A Bioactive Secosterol with an Unusual A- and B-Ring Oxygenation Pattern Isolated from an Indonesian Soft Coral *Lobophytum* sp.

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A secosterol with a gorgosterol side chain and an unusual oxygenation pattern on the A and B rings was isolated from an Indonesian soft coral *Lobophytum sp*. The A and B rings of the reported compound **1** have hydroxyl groups at C-3 and C-7 and an epoxide ring at C-5−C-6. The structure of **1** was solved by 2D NMR methods and by chemical shift analogy to the known secogorgosterol **2**. Compound **1** was found to have activity against human ovarian tumor and human leukemia cell lines.

Marine organisms provide one of the worlds richest sources of polyoxygenated sterols.[1] The origin of these sterols from marine invertebrates is complicated by the fact that they may be of dietary origin or produced by a symbiont and later modified biochemically in the invertebrate. The diversity of these sterols is displayed in the side chain, which is often indicative of the producing organism, and the oxygenation pattern of the A−D rings. The unique gorgosterol side chain, shown in Figure 1, has been found in soft corals (e.g., *Sarcophyton* sp.)[2] and some zooxantellae (class Dinophyceae) containing invertebrates.[3] It is thought that the dinosterol side chain (Figure 1), indicative of dinoflagellates (class Dinophyceae), is a possible precursor to the gorgosterol side chain.[4] The first novel 9,11-secosterol system containing the gorgosterol side chain found in the Octocorallia was compound **2**, isolated from *Pseudopterogorgia americana*.[5] Its structure was deduced by X-ray crystallography, but ^{1}H and ^{13}C NMR data for **2** were not reported until 1995.[6] Although it is common for sterols from *Lobophytum* to be 3β,5α,6β-hydroxylated, oxygenation at C-7 is rare but has been observed in soft corals of the genus *Xenia*.[1] In this paper, we report on the structural elucidation of a secosterol isolated from *Lobophytum* sp. with a gorgosterol side chain and oxygenation at C-3, C-5, C-6, C-7, C-9, and C-11.

The sample of *Lobophytum* sp. was collected in Indonesian waters and was preserved in the field as described in the Experimental Section. The sample was found to have a thick green layer just below the outer surface, indicating the possible presence of a photosynthetic symbiont, perhaps zooxanthellae. The organism was subjected to solvent extraction followed by partitioning the extract between a series of solvents with increasing polarity. The dichloromethane fraction showed cyclopropyl resonances in its ^{1}H NMR spectrum, and these were followed through the isolation procedure, involving size-exclusion chromatography, silica chromatography, and ODS−HPLC, to give 10.3 mg of **1** as a colorless oil.

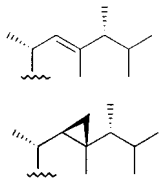
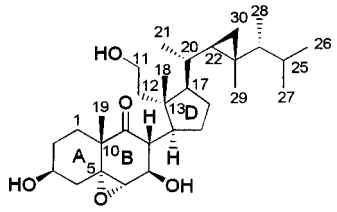


Figure 1. Dinosterol (top) and gorgosterol (bottom) side chains.



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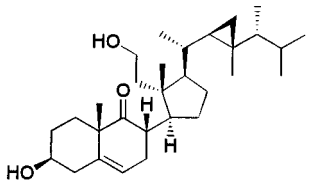

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The DEPT-135 and ^{13}C NMR spectra of **1** revealed the compound had 30 carbon atoms, five quaternary, 10 methines, eight methylenes, and seven methyl groups, thus suggesting it was of triterpenoid origin. The number of carbons with attached protons added up to $C_{30}H_{47}$. One C=O (δ 213.0 s), three CO (δ 69.2 d, 67.0 d, 59.1 t), and one epoxy group (δ 65.2 d, 62.7 s) suggested five oxygen atoms, and together with the low-resolution electrospray mass spectrum $(M + Na)^+$ at *m/z* 513, a molecular formula of $C_{30}H_{50}O_5$ was proposed. This was confirmed by the high-resolution electrospray mass spectrum (*m/z* 513.3541 $(M + Na)^+$ Δ 1.5 mmu of calcd. for $C_{30}H_{50}O_5Na$). The number of double-bond equivalents for this formula is six, ascribed to one carbonyl group, one epoxide ring, and four further rings. In the ^{1}H spectrum the doubled doublets at δ 0.46 and

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Table 1. NMR Data ($CDCl_3$) of **1** at 150/600 MHz

atom no.	^{13}C **1**	^1H (δ, mult, J, Hz) **1**	$^1H-^1H$ COSY	HMBC
1	31.2 t	1.80 m 1.24 m	2, 3	19
2	29.9 t	1.90 bd, 10.5 1.60 m	1, 3, 4	1, 4
3	69.2 d	3.70 dd,[a] 10.8, 6.4	1, 2, 4	1, 2, 4
4	40.0 t	2.12 t, 12.2 1.53 m	2, 3	2, 6
5	62.7 s			1, 4, 6, 19
6	65.2 d	3.40 s	7	4, 7, 8
7	67.0 d	4.05 d, 10.0	6, 8	6, 8, 14
8	49.3 d	2.50 dd, 2.8, 10.0	7, 14	6, 7, 14, 15
9	213.0 s			8, 14, 19
10	45.4 s			1, 2, 4, 6, 8
11	59.1 t	3.83 ddd, 5.8, 8.6, 10.2 3.73 ddd, 2.7, 6.4, 10.0	12	12
12	40.7 t	1.76 m 1.26 m	11	11, 17, 18
13	45.8 s			14, 15, 16, 17, 18, 20
14	43.0 d	2.78 ddd, 3.2, 9.0, 11.6	8, 15	7, 8, 12, 15, 16, 18
15	23.0 t	1.82 m 1.45 m	14, 16, 17	8, 14, 16
16	28.1 t	2.03 ddt, 6.2, 14.2, 9.7 1.39 m	15, 17	15, 17
17	50.3 d	1.65 q, 9.4	15, 16, 20	15, 16, 18, 20, 21, 22
18	17.8 q	0.71 s		12, 14, 17
19	18.1 q	1.29 s		1
20	35.2 d	1.03 m	17, 22	17, 21, 30
21	20.5 q	1.03 br s		17, 20, 22
22	31.9 d	0.20 dt, 8.7, 5.9	20, 30	17, 24, 29, 30
23	25.9 s			22, 24, 25, 28, 29, 30
24	50.7 d	0.25 dq, 8.8, 7.0	25, 28	22, 23, 25, 26, 27, 28, 29, 30
25	31.2 d	1.55 m	24, 26, 27	24, 26, 27, 28
26	22.0 q	0.94 d, 7.0	25	24, 25, 27
27	21.5 q	0.84 d, 7.0	25	24, 25, 26
28	15.3 q	0.91 d, 7.0	24	24, 25
29	14.3 q	0.87 s		22, 24, 30
30	21.3 t	0.46 ddd, 4.4, 9.7 −0.14 dd, 4.6, 5.6	22	22, 24, 29

[a] Severe overlap with H11′, only two J's can be determined.

−0.14 and doubled triplet at δ 0.20 are characteristic of a cyclopropyl group, accounting for one ring. Three rings thus remain to be accounted for, suggesting a secosterol-type of structure, and this was confirmed by the oxygenated methylene at ^{13}C δ 59.1.

The cyclopropyl ring and large number of methyls indicated a gorgosterol-type of side chain. The $^1H-^1H$ COSY spectrum connectivities from H-24−H-25, H-24−H-28, H-20−H-17, H-20−H-22, and H-22−H-30 were consistent with the structure of the side chain, and this was confirmed by correlations in the HMBC spectrum, Table 1, and by comparison with ^{13}C NMR literature values of **2**.[6] Further comparison of ^{13}C NMR shifts with the known secogorgosterol **2** delineated the D ring and C-11−C-12 side chain of **1**. This structural assignment was confirmed by $^1H-^1H$ COSY correlations and HMBC connectivities (Table 1).

The next task was to assemble the remaining subunits into a fused AB ring system typical of a secosterol, and this was achieved using $^1H-^1H$ COSY and HMBC data. The positioning of the ketone and remaining methyl in their normal secosterol positions, C-9 and C-19, respectively, was confirmed by the HMBC correlation from C-9 to H-19. This then gave the anchor points for the rest of the system. The quaternary carbon (δ 62.7 s) of the epoxide was fixed at the C-5 ring junction by its HMBC correlation with H-19. A chain of correlations C-9−H-8, C-8−H-6 places the epoxide

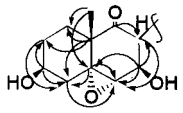

Figure 2. Selected HMBC correlations for the A and B rings of **1** (C−H).

methine (δ 65.2 d) at C-6. The correlations from C-7 to H-6 and H-8 confirm this and place the CHOH moiety at C-7. The final CHOH group (δ 69.2 d) was placed at C-3 because of correlations from C-3 to H-2 and H-4 and further correlations C-4−H-6 and C-5−H-4. Comparison of the ^{13}C NMR shifts between compounds **1** and **2** shows the main change to be in positions C-5, C-6, and C-7, and these have changed from δ 140.3, 121.4, 32.8 in **2** to δ 62.7, 65.2, 67.0 in **1**, respectively. Selected C−H HMBC correlations for the AB ring system are given in Figure 2, and a complete listing of all correlations observed is given in Table 1.

One unusual feature was the ^1H NMR signal for Me-21. Although its position as shown in **1** was in concurrence with all the 2D NMR data and literature values, in the ^1H spectrum its protons gave a singlet, suggesting it was attached to a quaternary carbon rather than the expected doublet if it was attached to the CH-20. This discrepancy can be accounted for by the signals being accidentally isochronous, Me-21 and CH-20 are both at δ 1.03. Literature ^1H NMR data in C_5D_5N for the gorgosterol side chain gives Me-21 at δ 1.09 and CH-20

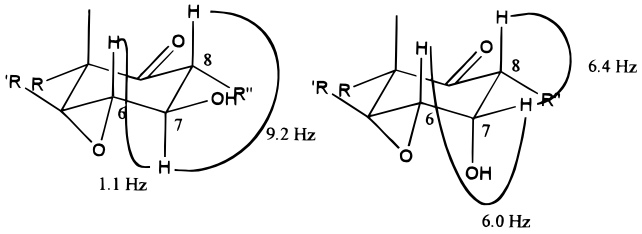

Figure 3. Calculated coupling constants for the two C-7 epimers of **1**. Observed values are ~0 and 10.0 Hz.

at δ 1.02. Attempts to change the chemical shifts of CH-20 and Me-21 in **1** by changing solvent and thus remove the isochronicity were unsuccessful.

The relative stereochemistry of the side chain, D ring, and C-11−C-12 side chain of **1** are identical to that reported for compound **2**, as the ^{13}C NMR shifts match very closely. The relative stereochemistry in the A and B rings was determined using coupling constants. Coupling constants measured from H-3−H-4/4′ are 6.4 and 10.8 Hz, and this is consistent with H-3 being axial and the OH being equatorial. Another axial−axial J value is measured between H-7 and H-8, indicating that the OH at C-7 is equatorial. H-6 shows no coupling to H-7, and therefore, the dihedral angle H-6−C-6−C-7−H-7 must be nearly 90°, indicating the epoxide ring to be 5α,6α, and the H-7 to be axial. That the stereochemistry at C-7 is correct is confirmed by molecular modeling on **1**. In the conformation shown, molecular modeling gives $J_{H6-H7} = 1.1$ Hz and $J_{H7-H8} = 9.2$ Hz (Figure 3a), whereas if the stereocenter at C-7 is inverted these values become 6.4 and 6.0 Hz, respectively (Figure 3b), compared to the measured values of ~0 and 10.0 Hz.[7] A correlation in the ^{1}H−^{1}H NOESY NMR spectrum (t_{mix} = 0.8 s) is observed between H-6 and H-8, indicating they must both be axial, confirming the epoxide ring stereochemical assignment.

Compound **1** was found to be mildly cytotoxic against A2780 human ovarian tumor cells and K562 human leukemia cells with IC_{50}'s of 6.3 and 7.1 μM, respectively.

Experimental Section

General Experimental Procedures. Mass spectra were obtained on a Finnigan Masslab Navigator (low-resolution electrospray) and a Finnigan Mat-95 (high-resolution electrospray). ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity 600 spectrometer at 600 and 150 MHz, respectively, in deuteriochloroform solution. HPLC separations were carried out using a Waters Associates Chromatography pump and monitored using a Waters R401 refractive index detector and an Alltech Econosphere ODS 10μm column.

Collection. The sample of *Lobothytum* sp. (order Alcyonacea, family Alcyoniidae, collection no. 96311) was collected in November 1996 at a depth of 7 m from a reef wall, Mayu Island, Molluca Sea, Indonesia (1° 19.699′ N; 126° 25.129′ E). A voucher specimen is preserved at the Marine Natural Products Laboratory, Department of Chemistry, University of Aberdeen (voucher no. 96311), and at the Nationaal Natuurhistorisch Museum in Leiden, The Netherlands. A full taxonomic description follows. The colony has crestlike lobes and a distinct stalk. The polyps have small dentate rods, 0.05−0.09 mm long, and clublike sclerites of about 0.15 mm long. The surface layer consists of crests with clubs, 0.10−0.20 mm long, the smaller ones with a central wart. The interiors of the crests contain spindles up to 0.35 mm long. The surface layer consists of a base with clubs, 0.10−0.15 mm long, many with a central wart. The interior of the base contains capstans 0.12−0.25 mm long. In addition, some oblong sclerites are present. The combination of spindles only in the interior of the crests, together with capstans only in the interior of the base, and the presence of many clubs with a central wart, seems to be unique in the genus *Lobophytum*. For this reason, the present specimen possibly represents a new species. However, the species of the genus *Lobophytum* show subtle differences in sclerites. Therefore, the specimen has to be compared with many other species of the genus in order ascertain this. Currently, the specimen is under examination by van Ofwegen.

Extraction and Isolation. The sample was preserved by immersion in a 1:1 EtOH/seawater mixture. After 24 h, the mixture was decanted and discarded, after which the organism was transported back to Aberdeen at ambient temperature. The organism was extracted with MeOH for 24 h (3×) and CH_2Cl_2 for 24 h (3×), and the concentrated extracts were combined. The crude oil was partitioned between water and CH_2Cl_2. The CH_2Cl_2 partition fraction was evaporated to give a crude oil that was partitioned between hexane and 10% aqueous MeOH. The MeOH fraction was phase adjusted to 50% aqueous MeOH and partitioned with CH_2Cl_2. The CH_2Cl_2 fraction was then subjected to Sephadex LH20 size-exclusion chromatography (1:1 CH_2Cl_2/MeOH), and the last fraction from this then underwent flash chromatography (hexane/EtOAc/MeOH gradient) followed by reversed-phase C-18 HPLC (20% aqueous MeOH followed by 10% aqueous MeOH) to give 10.3 mg of the pure compound (**1**).

3β,7β,11-Trihydroxy-5α,6α-epoxy-9,11-secogorgostan-9-one (1): colorless oil; $[\alpha]_D$ = 33.3° (*c* 0.1, $CHCl_3$); IR (CCl_4 soln) 3474 (OH), 3050, 2960, 2931, 2875, 1743 (C=O), 1678, 1551, 1541, 1459, 1371, 1260 (epoxide COC str), 1216, 1140, 1035, 808 (epoxide COC str), 777, 726 cm^{-1}; ^{1}H and ^{13}C NMR data in Table 1; LRESIMS *m/z* 1003 (10) $(2M + Na)^+$, 513 (100) $(M + Na)^+$; HRESIMS *m/z* 513.3541, calcd for $C_{30}H_{50}O_5Na$ 513.3556.

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