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RING D AROMATIC ERGOSTANE DERIVATIVES FROM *SALPICHROA ORIGANIFOLIA*

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Key Word Index—*Salpichroa origanifolia*; Solanaceae; withanolides; 27-nor-17(13 → 18)*abeo*-ergostanes.

Abstract—From the leaves of *Salpichroa origanifolia* collected in winter two new ergostane derivatives related to withanolides, 5,6α-epoxy-22-formyloxy-27-nor-17(13 → 18)*abeo*-5α-ergosta-2,13,15,17-tetraene-1,25-dione and 5α,6β-dihydroxy-22-formyloxy-27-nor-17(13 → 18)*abeo*-5α-ergosta-2,13,15,17-tetraene-1,25-dione were isolated and characterized by spectroscopic methods. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Salpichroa origanifolia (Lam.) Thell., the most widespread species of the genus *Salpichroa* Miers [1], has been shown to contain withanolides with a six-membered aromatic ring D. Salpichrolide A (**1**) was the only withanolide present in the plant during the flowering stage but other withanolides could be isolated when plants were collected before flowering [2, 3]. A few withanolides containing an aromatic ring D and possessing insect repellent properties have been found in the Peruvian 'shoofly' plant *Nicandra physaloides* [4, 5]; preliminary biological testing has shown that salpichrolide A (**1**) has strong antifeedant activity on larvae of *Tenebrio molitor* (R. D. Enriz and C. E. Tonn, Universidad Nacional de San Luis, Argentina, unpublished results). From leaves of *S. origanifolia* collected in winter, we isolated two new ergostane derivatives closely related to the withanolides present in this plant, named salpichrolide E (**2**) and salpichrolide F (**3**).

RESULTS AND DISCUSSION

The HR-EI mass spectrum of salpichrolide E (**2**) showed a molecular ion of *m/z* 450.2404 consistent with the formula $C_{28}H_{34}O_5$. A peak at *m/z* 404 corresponding to the loss of formic acid was observed in the EI mass spectrum together with peaks at *m/z* 307 and 143 which correspond to both fragments arising from the cleavage between C-20 and C-22. Compound **2** had ^{1}H and ^{13}C NMR spectra closely related to those of salpichrolide A (**1**) [2], the main difference being in the signals corresponding to the side chain. The ^{1}H NMR spectrum of **2** (Table 1) exhibited in the low field region, signals typical of a 2-en-1-one system without substituents at C-4 and those characteristic of the aromatic ring D. The singlet at δ 8.00, indicated the presence of a formyloxy group. The ^{13}C NMR spectrum showed the expected signal for the C-1 carbonyl carbon at δ 202.6 and the eight olefinic signals in the range δ 125–143. The latter resonances were assigned to the aromatic carbons (C-13 to C-18) and the double bond in ring A by comparison with the ^{13}C NMR spectrum of **1**. The methine resonance at δ 160.9 confirmed the presence of a formyloxy group and the carbonyl signal at 211.5 in conjunction with the signal at δ 2.11 in the ^{1}H NMR spectrum indicated the presence of a methyl ketone in the side chain. The COSY-45 spectrum showed the sequence of correlations along the side chain from H-21 to H-28 and confirmed the position of the formate group at C-22 (H-22 at δ 5.12). The stereochemistry at C-20 and C-22 may be assigned to be the same as in salpichrolide A (20*R*,22*S*) based on biosynthetic considerations (see below). However, the stereochemistry at C-24 could not be established from the spectroscopic data. The side-chain moiety of **2**, has been recently reported in nicaphysalins D and E, isolated from *Nicandra physaloides* [6], but in this case the stereochemistry at C-24 also remained undetermined.

The ^{1}H and ^{13}C NMR spectral data of salpichrolide F (**3**) were very similar to those of salpichrolide E and indicated that they differed in the substitution pattern of rings A/B. The main difference in the ^{1}H NMR spectrum of **3** (Table 1) was the downfield shift of H-6 (from δ 3.24 in **2** to δ 3.68 in **3**) and a small upfield shift of H-2 and H-3 characteristic of a 5α,6β-

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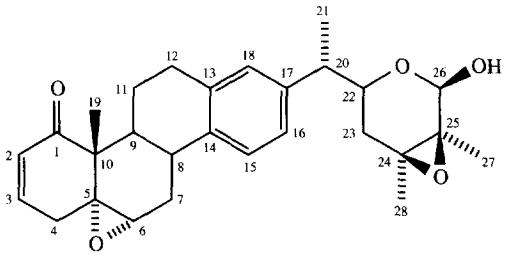

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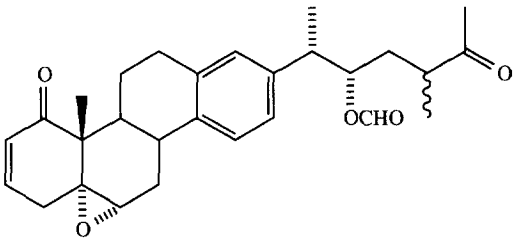

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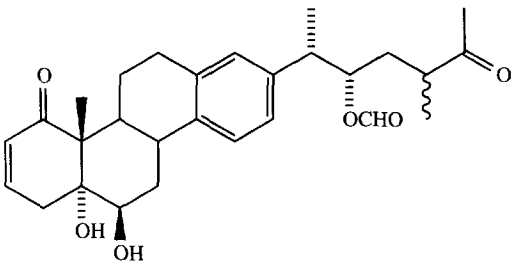

3

dihydroxy derivative [3]. The ^{13}C NMR spectrum (Table 2) showed the expected shifts for the signals of carbons of rings A and B. Mass measurements were in agreement with the proposed structure; the HR-EI mass spectrum of **3** did not show a molecular ion, but a peak at *m/z* 422.2454 was observed, consistent with the formula $C_{27}H_{34}O_4$ which corresponds to the loss of formic acid. The FAB-mass spectrum showed a base peak at *m/z* 489 $[M + K - H_2O]^+$ and the EI mass spectrum showed fragments at 325 and 143, corresponding to ions arising from the cleavage between C-20 and C-22.

Salpichrolide E (**2**) probably arises from salpichrolide A (**1**) by oxidative cleavage of the C-25–C-26 bond, with C-26 giving rise to the formyloxy group. Salpichrolide F (**3**) may be derived analogously from the corresponding 5α,6β-diol (salpichrolide C) [3] or by hydrolytic opening of the 5,6-epoxide in **2**. It is noteworthy that this type of side-chain has been found only in *Nicandra physaloides* and *S. origanifolia*, the

Table 1. ^1H NMR spectral data for relevant protons of compounds **2** and **3** (in $CDCl_3$ δ from TMS)

H	2	3
2	6.02 *dd* (10.0; 2.5)	5.94 *dd* (10.0; 2.3)
3	6.75 *ddd* (10.0, 5.0; 2.5)	6.65 *ddd* (10.0; 5.0; 2.3)
4α	1.90 *m*	2.15 *m*
4β	3.15 *dt* (19.3; 2.5)	3.35 *dt* (20.0; 2.3)
6	3.24 *d* (4.9)	3.85 *br s* ($W_{1/2}$ = 6)
7α	1.85 *m*	2.05 *m*
7β	2.60 *m*	2.35 *m*
15	7.13 *d* (8.1)	7.21 *d* (8.0)
16	7.02 *dd* (8.1; 1.5)	7.00 *dd* (8.0; 1.5)
18	6.92 *br s* ($W_{1/2}$ = 3)	6.95 *br s* ($W_{1/2}$ = 3)
19	1.39 *s*	1.39 *s*
20	2.90 *m*	2.85 *m*
21	1.26 *d* (7.0)	1.27 *d* (7.0)
22	5.12 *ddd* (10.1; 6.0; 2.4)	5.17 *ddd* (10.0; 6.2; 2.5)
23a	2.05 *m*	2.09 *m*
23b	1.35 *m*	1.35 *m*
24	2.57 *m*	2.57 *m*
26	2.11 *s*	2.11 *s*
28	1.06 *d* (7.0)	1.06 *d* (7.0)
HCOO	8.00 *s*	8.01 *s*

Coupling constants (in parentheses) in Hz.
Assignments are based on COSY-45 spectra and coupling constants.

Table 2. ^{13}C NMR spectral data of compounds **2** and **3** (in $CDCl_3$, δ from TMS)

C	2	3	C	2	3
1	202.6	n.o.	15	126.6	125.7
2	128.6	128.9	16	125.5	125.3
3	142.4	141.2	17	139.3	139.3
4	33.6	35.6	18	128.9	128.9
5	64.7	n.o.	19	14.9	14.9
6	59.0	75.0	20	43.3	43.4
7	30.6	34.4	21	17.6	17.7
8	33.2	32.4	22	76.0	75.6
9	36.4	38.4	23	35.0	35.0
10	48.8	n.o.	24	43.2	43.4
11	25.4	25.9	25	211.5	n.o.
12	30.4	29.7	26	28.6	29.3
13	138.2	138.7	28	17.6	17.7
14	137.3	138.4	HCOO	160.9	160.9

Assignments based in DEPT spectra and comparison with salpichrolides A and C.

n.o., not observed.

only plants known to contain aromatic ring D withanolides.

EXPERIMENTAL

Mps: uncorr. ^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) 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 at 70 eV by direct inlet, FAB-MS and HR-EIMS were measured on a VG ZAB-BEQQ mass spectrometer.

Plant material and isolation procedure. Whole *Salpichroa origanifolia* plants were collected in the surroundings of the University campus in Buenos Aires, Argentina. A voucher specimen is deposited at the Museo Botánico, Universidad de Córdoba under No. CORD 254. Fresh plants (1 kg) were triturated and extracted successively with Et_2O and EtOH at room temp. The residue (2 g) obtained after evapn of the combined extracts were chromatographed on Kieselgel 60-G. Elution with hexane–EtOAc mixtures of increasing polarity (100:0–0:100) afforded six fractions containing withanolides. The fractions eluted with hexane–EtOAc from 40:60 to 0:100 were further fractionated by flash chromatography on silica gel and purified by prep. TLC yielding salpichrolide E (**2**) (7 mg) and salpichrolide F (**3**) (1 mg).

Salpichrolide E (**2**). Crystals from EtOAc–hexane, mp 180–181°; UV λ_{max}^{MeOH} nm: 222, 268, 276; FTIR ν_{max}^{Film} cm^{-1}: 3422, 1715, 1684, 1456, 1379, 1182; ^1H and ^{13}C NMR: Tables 1 and 2; EIMS *m/z* (rel. int): 404 $[M - HCOOH]^+$ (9), 386 (1), 343 (1), 332 (1), 307 (14), 143 (11), 43 (100); FAB-MS (*m*-nitrobenzylalcohol, KCl), *m/z* (rel. int.): 489 $[M + K]^+$ (100); HR-EIMS *m/z*: 450.2406 $[M]^+$ (calcd for $C_{28}H_{34}O_5$: 450.2406).

Salpichrolide F (**3**). Amorphous solid; UV λ_{max}^{MeOH} nm: 220, 268, 276; FTIR ν_{max}^{Film} cm^{-1}: 3447, 1717, 1684, 1456; ^1H and ^{13}C NMR: Tables 1 and 2; EIMS *m/z* (rel. int.): 422 $[M - HCOOH]^+$ (7), 387 (2), 351 (3), 325 (11), 143 (10), 43 (100); FAB-MS (*m*-nitrobenzylalcohol, KCl), *m/z* (rel. int.): 489 $[M - H_2O + K]^+$ (100); HR-EIMS *m/z*: 422.2454 $[M - HCOOH]^+$ (calcd for $C_{27}H_{34}O_4$: 422.2457).

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