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Structure and biological evaluation of novel cytotoxic sterol glycosides from the marine red alga *Peyssonnelia* sp.

An-Shen Lin^a, Sebastian Engel^a, Benjamin A. Smith^b, Craig R. Fairchild^c, William Aalbersberg^d, Mark E. Hay^a, Julia Kubanek^{a,b,*}

^a School of Biology, Georgia Institute of Technology, Atlanta, GA 30332, USA

^b School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332, USA

^c Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ 08543, USA

^d Institute of Applied Sciences, University of the South Pacific, Suva, Fiji

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ABSTRACT

Bioactivity-guided fractionation of the extract from a Fijian red alga *Peyssonnelia* sp. led to the isolation of two novel sterol glycosides 19-O-β-D-glucopyranosyl-19-hydroxy-cholest-4-en-3-one (**1**) and 19-O-β-D-N-acetyl-2-aminoglucopyranosyl-19-hydroxy-cholest-4-en-3-one (**2**), and two known alkaloids indole-3-carboxaldehyde (**3**) and 3-(hydroxyacetyl)indole (**4**). Their structures were characterized by 1D and 2D NMR and mass spectral analysis. The sterol glycosides inhibited cancer cell growth with mean IC₅₀ values (for 11 human cancer cell lines) of 1.63 and 1.41 μM for **1** and **2**, respectively. The most sensitive cancer cell lines were MDA-MB-468 (breast) and A549 (lung), with IC₅₀'s in of 0.71–0.97 μM for **1** and **2**. Modification of the sterol glycoside structures revealed that the α,β-unsaturated ketone at C-3 and oxygenation at C-19 of **1** and **2** are crucial for anticancer activity, whereas the glucosidic group was not essential but contributed to enhanced activity against the most sensitive cell lines.

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1. Introduction

Red algae (Rhodophycota) have been a rich source for a large variety of novel bioactive natural products, including many terpenes and halogenated polyphenols.¹ Our efforts to discover new bioactive secondary metabolites from Fijian marine organisms have led us to collect over 300 red algal samples, representing more than 50 species found in the tropical South Pacific. Initial screening of fractionated extracts from *Peyssonnelia* sp. showed good anti-proliferation activity against human colon (HCT-116) and breast (MDA-MB-468) cancer cell lines, as well as moderate antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA).

Red algae of the genus *Peyssonnelia* are among those crustose algae that have pantropic distributions within coral reef habitats. *Peyssonnelia* spp. are typically found encrusting various hard substrates under ledges and in caves to deeper than 20 m.^{2,3} Some *Peyssonnelia* have been found as deep as 274 m, making them the deepest known photosynthetic organisms on earth.⁴ Given the difficulty of collection, there are few reports of chemical studies of *Peyssonnelia* spp. and their secondary metabolites remain largely unknown, although eight compounds have been reported representing terpene hydroquinone and fatty acid-derived structural

classes.^{5–7} The *Peyssonnelia* sp. examined in this study was collected in 2006 from 30 m deep reef overhangs near Tuvuca Island, Fiji. Bioassay-guided fractionation of its methanol extract yielded two new sterol glycosides, 19-O-β-D-glucopyranosyl-19-hydroxy-cholest-4-en-3-one (**1**) and 19-O-β-D-N-acetyl-2-aminoglucopyranosyl-19-hydroxy-cholest-4-en-3-one (**2**), and two known alkaloids, indole-3-carboxaldehyde (**3**) and 3-(hydroxyacetyl)indole (**4**). Synthetic modification of the sterol glycosides and anticancer testing of commercially available derivatives enabled examination of structure–activity relationships.

2. Results and discussion

2.1. Novel natural products

Freshly collected *Peyssonnelia* sp. was frozen until processed for extraction in the lab. Frozen tissue was ground and repeatedly extracted with methanol. The crude organic extract was subjected to cytotoxicity-guided fractionation using cell lines HCT-116 and MDA-MB-468, yielding compounds **1–4**.

The positive ESI-MS of **1** exhibited an [M+H]⁺ peak at *m/z* 563. The molecular formula of C₃₃H₅₄O₇ was established on the basis of its HR-ESI-MS [M+Na]⁺ ion at *m/z* 585.3689, suggesting seven degrees of unsaturation. Inspection of ¹H, ¹³C, HSQC, and COSY NMR spectra for **1** revealed four hydroxy signals at δ 6.43–7.44, one anomeric proton at δ 4.96, and six carbinol methine/methylene

* Corresponding author. Tel.: +1 404 894 8424; fax: +1 404 385 4440.

E-mail address: julia.kubanek@biology.gatech.edu (J. Kubanek).

protons signals at δ 4.00–4.60, in good agreement with the literature for a β -D-glucopyranoside moiety (Table 1).^{8,9} In the ^{13}C NMR spectrum, a carbonyl signal at δ 200.1 (C-3), a downfield shifted quaternary carbon at δ 167.7 (C-5), and an olefinic methine at δ 126.8 (C-4) suggested an α,β -unsaturated ketone. Considering the degrees of unsaturation and the remaining 27 carbons bearing three downfield shifted tertiary carbons at δ 56.9, 56.6, and 55.0 that resembled the resonances of C-17, C-14, and C-9 of cholesterol, a steroid structure with tetracyclic cyclopentanoperhydrophenanthrene nucleus was suggested (Fig. 1).¹⁰ The α,β -unsaturated ketone was assigned at C-3 based on HMBC correlations from δ 6.02 (H-4) to C-3, δ 34.0 (C-6), and δ 43.3 (C-10). Inspection of COSY and HMBC spectroscopic data concluded a 1,5-dimethylhexanyl (C-20–C-27) function, with C-17 connected to C-20 based on HMBC correlations from δ 1.06 (H-17) to δ 36.3 (C-20), and from δ 0.95 (H₂-21) to δ 56.9 (C-17) (Fig. 2). HMBC correlations from oxygenated methylene protons at δ 3.96 and 4.70 (H₂-19) to δ 34.7 (C-1), δ 167.7 (C-5), δ 55.0 (C-9) and C-10 concluded the aglycone structure of 19-hydroxy-cholest-4-en-3-one, a known aglycone consistent with previous reports.^{11,12} The glucosyl moiety was linked through C-19 based on HMBC correlations from H₂-19 to the anomeric carbon δ 105.6 (C-1').

ROESY cross peaks connecting H-19a and H-6a, H-6a and H-7a, H-7a and H-8, H-8 and CH₃-18, CH₃-18 and H-20 indicated the β configurations of H-19, H-8, Me-13, and H-19. Large coupling con-

stants (7.5–9.5 Hz) between adjacent protons from H-1' to H-5' of the sugar moiety were observed in CDCl₃ (Supplementary data), indicating a β -glucopyranoside structure. Thus, the structure 19-O- β -D-glucopyranosyl-19-hydroxy-cholest-4-en-3-one (**1**) of **1** was established as shown in Figure 1, with H-9 and H-14 oriented *alpha* as in cholesterol.

The positive ESI-MS of **2** exhibited a $[\text{M}+\text{H}]^+$ peak at m/z 604. The HR-ESI-MS molecular ion at m/z 602.3766 $[\text{M}-\text{H}]^-$ was appropriate for a molecular formula of C₃₅H₅₇NO₇ and suggested eight degrees of unsaturation. The UV spectrum of **2** showed the same maximal absorption wavelength at 242 nm as **1**, indicating a similar chromophore. In comparing the ^1H , ^{13}C , HSQC, HMBC, and COSY NMR spectroscopic data with those of **1**, identical signals associated with the α,β -unsaturated ketone, tetracyclic steroid, and 1,5-dimethylhexanyl functions revealed a same 19-hydroxy-cholest-4-en-3-one aglycone structure for **2**. The anomeric proton at δ 5.16 together with six proton signals at δ 4.00–4.57 suggested a glycosyl moiety similar to **1**. Among these carbinol methine and methylene protons, the signal at δ 4.41 (H-2') correlated with an upfield-shifted carbon signal at δ 57.9 (C-2') in the HSQC spectrum, indicating that this glycosyl moiety was an aminoglucoside.^{13,14} In addition, an acetyl function [^1H (δ 2.13) and ^{13}C (δ 170.7 and 24.0)] was identified. HMBC correlations from H-2' and NH (δ 8.96) to the carbonyl carbon at δ 170.7 indicated an *N*-acetylglucosamine structure.^{15,16} The linkage between the glycoside and aglycone

Table 1
NMR spectroscopic data (500 MHz, pyridine-*d*₅) for **1** and **2**^a

#	19-O- β -D-Glucopyranosyl-19-hydroxy-cholest-4-en-3-one (1)		19-O- β -D-N-Acetyl-2-aminoglucopyranosyl-19-hydroxy-cholest-4-en-3-one (2)	
	δ_{C}	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)
1	34.7	2.47 m, 1.72 m	34.3	2.48 m, 2.13 m
2	36.1	3.50 m, 2.47 m	35.7	3.00 td (15.5, 4.5), 2.42 m
3	200.1		199.5	
4	126.8	6.08 s	126.3	6.02 s
5	167.7		168.1	
6	34.0	2.58 td (14.0, 4.5), 2.15 dt (14.0, 2.0)	34.2	2.36 m, 1.60 m
7	33.0	1.63 m, 0.91 m	32.8	1.64 m, 0.87 m
8	36.5	1.40 m	36.3	1.35 m
9	55.0	0.92 m	54.9	0.86 m
10	43.3		43.1	
11	22.2	1.55 m, 1.48 m	22.3	1.50 m, 1.46 m
12	28.8	1.82 m, 1.2 4 m	28.8	1.79 m, 1.22 m
13	43.0		43.1	
14	56.6	0.84 m	56.6	1.01 m
15	24.5	1.55 m, 1.39 m	24.5	1.46 m, 1.38 m
16	40.7	1.94 dt (7.5, 2.0), 1.18 m	40.7	1.94 d (12.5), 1.04 d (11.5)
17	56.9	1.06 m	56.9	1.01 m
18	12.7	0.62 s	12.7	0.66 s
19	74.1	3.96 d (9.5), 4.70 d (9.5)	72.8	4.52 d (9.5), 3.84 d (9.5)
20	36.3	1.52 m	36.4	1.59 m
21	19.2	0.95 d (6.5)	19.2	0.93 d (6.5)
22	36.8	1.39 m, 1.02 m	36.8	1.35 m, 1.01 m
23	24.5	1.18 m, 0.92 m	24.5	1.13 m, 1.01 m
24	40.1	1.19 m, 1.19 m	40.1	1.13 m, 1.13 m
25	28.6	1.52 m	28.6	1.49 m
26	23.3	0.91 d (6.5)	23.3	0.88 d (6.5)
27	23.1	0.91 d (6.5)	23.1	0.88 d (6.5)
Glc 1'	105.6	4.96 d (8.0)	102.9	5.16 d (7.0)
2'	75.2	4.00 m	57.9	4.41 m
3'	79.4	4.22 m	76.4	4.42 m
4'	72.0	4.20 m	72.9	4.14 m
5'	79.1	4.03 m	79.2	4.00 m
6'	63.2	4.60 m, 4.42 m	63.2	4.57 dd (11.5, 5.0), 4.35 dd (11.5, 6.0)
OH-2'		7.44 d (5.0)		
OH-3'		7.29 br s		7.23 d (3.5)
OH-4'		7.17 d (3.5)		7.33 d (4.5)
OH-6'		6.43 t (6.0)		6.45 t (6.0)
NH				8.96 d (7.5)
OAc			170.7	
			24.0	2.13 s

^a br = broad; s = singlet; d = doublet; dd = doublet of doublets; m = multiplet.

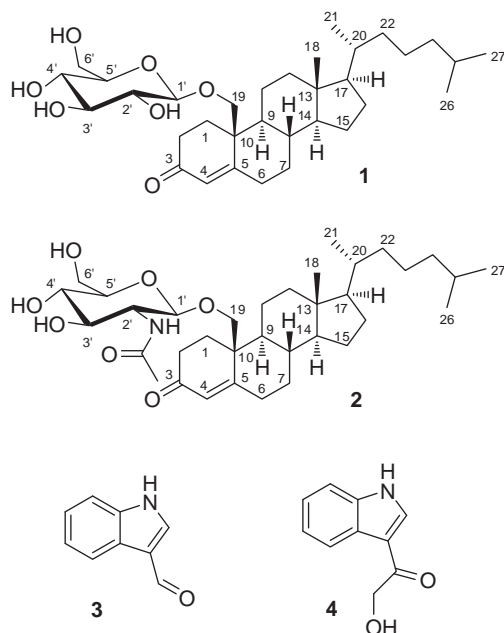


Figure 1. Novel natural products 19-*O*- β -D-glucopyranosyl-19-hydroxy-cholest-4-en-3-one (**1**) and 19-*O*- β -D-*N*-acetyl-2-aminoglucopyranosyl-19-hydroxy-cholest-4-en-3-one (**2**), and two known alkaloids, indole-3-carboxaldehyde (**3**) and 3-(hydroxyacetyl)indole (**4**) from *Peyssonnelia* sp.

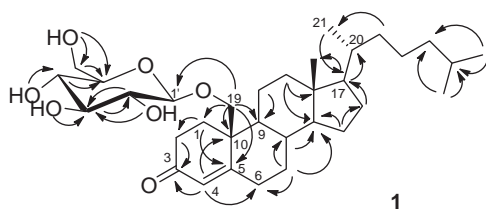


Figure 2. Key HMBC correlations of 19-*O*- β -D-glucopyranosyl-19-hydroxy-cholest-4-en-3-one (**1**).

was confirmed by HMBC correlations from both oxygenated methylene protons at δ 4.52 and 3.84 (H₂-19) to δ 102.9 (C-1') and from δ 5.16 (H-1') to δ 72.8 (C-19). Thus the structure of **2** was established as 19-*O*- β -D-*N*-acetyl-2-aminoglucopyranosyl-19-hydroxy-cholest-4-en-3-one.

Indole-3-carboxaldehyde (**3**) and 3-(hydroxyacetyl)indole (**4**) were isolated and their structures were identified by ¹H NMR and mass spectroscopic analysis in comparison with the literature.^{17,18}

2.2. Structural modification of sterol glycosides

Steroids contribute to a wide range of therapeutic applications in humans, such as cardiogenic, contraceptive and anti-inflammatory agents.¹⁹ Some synthetic steroids have been reported for their anticancer potential.^{20,21} Cholestene derivatives, however, were recently reported to exhibit weak to no cytotoxic activity.^{22,23} In order to uncover structure–activity relationships of these novel natural products, we carried out structural modifications by semi-synthesis. To obtain the cholest-4-en-3-ol analogs of **1–2**, the Luche reduction was used to reduce the α,β -unsaturated ketone of C-3 in **1** and **2**.²⁴ With sodium borohydride as reducing agent, this highly stereoselective synthesis was achieved in combination with CeCl₃ in MeOH, yielding 19-*O*- β -D-glucopyranosyl-cholest-4-en-3 β ,19-diol (**5**) from **1**, and 19-*O*- β -D-*N*-acetyl-2-

aminoglucopyranosyl-cholest-4-en-3 β ,19-diol (**6**) from **2**, each in 21–23% yield. To acquire the cholest-4-en-3-one aglycone from **2**, a mild condition of refluxing with pyridium *p*-toluenesulfonic acid (PPTS) in benzene was used, and 19-hydroxy-cholest-4-en-3-one (**7**), was acquired in 56% yield.²⁵ Commercially available 4-cholesten-3-one (**8**) was reduced to 4-cholesten-3 β -ol (**9**) in 86% yield by Luche reduction (Fig. 3).

2.3. Pharmacological evaluation

Both sterol glycosides **1** and **2** displayed moderate activity toward all human cancer cell lines tested with IC₅₀'s of 0.86–3.44 and 0.71–2.44 μ M, respectively (Table 2). More specifically, **1** and **2** displayed good cytotoxicity toward human breast cancer MDA-MB-468 with IC₅₀ = 0.71 and 0.86 μ M, respectively. Human lung cancer cell line A549, which is often more resistant to cytotoxins than other commonly used cells,²⁶ was inhibited by **1** and **2** with IC₅₀'s of 0.93 and 0.97 μ M, respectively.

Reduced derivatives **5** and **6** exhibited anticancer IC₅₀s from 6.09 to >25.0 μ M and from 6.12 to >25.0 μ M, respectively, against 11 human cancer cell lines (Table 2). Thus, the 3-keto sterol glycosides were 2.5–27 times more cytotoxic than their 3 β -hydroxy counterparts, indicating the importance of oxidation state at C-3. The importance of the glucopyranosyl moiety towards anticancer activity was less pronounced. Relative to **1–2**, their aglycone (i.e., 19-hydroxy-cholest-4-en-3-one (**7**)) exhibited slightly improved cytotoxicity toward human breast cancer cell line DU4775 and human prostate cancer cell line PC-3 with IC₅₀'s of 1.00 and 1.96 μ M, respectively, but higher IC₅₀ values for the other tested cell lines. Removal of the 19-hydroxy group to 4-cholesten-3-one (**8**) resulted in only moderate further declines in cytotoxicity, but as expected reduction to the 3 β -hydroxy form of the aglycone (i.e., 4-cholesten-3 β -ol (**9**)) led to complete loss of activity (Table 2).

Sterol glycosides **1** and **2** were further tested for their effects on cell cycle arrest and apoptosis against the A2780/DDP-S ovarian cancer cell line. Neither compound demonstrated any significant perturbation of the cell cycle or induction of PARP cleavage at 10 μ M for 23 h. A longer incubation time of 48 h also did not affect a change in cell cycle or apoptosis (data not shown).

Sterol glycosides **1** and **2** exhibited moderate antibacterial activity toward methicillin-resistant *S. aureus* with MIC's of 6.3 and 3.1 μ g/mL, respectively, and vancomycin-resistant *Enterococcus faecium*, IC₅₀ = 6.3 and 12.5 μ g/mL, respectively. Weak or no potency was detected in the assays against amphotericin-resistant *Candida albicans* (Table 3), *Mycobacterium tuberculosis* (>40 μ M, data not shown), and the malaria parasite *Plasmodium falciparum* (>40 μ M, data not shown).

3. Experimental

3.1. General experimental procedures

Optical rotations were acquired on a Jasco P-1010 spectropolarimeter. UV spectra were determined in MeOH with a Spectronic 21D spectrophotometer. NMR spectra were measured on a Bruker DRX-500 instrument, using a 5 mm broadband or inverse detection probe for ¹H, ¹³C, ¹H–¹H COSY, HSQC, HMBC, and ROESY experiments. LC-MS analyses were conducted using a Waters 2695 HPLC with Waters spectrometer with 2996 diode-array UV detection and Micromass ZQ 200 mass spectrometer with electrospray ionization in both positive and negative mode. LC-MS chromatography was achieved with an Xterra NS-C-18 3.5 μ m column measuring 2.1 \times 15 mm and gradient mobile phases of aqueous methanol with 0.1% acetic acid. High-resolution mass spectra were measured

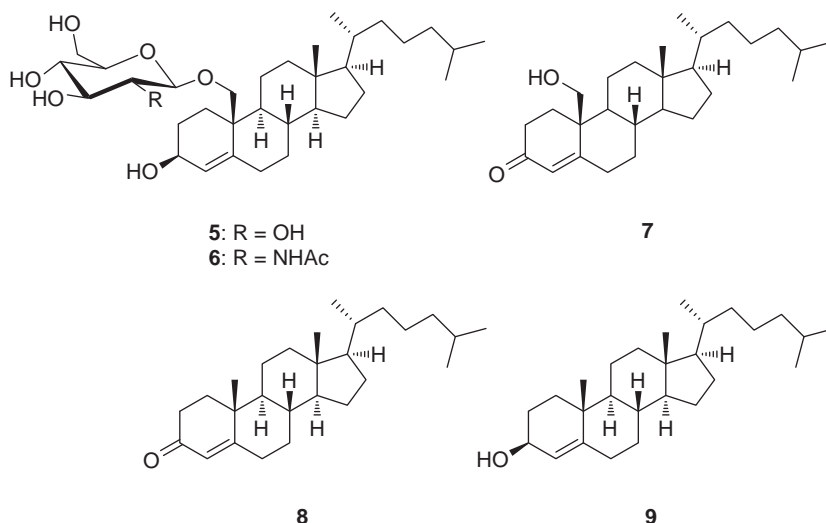


Figure 3. 19-*O*-β-*D*-glucopyranosyl-cholest-4-en-3β,19-diol (**5**), 19-*O*-β-*D*-*N*-acetyl-2-aminoglucopyranosyl-cholest-4-en-3β,19-diol (**6**), and 19-hydroxy-cholest-4-en-3-one (**7**) synthesized by modification of **1–2**; commercially available 4-cholesten-3-one (**8**) and its reduction product 4-cholesten-3β-ol (**9**).

Table 2
Cytotoxicities of *Peyssonnelia* sp. natural products and derivatives

Cell lines	IC ₅₀ (μM)						
	1	2	5	6	7	8	9
DU4775	1.29	2.44	6.09	6.12	1.00	6.74	>25.0
MDA-MB-468	0.86	0.71	6.69	7.13	3.99	6.09	>25.0
PC-3	3.44	2.40	>25.0	9.61	1.96	>25.0	>25.0
LNCaP-FGC	3.40	1.48	24.2	21.39	15.63	5.96	>25.0
HCT116	3.13	2.23	>25.0	>25.0	20.95	9.58	>25.0
MDA-MB-231	1.27	1.01	16.94	10.20	22.33	13.51	>25.0
Du145	1.31	1.35	>25.0	>25.0	5.05	20.30	>25.0
BT-549	1.26	1.24	11.87	10.06	—	—	—
A549	0.93	0.97	>25.0	>25.0	—	—	—
A2780/DDP-S	1.39	1.35	>25.0	8.98	—	—	—
CCRF-CEM	1.97	1.45	9.82	10.73	—	—	—
Mean ^a	1.63	1.41	16.71	11.98	7.09	12.45	>25.0
Cell line selectivity (IC ₅₀ max/IC ₅₀ min)	4.00	3.43	4.10	4.08	22.33	4.11	1.00

— Cell line not tested.

^a Mean IC₅₀ of all cancer cell lines tested (see the Experimental Section for details).

Table 3
Antibacterial and antifungal activities of 19-*O*-β-*D*-glucopyranosyl-19-hydroxy-cholest-4-en-3-one (**1**) and 19-*O*-β-*D*-*N*-acetyl-2-aminoglucopyranosyl-19-hydroxy-cholest-4-en-3-one (**2**)

Compd.	Antibacterial activity (μg/mL)		antifungal activity (μg/mL) ^a MIC
	MRSA MIC	VREF MIC	
1	6.3	6.3	25
2	3.1	12.5	25

^a Using amphotericin-resistant *Candida albicans*. MRSA = methicillin-resistant *Staphylococcus aureus*; VREF = vancomycin-resistant *Enterococcus faecium*.

using electrospray ionization with an Applied Biosystems QSTAR-XL hybrid quadrupole-time-of-flight tandem mass spectrometer and Analyst QS software. Semipreparative HPLC was performed using a Waters 2690 pump and 996 diode-array UV detector, controlled by Waters Millennium software. Compound purification by HPLC was performed on Agilent Zorbax SB-C-18 (5 μm, 9.4 × 250 mm) and Phenomenex Develosil C₃₀ RPAQUEOUS (5 μm, 4.6 × 250 mm) columns. All commercial chemicals were reagent grade. Optima grade (Fisher Scientific Co.) solvents were used for HPLC and LC-MS. NMR solvents were purchased from Cambridge Isotope Laboratories.

3.2. Algal material

Peyssonnelia sp. (Rhodophycota) was collected from a coral reef at 30 m depth near Tuvuca Island (S17° 39'28", W178° 50'36"), Fiji. Fresh tissue samples were frozen aboard the Vanuabalavu Fisheries vessel (TUI-NI-WASABULA) at −20 °C. All samples were transferred to a −80 °C freezer at University of the South Pacific until further processed for extraction in the laboratory. Multiple voucher samples were preserved in 10% aqueous formalin and are stored at the University of the South Pacific in Suva, Fiji and at Georgia Institute of Technology, Atlanta, GA USA with voucher identification ICBG-G-0349.

3.3. Extraction and isolation

Frozen *Peyssonnelia* sp. (227.0 g wet weight) was extracted with MeOH (500 mL) five times. The extracts were combined and reduced in vacuo to afford 4.23 g crude extract. The extracts were fractionated by HP20ss gel column chromatography and eluted sequentially with 1:1 MeOH/H₂O, 4:1 MeOH/H₂O, 100% MeOH, and 100% acetone (150 mL each) to give four fractions (F1–F4). Fraction F3 (363 mg) was separated by C₁₈ reversed-phase HPLC using an isocratic system of 95% MeOH and 5% H₂O to afford **1**

(8.0 mg) and **2** (7.6 mg); F2 (130 mg) was separated by C₁₈ reversed-phase HPLC using an isocratic system of 1:1 MeOH/H₂O to afford **3** (1.0 mg) and **4** (0.8 mg). Pure compounds (10 µg/mL) were analyzed by LC-MS to determine λ_{\max} and molecular mass, and quantified by ¹H NMR spectroscopy using 2,5-dimethylfuran as internal standard.²⁷

3.3.1. 19-O-β-D-Glucopyranosyl-19-hydroxy-cholest-4-en-3-one (1)

White amorphous solid (0.0035% wet mass); $[\alpha]_D^{23} +19.4$ (c 0.038, MeOH); UV (MeOH) λ_{\max} (log ϵ) 242 (3.77) nm; ¹H NMR (pyridine-d₅, 500 MHz) and ¹³C NMR (pyridine-d₅, 125 MHz) data, Table 1; 2D NMR data, Supplementary data; HRESIMS [M+Na]⁺ *m/z* 585.3689 (calcd for C₃₃H₅₄O₇Na, 585.3767).

3.3.2. 19-O-β-D-N-Acetyl-2-aminoglucopyranosyl-19-hydroxy-cholest-4-en-3-one (2)

White needles obtained from MeOH (0.0033% wet mass); $[\alpha]_D^{23} +11.4$ (c 0.038, MeOH); UV (MeOH) λ_{\max} (log ϵ) 242 (3.88) nm; ¹H NMR (pyridine-d₅, 500 MHz) and ¹³C NMR (pyridine-d₅, 125 MHz) data, Table 1; 2D NMR data, Supplementary data; HRESIMS [M+Na]⁺ *m/z* 626.4068 (calcd for C₃₅H₅₇NO₇Na, 626.4033).

3.4. Reduction of 1 and 2

NaBH₄ (0.0060 mmol) and CeCl₃·7H₂O (0.0060 mmol) were dissolved in 1.0 mL MeOH, and **1** (2.0 mg) was added. The reaction was stirred at room temperature for 3 h until no starting material was detected by TLC. Aqueous HCl was added to the reaction mixture, and then extracted with EtOAc (4 × 2 mL).¹⁹ The EtOAc layers were combined and dried in vacuo, and the products were purified by HPLC using an isocratic system of 9:1 MeOH/H₂O to afford **5** (0.47 mg, yield 23%). The same method was applied for **2** to afford **6** (0.43 mg, yield 21%).

3.4.1. 19-O-β-D-Glucopyranosyl-cholest-4-en-3β,19-diol (5)

White amorphous solid; ¹H NMR (pyridine-d₅, 500 MHz) δ 5.91 (br s, H-4), 4.90 (d, *J* = 8.0, H-1'), 4.54 (m, H-19a), 4.52 (m, H-3), 3.85 (m, H-19b), 2.30 (m, H-2a); HRESIMS [M+Na]⁺ *m/z* 587.3895 (calcd for C₃₃H₅₆O₇Na, 587.3923).

3.4.2. 19-O-β-D-N-Acetyl-2-aminoglucopyranosyl-cholest-4-en-3β,19-diol (6)

White needles obtained from MeOH; ¹H NMR (pyridine-d₅, 500 MHz) δ 5.89 (br s, H-4), 5.02 (d, *J* = 8.5, H-1'), 4.59 (d, *J* = 9.5, H-19a), 4.48 (m, H-3), 3.71 (d, *J* = 9.5, H-19b), 2.19 (m, H-2a); HRESIMS [M+Na]⁺ *m/z* 628.4214 (calcd for C₃₅H₅₉NO₇Na, 628.4189).

3.5. Aglycone hydrolysis

Pyridium *p*-toluenesulfonic acid (PPTS, 0.1 equiv) and **2** (1.6 mg) were dissolved in 2.0 mL benzene. The reaction was refluxed at 90 °C for 3.5 h until no starting material was detected by TLC. Water (10 mL) was added to the reaction mixture which was then extracted with EtOAc (5 × 2 mL). The EtOAc layers were combined and dried in vacuo, and the product was purified by HPLC using an isocratic system of 49:1 MeOH/H₂O to afford **7** (0.59 mg).

3.5.1. 19-Hydroxy-cholest-4-en-3-one (7)

White wax (0.59 mg); ¹H NMR (pyridine-d₅, 500 MHz) δ 6.38 (t, *J* = 5.0 Hz, OH), 6.18 (s, H-4), 4.27 (dd, *J* = 10.5, 4.0 Hz, H-19a), 4.13 (dd, *J* = 11.0, 5.5 Hz, H-19b), 3.18 (m, H-2a), 2.57 (m, H-6a), 2.50 (m, H-1a, H-2b), 2.26 (d, *J* = 13.5 Hz, H-6b), 1.98 (d, *J* = 12.5 Hz, H-16a), 1.84 (m, H-12a), 1.77–1.02 (m, 21H), 0.97 (d, *J* = 6.5 Hz, Me-21), 0.91 (d, *J* = 6.5 Hz, Me-26 and 27), 0.70 (s, Me-18); ¹³C NMR (pyri-

dine-d₅, 125 MHz) δ 199.5 (C-3), 168.4 (C-5), 126.5 (C-4), 76.3 (C-19), 56.5 (C-17), 56.3 (C-14), 54.5 (C-9), 44.4 (C-10), 42.7 (C-13), 40.4 (C-16), 39.7 (C-24), 36.5 (C-8), 36.3 and 36.1 (C-20 and 22), 35.9 (C-2), 34.2 (C-2), 33.9 (C-6), 32.6 (C-7), 28.5 (C-25), 28.3 (C-12), 24.3 and 24.2 (C-15 and 23), 22.9 (C-26), 22.7 (C-27), 21.9 (C-11), 18.8 (C-21), 12.3 (C-18); HRESIMS [M+Na]⁺ *m/z* 423.3230 (calcd for C₂₇H₄₄O₂Na, 423.3239).

3.6. Pharmacological assays

The pharmacological assays were performed as previously reported.^{28,29} Anticancer assays were conducted using 11 human cancer cell lines including breast (BT-549, DU4475, MDA-MB-468 and MDA-MB-231), colon (HCT-116), lung (A549), prostate (PC-3, LNCaP-FGC and DU145), ovarian (A2780/DDP-S) and leukemia (CCRF-CEM).³⁰ Antibacterial assays were performed using methicillin-resistant *S. aureus* (MRSA, ATCC 33591) and vancomycin-resistant *E. faecium* (VREF, ATCC 700221) as test pathogens.^{28,29} Antifungal assays were performed using amphotericin B-resistant *C. albicans* (ATCC 90873).^{28,29} Antitubercular activity was assessed against *M. tuberculosis* strain H37Rv (ATCC 27294) using the microplate alamar blue assay (MABA).³¹ Antimalarial activity was determined with a SYBR Green based parasite proliferation assay.^{28,29}

Cell cycle arrest and apoptosis experiments using the A2780/DDP-S ovarian cancer cell line were conducted for **1–2** at 1.0, 3.0 and 10 µM, over 24 and 48 h. Using flow cytometry analysis, cell cycle was assessed by propidium iodine staining of DNA and apoptosis was assessed by the presence of cleaved PARP (a marker of caspase activation in apoptotic cells).³²

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.10.010.

References and notes

- Blunt, J. W.; Copp, B. R.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2010**, *27*, 165.
- Littler, D. S.; Littler, M. M. *South Pacific Reef Plants*; Offshore Graphics: Washington, DC, 2003. p 90.
- Littler, D. S.; Littler, M. M. *Caribbean Reef Plants*; Offshore Graphics: Washington, DC, 2000. pp 86–90.
- Littler, M. M.; Littler, D. S. *Biol. unserer Zeit* **1994**, *24*, 330.
- Lane, A. L.; Mular, L.; Drenkard, E. J.; Shearer, T. L.; Engel, S.; Fredericq, S.; Fairchild, C. R.; Prudhomme, J.; Le Roch, K.; Hay, M. E.; Aalbersberg, W.; Kubanek, J. *Tetrahedron* **2010**, *66*, 455.
- McPhail, K. L.; France, D.; Cornell-Kennon, S.; Gerwick, W. H. *J. Nat. Prod.* **2004**, *67*, 1010.

7. Talpir, R.; Rudi, A.; Kashman, Y.; Loya, Y.; Hizi, A. *Tetrahedron* **1994**, *50*, 4179.
8. Lin, A.-S.; Chang, F.-R.; Wu, C.-C.; Liaw, C.-C.; Wu, Y.-C. *Planta Med.* **2005**, *71*, 867.
9. Kodai, T.; Umebayashi, K.; Nakatani, T.; Ishiyama, K.; Noda, N. *Chem. Pharm. Bull.* **2007**, *55*, 1528.
10. Seo, S.; Tomita, Y.; Tori, K.; Yoshimura, Y. *J. Am. Chem. Soc.* **1978**, *100*, 3331.
11. Ino, S.; Oinuma, H.; Yamakado, R.; Morisaki, M.; Furukawa, Y.; Hata, T. *Chem. Pharm. Bull.* **1991**, *39*, 3335.
12. Rabinowitz, M. H.; Djerassi, C. *J. Am. Chem. Soc.* **1992**, *114*, 304.
13. Yamada-Hada, J.; Hada, N.; Aoki, K.; Yamamoto, K.; Takeda, T. *Chem. Pharm. Bull.* **2004**, *52*, 473.
14. Boullanger, P.; Chevalier, Y.; Croizier, M.-C.; Lafont, D.; Sancho, M.-R. *Carbohydr. Res.* **1995**, *278*, 91.
15. Watanabe, K.; Tanaka, R.; Sakurai, H.; Iguchi, K.; Yamada, Y.; Hsu, C.-S.; Sakuma, C.; Kikuchi, H.; Shibayama, H.; Kawai, T. *Chem. Pharm. Bull.* **2007**, *55*, 780.
16. Ohtsuka, I.; Hada, N.; Ohtaka, H.; Sugita, M.; Takeda, T. *Chem. Pharm. Bull.* **2002**, *50*, 600.
17. Bernart, M.; Gerwick, W. H. *Phytochemistry* **1990**, *29*, 3697.
18. Tan, J.; Bednarek, P.; Liu, J.; Schneider, B.; Svatos, A.; Hahlbrock, K. *Phytochemistry* **2004**, *65*, 691.
19. Robbers, J. E.; Speedie, M. K.; Tyler, V. E. *Pharmacognosy and Pharmacobiotechnology*; Williams & Wilkins: Maryland, 1996. p 108.
20. Bandy, A. H.; Shameem, S. A.; Gupta, B. D.; Kumar, H. M. S. *Steroids* **2010**, *75*, 801.
21. Bandy, A. H.; Mir, B. P.; Lone, I. H.; Kumar, H. M. S. *Steroids* **2010**, *75*, 805.
22. Wang, W.; Lee, J.-S.; Nakazawa, T.; Ukai, K.; Mangindaan, R. E. P.; Wewengkang, D. S.; Rotinsulu, H.; Kobayashi, H.; Tsukamoto, S.; Namikoshi, M. *Steroids* **2009**, *74*, 758.
23. Jiang, C.-S.; Huang, C.-G.; Feng, B.; Li, J.; Gong, J.-X.; Kutan, T.; Guo, Y.-W. *Steroids* **2010**, *75*, 1153.
24. Bisogno, F. R.; Orden, A. A.; Pranzoni, C. A.; Cifuentes, D. A.; Giordano, O. S.; Sanz, M. K. *Steroids* **2007**, *72*, 643.
25. Frye, L. L.; Cusack, K. P.; Leonard, D. A. *J. Med. Chem.* **1993**, *36*, 410.
26. Miao, Z.-H.; Tong, L.-J.; Zhang, J.-S.; Han, J.-X.; Ding, J. *Int. J. Cancer* **2004**, *110*, 627.
27. Gerritz, S. W.; Seffler, A. M. *J. Comb. Chem.* **2000**, *2*, 39.
28. Kubanek, J.; Prusak, A. C.; Snell, T. W.; Giese, R. A.; Hardcastle, K. I.; Fairchild, C. R.; Aalbersberg, W.; Raventos-Suarez, C.; Hay, M. E. *Org. Lett.* **2005**, *23*, 5261.
29. Lane, A. L.; Stout, E. P.; Lin, A.-S.; Prudhomme, J.; Le Roch, K.; Fairchild, C. R.; Franzblau, S. G.; Hay, M. E.; Aalbersberg, W.; Kubanek, J. *J. Org. Chem.* **2009**, *74*, 2736.
30. Lee, F. Y. F.; Borzilleri, R.; Fairchild, C. R.; Kim, S. H.; Long, B. H.; Raventos-Suarez, C.; Vite, G. D.; Rose, W. C.; Kramer, R. A. *Clin. Cancer Res.* **2001**, *7*, 1429.
31. Falzari, K.; Zhu, Z.; Pan, D.; Liu, H.; Hongmanee, P.; Franzblau, S. G. *Antimicrob. Agents Chemother.* **2005**, *49*, 1447.
32. Litzenburger, B. C.; Kim, H.-J.; Kuitatse, I.; Carboni, J. M.; Attar, R. M.; Gottardis, M. M.; Fairchild, C. R.; Lee, A. V. *Clin. Cancer Res.* **2009**, *15*, 226.