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TWO NOVEL STEROIDS FROM *EUPHORBIA OFFICINARUM* LATEX

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Two novel steroids, 3β,7α-dihydroxy-4α,14α-dimethyl-5α-cholest-8-en-11-one (**2**) and 3β,7β-dihydroxy-4α,14α-dimethyl-5α-cholest-8-en-11-one (**3**) were isolated from the latex of *Euphorbia officinarum*. Their structures were established on the basis of NMR and MS studies.

INTRODUCTION

The latex of the Moroccan endemic plant *Euphorbia officinarum* L. (Euphorbiaceae) has been used in folk medicine to treat various skin and ophthalmologic diseases in low concentration due to its high toxicity [1]. Characterization of its components is necessary to fully develop its therapeutic potential.

Nine compounds with triterpenic and steroideal skeleton have been previously isolated [2] from the methanolic extract of the latex of this plant, which were identified as: lupeol, lupeol acetate, lanostenol, lanosterol, 24-methylene lanostenol, 4α,14α-dimethyl-24-methylen-5α-cholest-8-en-3β-ol, 4α,14α,24(*R*)-trimethyl-5α-cholesta-8,25(27)-dien-3β-ol, 4α,14α-dimethyl-5α-cholesta-8,24-dien-3β-ol and 4α,14α-dimethyl-5α-cholest-8-en-3β-ol (**1**). Further examination of this extract led to the isolation of two new steroids (**2** and **3**). This article deals with their structural elucidation.

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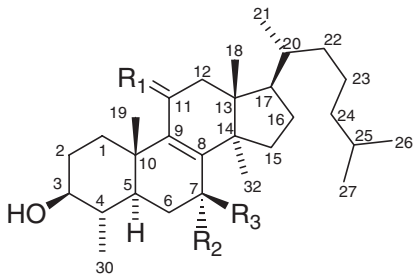

1 $R_1 = H_2$; $R_2 = R_3 = H$
2 $R_1 = O$; $R_2 = OH$; $R_3 = H$
3 $R_1 = O$; $R_2 = H$; $R_3 = OH$

RESULTS AND DISCUSSION

Both compounds (**2** and **3**) were obtained as white needles and they were assigned the molecular formula $C_{29}H_{48}O_3$ (HREIMS). The IR spectra of both compounds showed absorption bands for a γ-hydroxy-α,β-unsaturated ketone (ν_{max} 3474, 1642 cm^{-1}). The ^1H NMR and ^{13}C NMR of both compounds showed the presence of three tertiary and two secondary methyl groups, an isopropyl group [**2**: δ_H 0.86, 0.87 (each 3H, d); **3**: δ_H 0.87, 0.88 (each 3H, d)], nine methylene groups, five methine groups, two hydroxymethine groups [**2**: δ_H 3.17 (1H, ddd), 4.35 (1H, dd); δ_C 76.3 (d), 67.5 (d); **3**: δ_H 3.07 (1H, ddd), 4.41 (1H, dd); δ_C 76.0 (d), 68.7 (d)], a tetrasubstituted double bond [**2**: δ_C 160.5 (s), 140.6 (s); **3**: δ_C 160.0 (s), 141.9 (s)], and a conjugated ketone [**2**: δ_C 200.9 (s); **3**: 200.8 (s)]. A significative difference between these compounds lies in the coupling constants observed for one of the methine protons, assigned by 2D-NMR spectroscopy as H-7 [**2**: δ_H 4.35 (1H, dd, $J = 4.8$, 1.1 Hz); **3**: δ_H 4.41 (1H, dd, $J = 8.9$, 8.9 Hz)]. A molecular model analysis shows that a difference in the configuration of the hydroxyl group at C-7 justifies the observed coupling constants. In compound **2**, an α configuration for the hydroxyl group at C-7 implies a half chair configuration for ring B and leaves H-7β bisecting both H-6α and H-6β yielding small coupling constants; on the other hand, a β configuration for the hydroxyl group at C-7 leaves H-7β in *anti* and *gauge* relationships to H-6β and H-6α, respectively, causing larger coupling constants [3]. These assignations are supported by the HMBC and NOESY spectra of compound **2**. The NOESY data (Fig. 1) exhibited cross correlations for H-3α (with H-5α), H_3-18 (with H-20), H_3-32 (with H-12α and H-17α), and H-7β (with H-15, H-15′, H-6, H-6′). This analysis allows the assignment for **2** as 3β,7α-dihydroxy-4α,14α-dimethyl-5α-ergost-8-en-11-one and for **3** as 3β,7β-dihydroxy-4α,14α-dimethyl-5α-ergost-8-en-11-one. Further support for these assignments follows from the comparison of the ^1H NMR and ^{13}C NMR data of compound **2** with those of 3β,7α-dihydroxy-4α,14α-dimethyl-5α-ergosta-8,24(28)-dien-11-one [4] and the comparison of the ^1H NMR and ^{13}C NMR data of compound **3** with those of lucidenic acid N [5], which has an identical structure of the ring system with that of compound **3**.

Both lucidenic acid N and 3β,7α-dihydroxy-4α,14α-dimethyl-5α-ergosta-8,24(28)-dien-11-one have cytotoxic [5] and antitumor-promoting [4] activities, respectively, what suggest compounds **2** and **3** to be potentially bioactive.

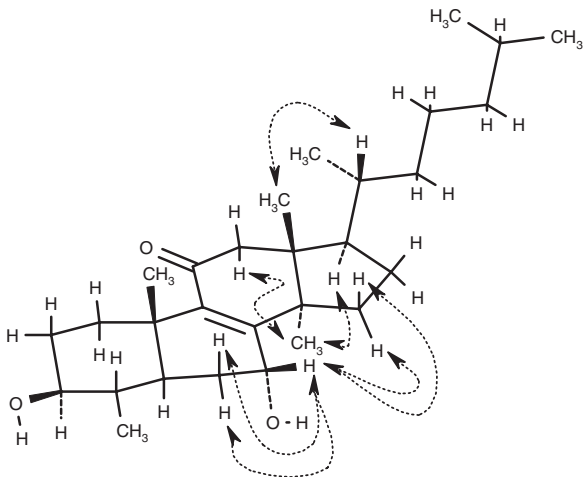

FIGURE 1 Selected NOESY correlations observed for compound **2**.

EXPERIMENTAL SECTION

General Experimental Procedures

Melting points were measured with a Reichert-Jung Kofler block and are uncorrected. Optical rotations were determined with a Perkin-Elmer 241 polarimeter. IR spectra were recorded on a Perkin-Elmer 881 spectrophotometer. ^{1}H and ^{13}C NMR measurements were obtained on a Varian INOVA 400 NMR spectrometer with $SiMe_4$ as internal reference. Mass spectra were recorded on VG Platform II and VG Autospec spectrometers at 70 eV. HPLC was performed with a Hitachi/Merck L-6270 apparatus equipped with a UV-Vis detector (L 4250) and a differential refractometer detector (RI-71). TLC was performed on 0.2 mm Merck Kiesegel 60 F_{254}, plates. Silica gel (Merck) was used for column chromatography. Purification by HPLC was accomplished using a silica gel column (LiChrospher® Si 60, 10 μm, 1 × 25 cm long).

Plant Material

Latex from *E. officinarum* was collected in May 1999 from plants of the North Atlantic coast of Agadir, Morocco. Latex was obtained by making repeated cuts along the stems of the plant, using a knife, and collecting the white milky exudates. A voucher specimen is deposited at the Herbarium of the laboratoire de Chimie des Substances Naturelles et Hétérocycles, Université Cadi Ayyad.

Extraction and Isolation

The latex (0.5 L) from *E. officinarum* was allowed to dry and the resulting coagulum (200 g) was extracted with MeOH (1.5 L), employing a Sohxlet apparatus. The MeOH solution was cooled to yield a precipitate (60 g) which was subjected to silica gel column chromatography. Elution of the column with hexane : ethyl acetate (95 : 5) afforded three fractions [fraction (weight after solvent removal)]: A (5 mg), B (4 g), C (53 g) and elution with hexane : ethyl acetate (90 : 10) yielded a fourth fraction

[D (16 mg)]. Analysis of fractions A–C by chromatography on silica gel with 10% $AgNO_3$ yielded already known compounds [2]. Purification of the more polar fraction D by HPLC, using hexane : ethyl acetate (72 : 28), yielded 3β,7α-dihydroxy-4α,14α-dimethyl-5α-ergost-8-en-11-one (**2**) (6 mg) and 3β,7β-dihydroxy-4α,14α-dimethyl-5α-ergost-8-en-11-one (**3**) (1 mg). The spectral data of these novel compounds are given below.

3β,7α-Dihydroxy-4α,14α-dimethyl-5α-cholest-8-en-11-one (***2***) White needles; m.p.: 158–160°C; $[\alpha]_D^{25}+99.0°$ (*c* 0.33, $CHCl_3$); UV (EtOH) λ_{max} 256.0 (ε 5900); IR (film) ν_{max} 3374, 1642 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 4.35 (1H, dd, $J=4.8$, 1.1 Hz, H-7β), 3.17 (1H, ddd, $J=11.2$, 9.6, 4.8 Hz, H-3α), 2.82 (1H, ddd, $J=13.2$, 3.6, 3.6 Hz, H-1β), 2.64 (1H, d, $J=17.2$ Hz, H-12α), 2.43 (1H, d, $J=17.2$ Hz, H-12β), 2.02 (1H, m, H-16), 1.86 (1H, m, H-15), 1.85 (1H, m, H-2), 1.80 (1H, m, H-6), 1.72 (1H, m, H-17α), 1.66 (1H, m, H-15′), 1.55 (1H, m, H-2′), 1.50 (1H, m, H-6′), 1.48 (1H, m, H-25), 1.41 (1H, m, H-4), 1.38 (1H, m, H-16′), 1.34 (1H, m, H-23), 1.35 (1H, m, H-20), 1.26 (3H, s, H-32), 1.2–1.1 (5H, H-1α, H-5α, H-23′, H-24, H-24′), 1.03 (3H, d, $J=6.7$ Hz, H-30), 1.02 (3H, s, H-19), 0.86 (6H, d, $J=6.7$ Hz, H-21, H-26), 0.85 (3H, d, $J=6.7$ Hz, H-27), 0.79 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 200.9 (s, C-11), 160.5 (s, C-8), 140.6 (s, C-9), 76.3 (d, C-3), 67.5 (d, C-7), 51.8 (t, C-12), 50.8 (s, C-14), 50.2 (d, C-17), 47.6 (s, C-13), 42.9 (d, C-5), 39.4 (t, C-24), 37.7 (s, C-10), 37.6 (d, C-4), 36.3 (d, C-20), 36.2 (t, C-22), 33.5 (t, C-1), 31.1 (t, C-2), 30.08 (t, C-15), 30.06 (t, C-6), 28.0 (d, C-25), 27.2 (t, C-16), 27.6 (q, C-32), 24.0 (t, C-23), 22.8 (q, C-26), 22.5 (q, C-27), 18.5 (q, C-21), 16.8 (C-18), 16.1 (q, C-19), 15.2 (q, C-30); HMBC cross-peaks C-1/H-5α, H_3-19; C-3/H-2, H-2′, H_3-30; C-5/H_3-19, H-7β; C-7/H-6, H-6′; C-8/H_3-32, H-6, H-6′; C-9/H_3-19, H-7β; C-11/H-12, H-12'; C-12/H_3-18; C-17/H_3-18, H_3-21; C-30/H-3α; EIMS *m*/*z* (70 eV) 444 (100) [M^+], 429 (50) [M^+-15], 420 (22) [M^+-18], 401 (15) [M^+-43], 292 (20), 263 (40), 237 (95); HREIMS *m*/*z* 444.3602 (calcd. for $C_{29}H_{48}O_3$, 444.3603).

3β,7β-Dihydroxy-4α,14α-dimethyl-5α-ergost-8-en-11-one (***3***) Amorphous solid; $[\alpha]_D^{25}+162.0°$ (*c* 0.1, $CHCl_3$); UV (EtOH) λ_{max} 260.0(ε 7600); IR (film) ν_{max} 3374, 1642 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 4.41 (1H, dd, $J=8.9$, 8.9 Hz, H-7α), 3.07 (1H, ddd, $J=11.0$, 9.8, 4.6 Hz, H-3α), 2.74 (1H, ddd, $J=13.2$, 3.6, 3.6 Hz, H-1β), 2.69 (1H, d, $J=17.2$ Hz, H-12α), 2.54 (1H, d, $J=17.2$ Hz, H-12β), 1.23 (3H, s, H-32), 1.18 (3H, s, H-19), 1.03 (3H, d, $J=6.7$ Hz, H-30), 0.9 (3H, s, H-18), 0.89 (3H, d, $J=6.7$ Hz, H-21), 0.88 (3H, d, $J=6.7$ Hz, H-26), 0.87 (3H, d, $J=6.7$ Hz, H-27); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 200.8 (s, C-11), 160.0 (s, C-8), 141.9 (s, C-9), 76.0 (d, C-3), 68.7 (d, C-7), 52.0 (t, C-12), 49.9 (s, C-14), 50.8 (d, C-17), 47.6 (s, C-13), 42.9 (d, C-5), 39.4 (t, C-24), 38.1 (s, C-10), 37.1 (d, C-4), 36.3 (d, C-20), 36.2 (t, C-22), 33.8 (t, C-1), 31.7 (t, C-2), 30.05 (t, C-15), 30.06 (t, C-6), 28.0 (d, C-25), 27.3 (t, C-16), 27.3 (q, C-32), 24.0 (t, C-23), 22.8 (q, C-26), 22.5 (q, C-27), 18.6 (q, C-21), 17.8 (C-19), 16.9 (q, C-18), 15.1 (q, C-30); EIMS *m*/*z* (70 eV) 444 (91) [M^+], 429 (78) [M^+-15], 426 (23) [M^+-18], 401 (17) [M^+-43], 292 (19), 263 (50), 237 (100); HREIMS *m*/*z* 444.3599 (calcd. for $C_{29}H_{48}O_3$, 444.3603).

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