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Novel polyhydroxysterols from the Red Sea marine sponge *Lamellodysidea herbacea*

Pierre Sauleau, Marie-Lise Bourguet-Kondracki *

Laboratoire de Chimie, UMR 5154 CNRS, Muséum National d' Histoire Naturelle, 63 rue Buffon, 75231 Paris Cedex 05, France

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Abstract

Chemical investigation of the dichloromethane extract of the Red Sea marine sponge *Lamellodysidea herbacea* led to the isolation of four novel polyhydroxysteroids: cholesta-8-en-3β,5α,6α,25-tetrol (**1**), cholesta-8(14)-en-3β,5α,6α,25-tetrol (**2**), cholesta-8,24-dien-3β,5α,6α-triol (**3**), and cholesta-8(14),24-dien-3β,5α,6α-triol (**4**). Their structures were identified through 1D and 2D NMR studies. Relative stereochemistries were established by analysis of chemical shifts, coupling constants, and NOESY correlations. Compounds **3**–**4** showed antifungal activity against *Candida tropicalis*, with an inhibition diameter of 13 and 11 mm at 10 μg/disc, respectively.

Keywords: Sponge; *Lamellodysidea herbacea*; Polyhydroxysterol; Antifungal activity

1. Introduction

In the most recent classification of Sponges [1], *Lamellodysidea* appears as a new genus, split off from *Dysidea*, because of the consistent presence of an encrusting basal plate and the lack of orientation of the skeleton with respect to the surface. Currently, the genus *Lamellodysidea* includes the two species *herbacea* and *chlorea*. Study of the marine sponge *Lamellodysidea chlorea*, collected off Australia, yielded the linear peptides dysinosins B-D, that were shown to be potent inhibitors of the blood coagulation cascade serine proteases factor VIIa and thrombin [2]. Previously, dysinosin-A had been isolated as a novel inhibitor of factor VIIa and thrombin from an Australian sponge of a new genus and species in the family Dysideidae [3]. Recent chemical investigations by our group of the dichloromethane extract of the marine sponge *Lamellodysidea herbacea* (Order Dictyoceratida, Family Dysideidae), collected in the Red Sea, have led to the isolation of seven new polychlorinated pyrrolidinone derivatives, in addition to the known dysidamide, dysidamide B and dysidamide C [4].

In our continuing study on the dichloromethane extract of the marine sponge *Lamellodysidea herbacea*, we isolated from the polar fractions four novel polyhydroxysterols: cholesta-8-en-3β,5α,6α,25-tetrol (**1**), cholesta-8(14)-en-3β,5α,6α,25-tetrol (**2**), cholesta-8,24-dien-3β,5α,6α-triol (**3**), and cholesta-8(14),24-dien-3β,5α,6α-triol (**4**).

Marine sponges of the genus *Dysidea* have already yielded a wide range of polyhydroxysterols [5–11]. Most of them have been proven to be cytotoxic on different tumor cell lines [4–9]. More recently, three new polyoxygenated sterols were found to inhibit the binding of [I125] interleukin-8 to the human recombinant IL-8 receptor type A [11].

Compounds **1**–**4** exhibited no antibacterial activity against both *S. aureus* and *E. coli* strains at 100 μg/disc, no cytotoxic activity on KB cells at 5 μg/ml, and no anti-PLA_2 activity up to a concentration of 100 μg/ml. However, compounds **3**–**4** showed antifungal activity against *Candida tropicalis*, with an inhibition diameter of 13 and 11 mm at 10 μg/disc, respectively. In contrast, compounds **1**–**2** remained inactive against *C. tropicalis* at 100 μg/disc.

* Corresponding author. Tel.: +33 1 40 79 56 06; fax: +33 1 40 79 31 35.
E-mail address: bourguet@mnhn.fr (M.-L. Bourguet-Kondracki).

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2. Experimental

2.1. General methods

Silica gel column chromatographies were carried out using Kieselgel 60 (230–400 mesh, E. Merck). Fractions were monitored by TLC using aluminium-backed sheets (Si gel 60 F254, 0.25 mm thick). Analytical reversed-phase HPLC (Kromasil RP18 column K2185, 4.6 × 250 mm, MeOH/H_2O) was performed with a L-6200A pump (Merck-Hitachi) equipped with a UV–vis detector ($\lambda = 210$ nm) L-4250C (Merck-Hitachi) and a chromato-integrator D-2500 (Merck-Hitachi).

Melting points were determined on a Reichert apparatus and are uncorrected. Optical rotations were recorded on a Perkin-Elmer 341 polarimeter. UV spectra were recorded on an Uvikon 930 spectrophotometer. IR spectra were recorded on a Nicolet IMPACT 400D FT-IR spectrophotometer.

Mass spectra were recorded on an API Q-STAR PULSAR I (Applied Biosystem) and on a JEOL MS 700BE for low and high- resolution, respectively.

^{13}C NMR spectra were obtained using a Bruker AC300 at 75.47 MHz; ^{1}H NMR spectra 1D and 2D (COSY, HSQC, HMBC, NOESY) were obtained using a Bruker AVANCE 400.

2.2. Collection, extraction, and isolation of polyhydroxysterols

Specimens of *Lamellodysidea herbacea* (Keller, 1889) (Order Dictyoceratida, Family Dysideidae) were collected by J. Vacelet in the Red Sea at a depth of 20 m in January 1985 during the Ardoukoba expedition. A voucher specimen has been deposited at the Muséum d' Histoire Naturelle in Marseille as collection number MHNM 13660.

The air-dried sponge (288 g) was extracted with 3L methanol at room temperature for 3 days. The concentrated extract (60 g) was partitioned into hexane-, CH_2Cl_2-, MeOH-, and H_2O-soluble fractions.

The CH_2Cl_2-soluble extract was then fractionated by rapid filtration on a silica gel column (Merck silica gel, 70–230 mesh) using dichloromethane with increasing amounts of methanol as eluent. The fraction eluted with 50% MeOH (9.7 g) was passed through a silica gel 60 silanised column, and eluted with MeOH/H_2O 60: 40, to yield six fractions. The fourth fraction (3.2 g), which gave positive spots to the Liebermann reagent, was chromatographed on a silica gel column, using CH_2Cl_2/MeOH 95:5 as eluent, to yield six subfractions. Subfraction 3 (500 mg) was purified on reverse-phase HPLC using MeOH/H_2O 85:15 (flow rate: 0.8 ml/min), and yielded compound **3** (5.7 mg, at $t = 15$ min) and compound **4** (5.9 mg, at $t = 18$ min). Subfraction 4 (100 mg) was subjected to successive reversed-phase HPLC, using MeOH/H_2O 80:20 as eluent (flow rate: 0.75 ml/min), and yielded compound **1** (8.3 mg, at $t = 9$ min) and compound **2** (2.9 mg, at $t = 10$ min).

2.3. Determination of antifungal activity

Antifungal activity was tested against *Candida tropicalis* (ATCC 66029), provided by Institut Pasteur, by the paper disc method. The yeast *Candida tropicalis* was grown on Sabouraud dextrose agar medium (Sanofi Diagnostic Pasteur) in Petri plates. Compounds were dissolved in MeOH. Growth inhibitions were recorded after 24 h at 29 °C.

2.4. Cholesta-8-en-3β,5α,6α,25-tetrol (*1*).

White needles; mp 225 °C; $[\alpha]_D^{25} = +16.0$ (*c* 0.83, MeOH); UV (MeOH) λ_{max} 219 nm ($\varepsilon = 1585$); IR ν cm^{-1}: 3316, 2951, 2932, 2862, 1370, 1359, 1145, 1044, 908; HRCIMS: $[M + NH_4]^+$ found at *m/z* 452.3729 (Δ −1.1 mmu) for $C_{27}H_{50}O_4N$; ^{1}H and ^{13}C NMR: see Tables 1 and 2. 0.0083 g, 0.003%.

2.5. Cholesta-8(14)-en-3β,5α,6α,25-tetrol (*2*).

White needles; mp 168 °C; $[\alpha]_D^{25} = +18.0$ (*c* 0.29, MeOH); UV (MeOH) λ_{max} 229 nm ($\varepsilon = 3981$); HRCIMS: $[M + NH_4]^+$ found at *m/z* 452.3735 (Δ −0.5 mmu) for $C_{27}H_{50}O_4N$; ^{1}H and ^{13}C NMR: see Tables 1 and 2; 0.0029 g, 0.001%.

2.6. Cholesta-8,24-dien-3β,5α,6α-triol (*3*).

White needles; mp 115 °C; $[\alpha]_D^{25} = +17.3$ (*c* 0.57, MeOH); UV (MeOH) λ_{max} 212 nm ($\varepsilon = 1585$); HRCIMS: $[M + NH_4]^+$ found at *m/z* 434.3632 (Δ −0.2 mmu) for $C_{27}H_{48}O_3N$; ^{1}H and ^{13}C NMR: see Tables 1 and 2; 0.0057 g, 0.002%.

2.7. Cholesta-8(14),24-dien-3β,5α,6α-triol (*4*).

White needles; mp 161 °C; $[\alpha]_D^{25} = +18.8$ (*c* 0.59, MeOH); UV (MeOH) λ_{max} 221 nm ($\varepsilon = 1995$); HRCIMS: $[M + NH_4]^+$ found at *m/z* 434.3631 (Δ −0.4 mmu) for $C_{27}H_{48}O_3N$; ^{1}H and ^{13}C NMR: see Tables 1 and 2; 0.0059 g, 0.002%.

3. Results and discussion

Compound **1**, the most abundant and polar compound of the series, was isolated in the form of white needles. The HRCIMS gave a pseudomolecular ion peak at *m/z* 452.3729 for $[M + NH_4]^+$ (observed for $C_{27}H_{50}O_4N$, Δ −1.1 mmu), indicating the presence of five unsaturations in the molecule. The presence of hydroxyl groups was suggested by a strong absorption at 3316 cm^{-1} in the IR spectrum. The ^{13}C NMR spectrum confirmed the presence of 27 carbons, including one tetrasubstitued double bond (δ 133.7 and δ 128.9), two oxygenated quaternary carbons (δ 77.5 and δ 71.4), and two oxymethine carbons (δ 69.4 and δ 67.9). The steroid