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Polyoxygenated *Dysidea* Sterols That Inhibit the Binding of [I125] IL-8 to the Human Recombinant IL-8 Receptor Type A

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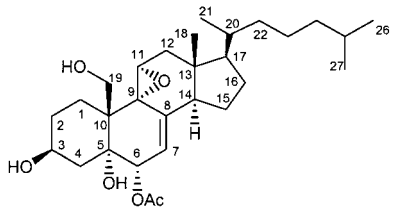
The combined CH_2Cl_2 and MeOH crude extract of a new species of the marine sponge *Dysidea*, collected in Northern Australia was found to inhibit the binding of [I125] interleukin-8 [IL-8] to the human recombinant IL-8 receptor type A at 500 μg/mL. Bioassay-guided fractionation led to the isolation of three new polyoxygenated sterols **3**, **4**, and **5**. Their structures were assigned on the basis of 1D and 2D NMR experiments, and relative stereochemistries were established by ROESY correlations and analysis of coupling constants. The IC_{50} values for inhibition of IL-8Ra for sterols **3**, **4**, and **5** were 20, 5.5, and 4.5 μM, respectively.

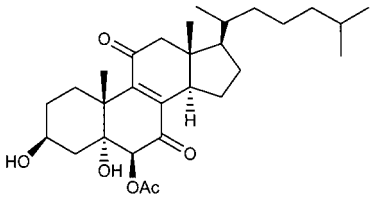
Marine sponges are well-known as a rich source of bioactive steroid compounds ranging in size and type of carbon skeleton and oxygenation patterns.[1] The genus *Dysidea* (Dictyoceratida) has provided a wide array of polyoxygenated sterols, including the first example of a 9,11-epoxide present in **1**[2] and the rare Δ^8, 11-keto functionality as in the cytotoxic sterol **2**.[3] In this report, the isolation of three new polyoxygenated sterols (**3**−**5**) from a new species of *Dysidea* using bioassay-guided fractionation for inhibition of the binding of [I125] interleukin (IL-8) to the human recombinant IL-8 receptor type A is described.

Flash chromatography of the combined CH_2Cl_2 and MeOH extracts of the *Dysidea* sp. on Si gel yielded six fractions, three of which were active. Sterol **3** was present in the most polar fraction. The other two active fractions were pooled and further chromatographed on a silica HPLC column using isocratic elution of hexane/EtOAc, 70:30, yielding **4** and **5**.

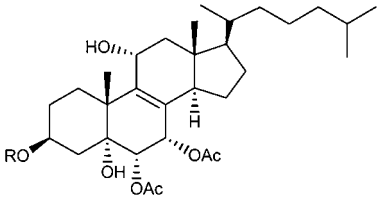
The molecular formula of sterol **3** was established as $C_{31}H_{50}O_7$ based on high-resolution measurements (positive ESI) *m*/*z* 557.3460 (calcd for $[C_{31}H_{50}O_7 + Na]^+$ 557.3448). The ^{1}H NMR spectrum of **3** (Table 1) showed two acetate methyl singlets (δ 2.10 and 2.06), two aliphatic methyl singlets (δ 1.26 and 0.58), and three aliphatic methyl doublets (δ 0.93, 0.88, and 0.87). Four oxygenated one-proton signals were present at δ 5.61, 5.35, 4.46, and 4.10. The ^{13}C NMR spectrum of **3** (Table 1) showed the presence of 31 carbons: five oxygenated carbons (four methines and one quaternary), one tetrasubstituted double bond (142.8 and 132.2 ppm), seven methyl groups, two aliphatic quaternary carbons, 13 aliphatic methylene and methine carbons, and two acetate carbons. One-bond correlations between protons and carbons were obtained by HMQC experiments, and the connectivity of the carbon framework was based on HMBC and COSY correlations (Table 1). Correlations from the oxygenated methines at δ 5.35 (H-6) and 5.61 (H-7) to each of the carbonyls 7-OCOCH_3 (170.1 ppm) and 6-OCOCH_3 (170.4 ppm) established a 6,7 oxygenation pattern. HMBC correlations from H-2a (δ 1.55), H-4a (δ 1.40), and H-4b (δ 2.07) to the oxygenated carbon at 66.7 ppm and corresponding COSY correlations to its attached methine proton (δ 4.10) indicated that an alcohol group was attached to C-3. Correlations from the oxygenated methine H-11 (δ 4.46) to C-8, C-9, and C-13 indicated



(1)



(2)



(3) R=H

(4) R=Ac

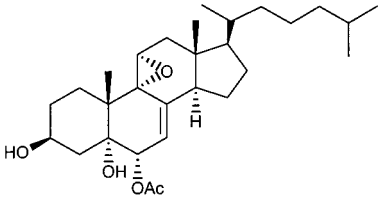


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Table 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR Data and HMBC and COSY Correlations for Sterols **3**, **4**, and **5** in $CDCl_3$

	3				**4**				**5**			
position	^{13}C	^{1}H	HMBC	COSY	^{13}C	^{1}H	HMBC	COSY	^{13}C	^{1}H	HMBC	COSY
1	29.5	2.33 dt (3.6, 13.8)	C2, C10, C19	H1a, H2a	29.0	2.38 m	C2, C10	H1a, H2b	25.1	2.03 m	C2, C10	H1a, H2b
		1.68 m	C2, C19	H1b, H2a, H2b		1.70 m	C2, C3	H1b		0.98 m	C3	H1b, H2a, H2b
2	30.5	1.96 m	C1	H1a, H2a	26.3	2.01 m		H1b, H2a	29.9	1.88 m		H1b, H2a
		1.55 m	C1, C3	H1a, H1b, H2b, H3				H3				H3
						1.59 m		H2b		1.46 m		H2b, H3
3	66.7	4.10 sept (5.4)		H2a, H4a, H4b	69.7	5.15 sept (5.4)	3-OCOCH$_3$	H2b, H4a, H4b	66.7	4.04 sept (5.2)		H2a, H2b, H4a, H4b
4	36.4	2.07 m	C2, C3, C5, C6, C10	H3, H4a	32.7	2.08 m	C2, C3, C5, C10	H3, H4a	38.9	2.04 m	C2, C3, C10	H3, H4a
		1.40 m	C2, C3	H3, H4b		1.50 m	C3	H3, H4b		1.43 m	C2, C3	H3, H4b
5	75.6				75.1				74.9			
6	69.7	5.35 d (6)	C7, 6-OCOCH$_3$	H7	69.4	5.35 d (6)	C7, 6-OCOCH$_3$	H7	73.5	5.39 t (2)	C7, C8, 6-OCOCH$_3$	H7
7	67.2	5.61 d (6)	C5, C6, C8, C9, C14, 7-OCOCH$_3$	H6	66.8	5.61 d (6)	C5, C6, C8, C9, C14, 7-OCOCH$_3$	H6	122.0	5.33 t (2)	C5, C9, C14	H6, H14
8	132.2				131.9				139.8			
9	142.8				142.3				62.6			
10	44.3				44.0				38.9			
11	66.1	4.46 dd (4.2, 8)	C8, C9, C13	H12a, H12b	65.9	4.45 br s		H12a, H12b	53.8	3.16 d (5.4)	C9, C12, C13	H12b
12	49.4	2.40 dd (8, 13.8)	C9, C11, C13	H11, H12a	49.2	2.39 m	C9, C11, C14	H11, H12a	40.0	2.15 m	C13, C14, C18	H11, H12a
		1.68 dd (4.2, 13.8)	C14, C18: C17, C18	H11, H12b		1.68 m	C11, C13, C17, C18	H11, H12b, H18		1.88 m	C9, C11, C14, C17, C18	H12b, H18
13	47.4				47.1				43.7			
14	48.2	2.53 dd (7.6, 12.4)	C8, C9, C12, C13, C16, C18	H15a, H15b	47.9	2.56 m	C8, C9, C13, C15, C18	H15a, H15b	46.6	2.39 br t (9)	C7, C8, C13, C15, C18	H15b
15	22.6	1.48 m		H14, H16b	22.3	1.47 m	C13	H14, H15a, H19b	22.1	1.60 m	C13	H14, H16
		1.38 m	C14, C15, C17	H14		1.38 m	C16	H14, H15b		1.33m		
16	28.5	1.92 m	C13, C16, C17, C20	H16a, H15b	28.3	1.92 m		H15b, H16a	29.0	1.48 m		H17
		1.34 m		H16b		1.35 m		H15b				
17	56.1	1.36 m			55.9	1.36 m			56.4	1.27 m	C13, C18	H16, H21
18	13.7	0.58 s	C12, C13, C17		13.5	0.58 s	C12, C13, C14, C17	H12a	13.8	0.59 s	C12, C13, C14, C17	H12a
19	24.4	1.26 s	C1, C5, C9, C10		24.0	1.27 s	C1, C5, C9, C10		20.4	1.23 s	C1, C5, C9, C10	
20	36.2	1.33 m		H21	35.9	1.35 m		H21	35.6	1.35 m	C16, C17, C21	H21
21	18.6	0.93 d (6)	C17, C20	H20	18.3	0.93 d (5.4)	C20	H20	18.4	0.90 d (6)	C17, C20	H20
22	36.1	1.32 m		H22a, H22b	35.8	1.32 m			35.8	1.01 m		H20, H23b, H24
		1.00 m		H23b								
23	24.0	1.34 m		H22a	23.8	1.33 m		H24a, H24b	23.8	1.34 m		H22, H24
		1.15 m	C25			1.20 m				1.27 m		
24	39.7	1.16 m	C23		39.4	1.18 m	C25	H23b, H24a, H25	39.5	1.14 m	C23	H22
		1.10 m				1.00 m		H23b, H24a				
25	28.2	1.53 m	C23, C24, C26	H26, H27	28.0	1.52 m	C24	H24b, H26, H27	28.0	1.52 m	C24, C26, C27	
26	23.0	0.88 d (2.4)	C24, C25, C27	H25	22.5	0.88 d (2.4)	C24, C25, C27	H25	22.7	0.88 d (2.4)	C24, C25, C27	H25
27	22.7	0.87 d (2.4)	C24, C25, C26	H25	22.8	0.87 br s	C24, C25, C26	H25	22.5	0.87 d (2.4)	C24, C25, C26	H25
6-OCOCH$_3$	170.4				170.4				171.0			
7-OCOCH$_3$	170.1				170.2							
6-OCO*CH*$_3$	21.1	2.10 s			21.3	2.03 s	6-OCOCH$_3$		21.2	2.17 s	6-OCOCH$_3$	
7-OCO*CH*$_3$	20.8	2.06 s			20.6	2.07 s	7-OCOCH$_3$					
3-OCOCH$_3$					170.0							
3-OCO*CH*$_3$					20.9	2.11 s	3-OCOCH$_3$					

that another alcohol group was attached to C-11. The quaternary carbon at 75.6 ppm was established as C-5 from HMBC correlations from H-4b, H-7, and H-19. COSY correlations established the remaining steroid nucleus. ROESY correlations from the methine doublet at δ 5.35 (H-6) to the methine at δ 5.61 (H-7) ($J_{6,7}$ = 6 Hz), to the methyl at δ 1.26 (H-19), and to methylene at δ 1.40 (H-4a) established those groups on the β face of the molecule. The methyl group at δ 0.58 (H-18) showed strong ROESY correlations to the methylene at δ 1.34 (H-16a), to the methyl at δ 0.93 (H-21) and to the methylene at δ 2.40 (H-12b). The latter showed strong correlation to the methine at δ 4.46 (H-11), which, in turn, showed correlation back to the methyl at δ 1.26 (H-19), thus establishing those groups on the same β face. The methylene at δ 1.67 (H-12a) showed correlations to the bridgehead methine at δ 2.53 (H-14) and the methine at δ 1.36 (H-17) correlated to the methylene at δ 1.92 (H-16b). These were thus placed on the α face of the molecule, establishing the side chain at C-17 on the β face. Also on the α face was the oxygenated methine at δ 4.10, with correlations to the methylene protons at δ 2.07 (H-4b), 1.96 (H-2b), and 2.33 (H-1b).

The structures of sterols **4** and **5** were also determined by 1D and 2D NMR experiments and were supported by MS analysis. Their molecular formulas were established as $C_{33}H_{52}O_8$ and $C_{29}H_{46}O_5$ by high-resolution measurements *m/z* 599.3569 $[C_{33}H_{52}O_8 + Na]^+$ (calcd for $[C_{33}H_{52}O_8 + Na]^+$ 599.3554) and *m/z* 497.3244 $[C_{29}H_{46}O_5 + Na]^+$ (calcd for $[C_{29}H_{46}O_5 + Na]^+$ 497.3237). The ^{1}H and ^{13}C NMR spectra of **4** were very similar to those of **3**, with the coincidence of many chemical shifts (see Table 1). The major difference was the presence of an extra acetate group at C-3, which resulted in changes in chemical shifts for the proton from δ 4.10 (alcohol) to δ 5.15 (acetate) and for the carbon from 66.7 ppm (alcohol) to 69.7 ppm (acetate). Other carbons around C-3 were also affected, such as C-2 and C-4 (see Table 1). The ROESY spectrum of **4** was consistent with that of **3** and confirmed their relative stereochemistry. The ^{1}H and ^{13}C NMR spectra of **5** were again fairly similar to those of **3** and **4**. The major differences were the presence of only one acetate group and one olefinic proton triplet at δ 5.33, which established a trisubstituted Δ^7. The presence of an epoxide ring was indicated by the oxygenated proton doublet at δ 3.16 (C-11 at 53.8 ppm), which showed HMBC correlations to the oxygenated quaternary C-9 (62.6 ppm), the bridgehead C-13 (40.0 ppm), and methylene C-12 (40.0 ppm). ROESY correlations observed in the spectrum of **5** were consistent with those observed for **3** and **4**. The assignment of the epoxide ring on the α face of the molecule was based on correlations observed between the oxygenated methine at δ 3.16 (H-11) to the methyl group at δ 1.23 (H-19) and to the methylene protons at δ 0.98 (H-1a) and δ 2.15 (H-12b).

Sterol **5** may be the precursor of the other two sterols, via acetate addition at C-7, double-bond migration, and epoxide-ring opening. Sterols **3**, **4**, and **5** inhibited the binding of [I125] IL-8 to the human recombinant IL-8 receptor type A in a competitive fashion. The IC_{50} values for inhibition of IL-8Ra for sterols **3**, **4**, and **5** were 20, 5.5, and 4.5 μM, respectively.

Experimental Section

General Experimental Procedures. NMR spectra were recorded on a Varian Unity INOVA at 599.926 MHz for ^{1}H and 149.98 MHz for ^{13}C. ^{1}H and ^{13}C were referenced to the peak solvent ($CDCl_3$) δ 7.26 and 77.3 ppm or (DMSO-d_6) δ 2.49 and 39.5 ppm, respectively. Standard parameters were used for 1D and 2D NMR spectra, which included ^{1}H, ^{13}C, DEPT, gradient COSY, HMQC, HMBC, and ROESY. UV spectra were recorded on a GBC 916 UV−vis spectrometer, and IR spectra were recorded on a Perkin-Elmer 1725X FT-IR spectrometer. Optical rotation was measured on a JASCO P-1020 polarimeter. Davisil silica powder (30−40 μm) was used for packing the semipreparative AP-1 Waters glass column (10 × 100 mm). Rainin 3 μm silica analytical HPLC column (4.6 × 100 mm) was used for analytical and semipreparative chromatography. A Waters 600 pump with a 717 Autosampler connected to an Alltech 500 evaporative light-scattering detector and a Waters 410 differential refractometer detector were used for analytical and semipreparative HPLC separations. Low-resolution mass spectra were measured on a Fisons VG Platform II, using positive electrospray ionization mode.

Animal Material. The *Dysidea* sample, which appears to represent a new species, was collected off Lizard Island, North Queensland, Australia. A voucher sample (QMG304134) has been lodged at the Queensland Museum, South Brisbane, Australia. Taxonomy: Porifera; Demospongiae; Dictyoceratida; Dysideidae; *Dysidea* new species (QM species number #1519). Description: shape, ranging from thinly encrusting to erect, arborescent, shrub-like, producing distinct lobate, flat lamellate, ridge-like or erect fingers superficially resembling an alga. Color: live coloration dull gray-green, yellowish-green, or greenish with yellow tips. Oscules: small, less than 3 mm in diameter, scattered on apexes of digits. Texture: very soft, compressible, mucus; produces copious amounts of a dark brown to purple-brown pigment after collection. Surface: microconulose, with small conules interconnected by ridges, producing a furry surface, and with soft ridges and shallow grooves running longitudinally along branches, producing a macroscopically angular surface. Ectosomal skeleton: membranous, with minimal surface detritus, but with ascending primary spongin fibers cored by sand grains and spicule fragments protruding through the surface and forming surface microconules; areas between conules free of detritus. Choanosomal skeleton: distinct primary and secondary spongin fibers. Primary fibers mainly ascending, up to 120 μm in diameter, depending on the detritus contained within, and secondary fibers mainly transverse, up to 60 μm in diameter, together forming an irregular but more-or-less relatively evenly spaced reticulation throughout, with fibers only occasionally branching and rejoining, forming very large meshes (>1 mm diameter). Primary fibers fully cored with small and large detritus (sand grains, foreign spicule fragments, diatoms, and molluscan shell fragments), secondary fibers often with only a thin axial core of detritus. Spongin fibers heavy, with relatively dense but mostly unpigmented collagen, distinct from collagenous mesohyl. Mesohyl composed of very dense collagen, lightly or heavily pigmented yellowish-brown, with few scattered detritus particles; choanocyte chambers are oval, eurypylous 90−140 μm long and 40−60 μm wide. From the literature of Australasian and New Caledonian *Dysidea*, this taxon is probably new to science.

Extraction and Isolation. The freeze-dried and ground sponge (4.468 g) was exhaustively extracted with CH_2Cl_2 followed by MeOH and the extracts combined (304.5 mg). The crude extract was filtered through a plug of charcoal (300 mg) using CH_2Cl_2 followed by MeOH as eluent. The filtered crude extract (177.8 mg) was fractioned on a Waters AP-1 silica column using stepped gradient elution: 100% CH_2Cl_2, 50% CH_2Cl_2/EtOAc, 100% EtOAc, 20% MeOH/EtOAc, 50% MeOH/EtOAc, and 100% MeOH for 60 min at 4 mL/min. Six fractions were collected, of which fractions 3 to 5 were active. Fraction 5 consisted of pure sterol **3** (18.4 mg). Fractions 3 and 4 were combined and further fractionated on analytical silica HPLC column using isocratic elution of hexane/EtOAc, 70:30, in 25 min. The separation was optimized using an ELSD for detection, and the collection of fractions was performed using a differential refractometer detector. Sterol **4** (1.3 mg) eluted at 8 min, and sterol **5** (2.3 mg) eluted at 17 min.

Cholest-8-ene-3*β*,5α,6α,7α,10α-pentol 6,7-diacetate (3): white powder (18.4 mg, 0.41%): $[\alpha]^{24}_D$ 589° (0.680 g/100 mL, $CHCl_3$) = +74°; UV (CH_2Cl_2) λ_{max} (log ϵ) 231 nm (1.13), 250

nm (0.30); IR ν_{max} (NaCl cell) 3416, 2951, 1730, 1653, 1457, 1374, 1243, 1048, 741 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; (+)LRESMS *m/z* 557 $[C_{31}H_{50}O_7 + Na]^+$; (+)HRESMS *m/z* 557.3460 $[C_{33}H_{52}O_8 + Na]^+$ (calcd for $[C_{33}H_{52}O_8 + Na]^+$ 557.3448).

Cholest-8-ene-3*β*,5α,6α,7α,10α-pentol 3,6,7-triacetate (4): white powder (1.3 mg, 0.03%); $[\alpha]^{24}_D$ 589° (0.031 g/100 mL, $CHCl_3$) = +21°; UV (CH_2Cl_2) λ_{max} (log ϵ) 231 nm (2.265), 244 nm (1.273); IR ν_{max} (NaCl cell) 3438, 2959, 2106, 1715, 1651, 1456, 1373, 1243, 1028, 738 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; (+)LRESMS *m/z* 599 $[C_{33}H_{52}O_8 + Na]^+$; (+)HRESMS *m/z* 599.3569 $[C_{33}H_{52}O_8 + Na]^+$ (calcd for $[C_{33}H_{52}O_8 + Na]^+$ 599.3554).

9α,11α-Epoxycholest-7-ene-3*β*,5α,6α-triol 6-acetate (5): white powder (2.3 mg, 0.05%); $[\alpha]^{24}_D$ 589° (0.131 g/100 mL, $CHCl_3$) = +32°; UV (CH_2Cl_2) λ_{max} (log ϵ) 231 nm (0.657), 244 nm (0.474); IR ν_{max} (NaCl cell) 3362, 2928, 1740, 1666, 1467, 1371, 1236, 1039, 736 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; (+)LRESMS *m/z* 497 $[C_{29}H_{46}O_5 + Na]^+$; (+)HRESMS *m/z* 497.3244 $[C_{29}H_{46}O_5 + Na]^+$ (calcd for $[C_{29}H_{46}O_5 + Na]^+$ 497.3237).

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