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Blancasterol, A Cytotoxic 9,11-Secosteroid Isolated from the Northeastern Pacific Marine Sponge *Pleraplysilla* sp.

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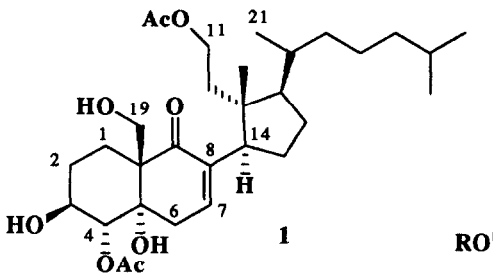
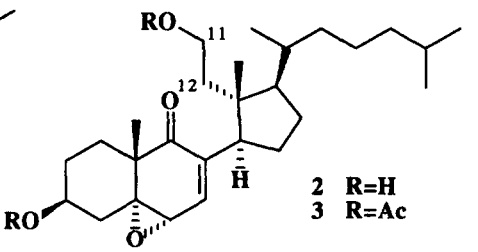
Abstract: *Blancasterol (1), a cytotoxic 9,11-secosteroid, has been isolated from the marine sponge Pleraplysilla sp. collected off the coast of Vancouver Island. The structure of blancasterol (1) was solved by analysis of spectroscopic data*

Marine sponges continue to be a rich source of highly functionalized steroids that display interesting biological activities. Recent examples include: herbasterol,[1] which is icthyotoxic; the xestobergsterols[2] and contignasterol,[3] which are potent inhibitors of histamine release from rat mast cells induced by anti-IgE; and the kiheisterones[4] and glaciasterols,[5] which are cytotoxic to human cancer cell lines in vitro. As part of an ongoing investigation of the chemistry of Northeastern Pacific marine invertebrates,[6] we have examined extracts of an undescribed species of sponge belonging to the genus *Pleraplysilla*[7] that was collected at Botanical Beach on the west coast of Vancouver Island, British Columbia. The extracts of *Pleraplysilla* sp. yielded furodysinin,[8] furodysin[8] and nakafuran-8,[9] three previously described sesquiterpenoids, as well as the new cytotoxic 9,11-secosteroid, blancasterol (**1**). Herein we describe the isolation and structure elucidation of blancasterol (**1**).



1



2 R=H
3 R=Ac

Blancasterol (**1**) was isolated as an amorphous white solid that gave a M+ H peak in the HRFABMS at m/z 551.36006 Da corresponding to a molecular formula of $C_{31}H_{51}O_8$ (ΔM 3.01 ppm). Well-resolved resonances for all thirty one carbon atoms in the molecular formula were observed in the ^{13}C NMR spectrum

(See Table 1). Resonances at δ 199.7 (C), 138.2 (CH) and 137.3 (C) were assigned to an αβ-unsaturated ketone and resonances at δ 171.8 and 171.0 were assigned to ester carbonyls. The lack of ^{13}C NMR evidence for additional unsaturated functionality in blancasterol indicated that the molecule was tricyclic. A detailed analysis of the ^{1}H and ^{13}C NMR data collected for **1** (Table 1) indicated that it was a 9,11-secosteroid, closely related to the previously reported glaciasterol B (**2**).

A pair of methyl singlets observed at δ 2.00 and 2.20 in the ^{1}H NMR spectrum of **1** and the ester carbonyl resonances observed in the ^{13}C NMR spectrum were assigned to two acetate functionalities. Subtraction of the four acetate carbons from the total of thirty one carbons in the molecular formula left the twenty seven carbons present in a standard steroidal skeleton. Resonances at δ 0.88 (d, J=6.6 Hz, 6H) and 1.00 (d, J=6.7 Hz, 3H) in the ^{1}H NMR spectrum and the ^{13}C resonances to which they were correlated in the HMQC spectrum (δ 22.5, 22.8 and 19.7) had chemical shifts characteristic of the Me26, Me27 and Me21 groups of a saturated cholestane-like steroid side chain. The resonances assigned to the remaining carbon atoms in the side chain of blancasterol (**1**) (δ 34.8 (C20), 35.5 (C22), 24.4 (C23), 39.5 (C24), and 28.5 (C25)) also had very similar chemical shifts to the literature values reported for cholesterol (δ 36.0 (C20), 18.9 (C21), 36.4 (C22), 24.1 (C23), 39.7 (C24), 28.1 (C25), 22.6 (C26), and 22.8 (C27)),[10] confirming the proposed side chain substructure.

COSY and HMQC data for **1** identified an isolated proton spin system consisting of mutually coupled resonances at δ 1.40 (H12), 1.62 (H12'), 4.05 (H11) and 4.22 (H11') that were assigned to two sets of methylene protons attached to adjacent carbons (δ 62.0 (C11), 37.1 (C12)). Both the ^{1}H and ^{13}C NMR chemical shifts observed for this ethyl fragment in blancasterol (**1**) were very similar to the corresponding chemical shifts for the C11/C12 fragment in glaciasterol B diacetate (**3**) (^{1}H: δ 1.23 (H12), 1.69 (H12'), 4.10 (H11) and 4.19 (H11'); ^{13}C: δ 61.1 (C11), 36.6 (C12)). The C12 resonance at δ 37.1 in **1** showed a HMBC correlation to a methyl resonance at δ 0.74 (s) in the ^{1}H NMR spectrum which was assigned to Me18. The Me18 proton resonance (δ 0.74) showed additional HMBC correlations to a quaternary carbon at δ 45.8, assigned to C13, and to a pair of methine carbon resonances at δ 42.7 and 50.8, assigned to C14 and C17, respectively. A proton resonance at δ 1.00 (d, J=6.7 Hz), assigned to Me21 in the steroid side chain, also showed a HMBC correlation to the C17 resonance (δ 50.8). The HMQC spectrum showed a correlation from the C14 resonance at δ 42.7 to the H14 resonance at δ 3.28. ROESY correlations observed between the H14 resonance (δ 3.28) and the H11 (δ 4.05)/ H11' (4.22) resonances established the normal trans relationship between Me18 and H14.

The substitution pattern on the A/B ring system of blancasterol was also deduced from analysis of the NMR data. A correlation in the HMQC spectrum showed that one of the olefinic carbons (δ 138.2: C7) of the αβ-unsaturated ketone was attached to a proton at δ 6.44 (H7). Correlations were observed in the COSY spectrum from this olefinic proton resonance (δ 6.44: H7) to a pair of geminal methylene proton resonances at δ 2.35 (H6) and 2.56 (H6'). The observed coupling constant of 5.8 Hz between the resonances at δ 6.44 and 2.35 (H7/H6) was appropriate for vicinal coupling, indicating that the olefinic proton (H7) had to be attached to the β carbon of the αβ-unsaturated ketone, which in turn had to be attached to an aliphatic methylene carbon (C6). HMBC correlations were observed from the β-olefinic proton resonance at δ 6.44 (H7) to the ketone carbonyl resonance at δ 199.7 (C9), to the C14 resonance at 42.7, and to a quaternary carbon resonance at 76.7 (C5). H14 (δ 3.28) was in turn correlated to the ketone (δ 199.7: C9) and α-olefinic carbon (137.3: C8) resonances in the HMBC spectrum. This set of HMQC and HMBC correlations confirmed that C14 was attached to the α carbon

(C8) of the unsaturated ketone fragment and demonstrated that there was an oxygen substituent attached to C5. A pair of one proton doublets at δ 3.91 (J=11.2 Hz: H19) and 4.05 (J=11.2 Hz: H19') in the ^{1}H NMR spectrum, that were correlated to a carbon resonance at δ 61.5 in the HMQC spectrum, were assigned to a hydroxymethyl functionality. The hydroxymethyl resonance at δ 3.91 (H19) was correlated to the ketone carbonyl at δ 199.7 (C9) and to a methylene carbon at 19.7 (C1) in the HMBC spectrum.

HMQC correlations indicated that C1 (δ 19.7) was attached to protons with chemical shifts of δ 1.88 and 2.26 ($H1_{ax}$ and $H1_{eq}$). A combination of COSY and HMQC data provided evidence for extension of this fragment in a linear sequence through an adjacent methylene (C2: ^{1}H δ 1.62, 2.09; ^{13}C δ 26.0) and two methine carbons (C3: ^{1}H δ 4.01; ^{13}C δ 69.9 and C4: ^{1}H δ 4.98; ^{13}C δ 78.8). A HMBC correlation between the H4 resonance at δ 4.98 and one of the ester carbonyl resonances (δ 171.0) demonstrated the attachment of an acetoxy functionality to C4. ROESY data, collected in $CDCl_3/C_6D_6$ 20:1 to improve dispersion of the resonances, showed that the H4 resonance (δ 4.93) was correlated to the H6β (δ 2.46) and H19 (δ 3.83) resonances, and that the H6β resonance (δ 2.46) was also correlated to both the H19 (δ 3.83) and H19' (δ 3.98) resonances. The observed ROESY correlations demonstrated that both H4 and the C19 hydroxymethyl were axial and that the two six membered rings were trans fused.

The location of the two acetate functionalities at C4 and C11 in blancasterol (**1**) confirmed that the oxygen substituents at C3, C5 and C19, which were indicated by the ^{13}C NMR chemical shifts of the three carbons (δ 69.9(C3), 76.7(C5), 61.5(C19)), had to be alcohol functionalities. A coupling constant of 9.5 Hz was observed between H3 and H4 which was consistent with diaxial coupling and 3β hydroxyl, 4α acetoxy substitution. The trans fusion of the A and B rings revealed by the ROESY data required an α hydroxyl substituent at C5. We have assumed that blancasterol has the normal steroidal configurations at C17 and C20 as shown in **1**. The similarity in chemical shifts of the side chain carbons in **1** and in cholesterol supports this assumption.

Blancasterol (**1**) showed in vitro cytotoxicity against L1210 murine leukemia (ED_{50} 8 μg/mL), drug sensitive MCF-7 human breast cancer (ED_{50} 3 μg/mL), and drug resistant MCF-7 Ad^r human breast cancer (ED_{50} 10 μg/mL) cell lines. The cytotoxicity of blancasterol (**1**) parallels that of the glaciasterol diacetates[5] (i.e. **3**: L1210 murine leukemia (ED_{50} 3 μg/mL), MCF-7 human breast cancer (ED_{50} 3μg/mL), and MCF-7 Ad^r human breast cancer (ED_{50} 2 μg/mL))[11] demonstrating that the epoxide functionality in the B ring of the glaciasterols is not required for cytotoxicity. It is interesting to note that although blancasterol (**1**) and the glaciasterol diacetates (i.e. **3**) are not extremely potent in vitro cytotoxins they are nearly equally effective against the drug sensitive MCF-7 and the drug resistant MCF-7 Ad^r human breast cancer cell lines, suggesting that they may be able to circumvent the normal mechansism of multidrug resistance.

EXPERIMENTAL

Collection and Isolation: A small sample of *Pleraplysilla* sp. (2g dry weight) was collected from an exposed rock during a low tide. The freshly collected sponge was frozen over solid CO_2 on site and kept frozen until it was lyophilized. Multiple extractions of the dried sponge with ethyl acetate followed by concentration of the combined extracts in vacuo gave a crude green oil (70mg). Fractionation of the crude oil via silica gel step gradient flash chromatography gave pure samples of furodysin, furodysinin, nakafuran-8 and blancasterol (**1**) (2mg). The known sesquiterpenoids were identified by comparison of their ^{1}H NMR data with literature

values.[8,9] **Blancasterol (1):** isolated as an amorphous white solid; UV (MeOH) λ_{max} (ε) 288 nm (1,050), 240 nm (5,100); CD (MeOH) $(\Theta)_{235}$ 15,600, $(\Theta)_{266}$ -670, $(\Theta)_{330}$ 556; ^1H NMR ($CDCl_3$, 400MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 125MHz) see Table 1; EILRMS m/z (relative intensity) 532 (1), 490 (3), 430 (2), 412 (2), 269 (21); FABHRMS (M + H) m/z 551.36006 ($C_{31}H_{51}O_8$, ΔM 3.01 ppm).

Table 1. NMR Data for blancasterol (1). Recorded in $CDCl_3$ at 400MHZ (^1H) and 125 MHz (^{13}C).

Carbon no.	δ ^1H	δ ^{13}C	Carbon no.	δ ^1H	δ ^{13}C
1_{eq}	2.26,m	19.7	15	1.73, m	29.7
1_{ax}	1.88,ddd,J=3.3,13.2,14.8 Hz		15'	1.46, m	
2	2.09,m	26.0	16		28.0
2'	1.62,m		17		50.8
3	4.01,m	69.9	18	0.74, s	16.7
4	4.98,d,J=9.5 Hz	78.8	19	3.91, d, J=11.2 Hz	61.5
5		76.7	19'	4.05, d, J=11.2 Hz	
6	2.35, dd, J=5.8,19.8 Hz	34.5	20	1.45, m	34.8
6'	2.56, dd, J=2.4,19.8 Hz		21	1.00, d, J=6.7 Hz	19.7
7	6.44, dd, J=2.4,5.8 Hz	138.2	22	1.83, m	35.5
8		137.3	22'	1.67, m	
9		199.7	23		24.4
10		56.3	24	1.83, m	39.5
11	4.05, m	62.0	24'	1.73, m	
11'	4.22, ddd, J=5.3, 10.6, 15.8 Hz		25	1.52, m	28.5
12	1.40, m	37.1	26	0.88, d, J=6.6 Hz	22.8
12'	1.62, m		27	0.88, d, J=6.6 Hz	22.5
13		45.8	OAc	2.00, s	20.9, 171.0
14	3.28, t, J=11.6 Hz	42.7	OAc	2.20, s	21.1, 171.8

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