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16β-Hydroxy-5α-cholestane-3,6-dione, a Novel Cytotoxic Oxysterol from the Red Alga *Jania rubens*

Leïla Ktari, Alain Blond and Michèle Guyot*

Laboratoire de Chimie des Substances Naturelles, associé au CNRS, Muséum National d'Histoire Naturelle, 63 rue Buffon, F-75005 Paris, France

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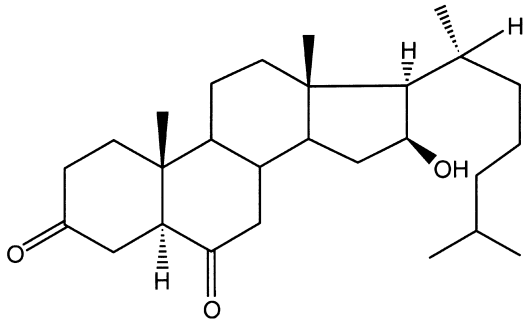
Abstract—A new cytotoxic oxysterol, 16β-hydroxy-5α-cholestane-3,6-dione was isolated from the red alga *Jania rubens*. Its structure was established by spectroscopic method. The ID_{50} value was 0.5 μg/mL.

In our ongoing program devoted to the search for bioactive compounds from marine origin, algae collected along the Tunisian coast, near Bizerte, were subjected to a panel of biological assays. The dichloromethane extract of the red alga *Jania rubens* was found to be significantly cytotoxic towards the KB tumor cell line which is valuable for the screening of anticancer agents.[1] *J. rubens* (Linnaeus) Lamouroux is a widely distributed species, which has been the object of only few studies: amino acids and proteins analysis,[2] chlorophylls and carotenoids content,[3] and isolation of 7-ketocholesterol.[4] Antitumorigenic activity of an extract of *J. rubens* was reported [5] as well as an antifungal activity,[6] but to our knowledge, no active compounds have been described to date. In this paper we describe isolation and structure elucidation of a new cytotoxic oxysterol, 16β-hydroxy-5α-cholestane-3,6-dione.

Dry specimens of *J. rubens* (200 g) were extracted with dichloromethane and then dichloromethane:MeOH 1:1. The dichloromethane extract was cytotoxic towards KB cells (ID_{50} 5 μg/mL). Chromatography on a silica gel column eluted with a dichloromethane–methanol gradient gave a fraction eluted with 5% methanol, which retained cytotoxic activity. This fraction was submitted to a preparative TLC on silica gel (dichloromethane–acetone 95:5) to give pure **1** (1 mg), which showed an ID_{50} value of 0.5 μg/mL towards KB cells. Compound **1** was obtained as a white powder, $[\alpha]_D$ −2 (c, 0.1, CH_2Cl_2), mp 125–126 °C, MS (EI) *m/z* 417 (M + H), HRMS furnished the molecular formula $C_{27}H_{44}O_3$.



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^{1}H NMR data were typical of a sterol, and ^{13}C NMR indicated 27 carbons, of which two carbonyls were at δ 211.5 and 209.2 ppm, and a secondary alcohol at δ 72.1 ppm. Localization of the two carbonyl groups at C-3 and C-6 and the hydroxyl group at C-16 was deduced from HMQC and HMBC correlations and COSY experiments, which allowed assignments of all carbons and protons of the molecule. HMBC correlations of methyl 18, 19 and 21 allowed fixing of carbons C-5 at δ 57.5, C-9 at δ 53.4, C-10 at δ 41.5, C-13 at δ 43.2, C-14 at δ 54.5, C-17 at δ 61.4 and C-20 at δ 29.5. Proton H-5 that resonates at δ 2.57 (^{1}H) and a methylene group at δ 2.55–2.33 (37.0) showed correlations with the two carbonyl groups. Two other deshielded methylene groups (δ 2.42–2.32, 37.3) and (δ 2.37–1.93, 46.3) gave a correlation with the carbonyls at δ 211.5 and δ 209.2 ppm respectively. These data suggested a diketo-3,6 skeleton. The hydroxyl group was easily located at C-16 thanks

*Corresponding author. +33-1-4079-3142; fax: +33-1-4079-3147; e-mail: guyot@mnhn.fr

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to the COSY spectrum which shows cross peaks of Me-21 with H-20 at δ 1.84, of proton H-20 with H-17 at δ 1.03, and of H-17 with proton at δ 4.36. Proton H-16 was also coupled with a methylene group resonating at δ 1.2–2.17 which enabled identification of protons at C-15. These data **1** allowed to be assigned the following structure, 16-hydroxy-diketo-3,6-cholestane. The stereochemistry was assigned on the basis of NOESY data. An NOE was observed between H-16 and H-17, which served to assign a β orientation to the hydroxyl group. This β orientation was confirmed by comparing ^{1}H NMR data of **1**, δ 4.36, W/2 = 20 Hz, with those given for guggulsterol.[7] An NOE was also observed between H-16 and H-15α at δ 2.17, other NOEs served to identify H-1β, H-4β and H-12β (see Table 1).

Diketo-3,6 sterols are not common as natural products. The only two examples found in the literature, 5-α-cholestane-3,6-dione and 11-hydroxy-5-α-cholestane-3,6-dione, were isolated from the red alga *Acantophora spicifera*,[8,9] but no activity was reported for these compounds.

Experimental

General experimental procedure. ^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker Avance 400 spectrometer with standard pulse sequences operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C, respectively. The chemical shift values are reported as ppm units and the coupling constants in Hz. The programs used for J_{mod}, NOESY, HMQC, HMBC ($J = 7$ Hz) experiments were those of the Bruker manual (1991). HRMS (positive mode) was measured on a Jeol 700 spectrometer (Ecole Normale Supérieure Paris) and EIMS, CIMS on a Nermag R 10. Optical rotations were measured with a Perkin–Elmer 341 polarimeter with a sodium lamp (λ = 589 nm) in a 10-cm microcell. Silica gel column chromatography was carried out using Kieselgel 60 (230–400 mesh, E. Merck). Fractionations were monitored by TLC using aluminum-backed sheets (silica gel 60 F-254, 0.25 mm thick) with visualization under UV (254 and 366 nm) and Lieberman spray reagent. All the solvents were distilled prior to their use.

Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) data of compound **1**, $CDCl_3$, δ ppm

Attribution	^{1}H	^{13}C	COSY	HMBC
1α	1.58	38.0	H_2-2	
1β	2.05			
2	2.32–2.42	37.3	H_2-1	
3		211.5		
4α	2.33	37.0		C-5, C-3, C-10
4β	2.55			
5	2.57	57.5		C-3, C-6, C-10, C-19
6		209.2		
7α	2.37	46.3		C-6, C-8,
7β	1.93			
8	1.93	37.6	H-9, H-14	
9	1.32	53.4		
10		41.5		
11	1.6–1.45			
12α	1.15	39.4		
12β	2.05			
13		43.2		
14	1.13	54.5		
15α	2.17	36.2		
15β	1.2			
16	4.36	72.1	H-17, H-15	
17	1.03	61.4	H-20	
18	0.88	12.6[a]		12, 13, 14, 17
19	0.94	12.8[a]		1, 10, 5, 9
20	1.84	29.5	CH_3-21	
21	0.96	18.1		17, 20, 22
22	1.1	36.3		
23	1.22	23.4		
24	1.15	39.2		
25	1.53	28.5		26, 27
26-27	0.86–0.84	12.5	H-25	25, 24

[a]Assignments can be reversed.

Algal material. *Jania rubens* (Linnaeus) Lamouroux was collected in winter along the Tunisian coast near Bizerte. The seaweed was washed with seawater, and then with distilled water to remove epiphytic growths and salt excess. The algae were allowed to dry in air, protected from light, for two weeks.

Extract preparation. Dichloromethane and methanol used for preparation of the extracts were of analytical grade and redistilled prior to use. The samples were cut into small pieces and extracted first with CH_2Cl_2 (3 times), and secondly with a MeOH:CH_2Cl_2 (1:1) mixture for 12 to 16 h at room temperature, in a flask covered with aluminum foil. The extracts (110 mg and 800 mg, respectively) were concentrated under reduced pressure and preserved at −32 °C until use.

In vitro antitumor activity assay. KB cells (human buccal epidermal carcinoma) were provided by Rhône-Poulenc-Rorer. For the bioassay we used the method of Arisawa et al.[10] with slight modifications. The cell suspension (3×10^3/mL) was placed in 96-well tissue culture microplates. Samples were dissolved in 0.2% DMSO or 0.2% ethanol and added to the cell suspension at 10 μg/mL. KB cells were counted by using neutral red as dye and absorbances were measured at 540 nm in a microplate reader (Ceres 900-Bio-Tek Instruments).

The crude extracts, chromatographic fractions and pure compound were assayed in triplicate and an average of the three values was calculated to give the results (% of inhibition or ID_{50}).

Purification of compound 1. The dichoromethane extract (110 mg) was purified on a silica gel column using dichloromethane–methanol mixtures of increasing polarity. The fraction eluted with 5% methanol retained cytotoxicity and was further chromatographed on a silica gel column eluted with dichloromethane–acetone mixtures. The active fraction (40 mg) eluted with dichloromethane–acetone 95:5 was purified by a preparative TLC on silica gel (dichloromethane–acetone 95:5) to give pure **1** (1 mg), mp 125–126 °C, HRMS m/z 417.3363 (calcd for $C_{27}H_{44}O_3$ 417.3369), $[\alpha]_D^{22}$ −2 (c, 0.1, CH_2Cl_2).

References

1. Shoemaker, R. H.; Abbot, B. J.; Macdonald, M. M.; Mayo, J. G.; Venditti, J. M.; Wolpert-de Filippes, M. K. *Cancer Treat. Rep.* **1982**, *67*, 97.
2. Soliman, F. M. E. I.; Tohamy, S. F.; Fathy, M. M.; Ramadan, A.; Afyfy, N. A.; Sanad, O. A. *J. Drug Res.* **1994**, *21*, 155.
3. Hegazi, M. M.; Perez-Ruzafa, A.; Almela, L.; Candela, M. E. *J. Chromatogr. A*. **1998**, *829*, 153.
4. Anjaneyulu, V.; Suresh Babu, J.; Rao, K. N. *Acta Cienc. Indica Chem*. **1993**, 19, 80 (CA122:286139).
5. El-Masry, M. H.; Mostafa, M. H.; Ibrahim, A. M.; El-Naggar, M. M. A. *FEMS Microbiol. Lett*. **1995**, *128*, 151.
6. Ballesteros, E.; Martin, D.; Uriz, M. J. *Bot. Mar*. **1992**, *35*, 481.
7. Cimino, G.; De Rosa, S.; De Stephano, S.; Sodano, G. *Experientia* **1984**, *40*, 246.
8. Wahidulla, S.; D'Souza, L.; Patel, J. *Phytochemistry* **1987**, *26*, 2864.
9. Prakash, O.; Roy, R.; Bhakuni, D. S.; Wahidulla, S.; Kamat, S. Y. *J. Nat. Prod.* **1989**, *52*, 686.
10. Arisawa, M.; Hayashi, K.; Nikaido, T.; Koike, K.; Fujita, D.; Nunomura, N.; Tanaka, M.; Sasaki, T. *Int. J. Pharm.* **1997**, *35*, 6.