sub>5</sub>D<sub>5</sub>N) δ 7.20 (1H, d, *J*=8.0 Hz, H-15), 7.12 (1H, d, *J*=8.0 Hz, H-16), 6.88 (1H, br s, H-18), 6.06 (1H, m, H-2), 5.82 (1H, ddd, *J*=10.0, 5.0, and 2.0 Hz, H-3), 4.14 (1H, m, H-22), 3.98 (1H, d, *J*=5.0 Hz, H-1), 3.90 (1H, br s, OH-26), 3.66 [4.01] (1H, br s, H-26), 3.09 (1H, br s, H-6), 1.40 [1.66] (6H, s, H<sub>3</sub>-27 and H<sub>3</sub>-28), 1.06 [1.02] (3H, s, H<sub>3</sub>-19); <sup>13</sup>C nmr (CDCl<sub>3</sub>) δ 140.25 (C-17), 138.53 (C-13), 137.07 (C-14), 128.92 (C-3), 128.23 (C-18), 126.92 (C-16), 125.65 (C-2), 125.47 (C-15), 74.06 (C-22), 70.85 (C-1), 65.21

(C-26), 65.12 (C-25), 65.26 (C-24), 64.13 (C-5), 56.32 (C-6), 45.71 (C-20), 39.43 (C-10), 36.65 (C-23), 35.21 (C-9), 32.43 (C-4), 32.32 (C-8), 31.12 (C-7), 30.67 (C-12), 22.03 (C-11), 20.12 (C-28), 17.01 (C-21), 16.43 (C-27), 15.10 (C-19); *eims m/z* 310 (8.3), 309 (20.5), 292 (7.4).

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