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Shagenes A and B, New Tricyclic Sesquiterpenes Produced by an Undescribed Antarctic Octocoral

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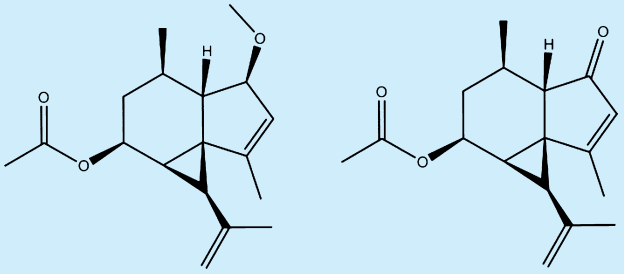
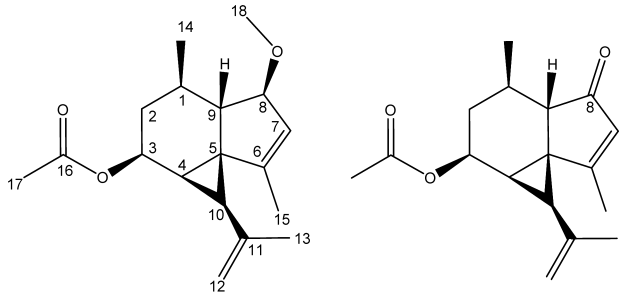
Supporting Information

ABSTRACT: The isolation and characterization of two new tricyclic sesquiterpenoids, shagenes A (**1**) and B (**2**) are presented. These compounds were isolated from an undescribed soft coral collected from the Scotia Arc in the Southern Ocean. One- and two-dimensional NMR spectroscopy and mass spectrometry provided the data necessary to characterize the compounds and their relative stereochemical configurations. Exploration of the bioactivity of shagenes A and B found **1** active against the visceral leishmaniasis causing parasite, *Leishmania donovani*, with no cytotoxicity against the mammalian host.



Sessile marine organisms such as sponges and corals are well-known for their chemically diverse natural products. It is common for these organisms to lack physical or behavioral defenses, therefore creating a necessity for the production of chemical defenses. From 1985 to 2008, cnidarians were second only to porifera in new marine natural products reported each year.[1] The largest class of cnidarians, Anthozoa, are almost solely responsible for producing the natural products isolated from this phylum with greater than 80% of the compounds identified coming from the subclass Octocorallia.[2] Many of these secondary metabolites have been investigated for their ecological roles in feeding deterrence, allelopathy, and antifouling, providing evidence of widespread bioactivity.[3] This has led many researchers to study their potential in drug discovery; however, the majority of published octocoral chemistry originates in tropical shallow waters with only a few dozen exceptions for deeper cold-water corals (>100 m).[4,5]

Important metabolites for drug discovery that have been identified from soft corals include the potent anticancer diterpene glycosides eleutherobin[6] and sarcodictyin A[7] and the anti-inflammatory pseudopterosins.[8] Terpenoids such as these comprise approximately 92% of all reported octocoral secondary metabolites.[2] Because terpenes are common in plant and algal extracts, it was once thought that the origin of octocoral terpenes was symbiotic microalgae called zooxanthellae.[9] However, more recent results favor soft corals producing their own terpenoid metabolites;[10,11] consider, for example, octocorals from depths of more than 100 m, where light has limited penetration through the water column and photosynthetic microalgal symbionts are absent, which are rich sources of terpenoids.[12] This paper presents the new terpenoid skeleton of shagene A and B (**1** and **2**)[13] from a previously undescribed genus of octocoral collected via trawling near the South Georgia Islands.[14] Shagenes are reminiscent of tamariscene, from the liverwort *Frullania tamarsci*, which bears the same fused 3/6/5 tricyclic ring system but displays a different methylation pattern on that ring system.[15]



Other cold-water sesquiterpenoids isolated from corals include the paesslerins,[16] ainigmaptilones,[17] and alcyopterosins,[18] all of which have shown moderate cytotoxicity toward either human cancer cell lines or microbial pathogens. Shagene A (**1**) demonstrates 5 μM half maximal inhibitory concentration (IC_{50}) of the infected macrophage stage of the visceral leishmaniasis causing parasite,[19] *Leishmania donovani*, showing a 10-fold increase in activity over the axenic amastigote form (54 μM) with no cytotoxicity up to 100 μg/mL (345 μM) on

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Table 1. $^{1}H^{a}$ and $^{13}C^{b}$ NMR Data for Shagenes A (1) and B (2) in $CDCl_3$

	shagene A (1)			shagene B (2)		
position	δ_C	δ_H (m, *J*, Hz)	HMBC	δ_C	δ_H (m, *J*, Hz)	HMBC
1	33.2	1.19 (1H, m)	2, 8, 9, 14	32.2	1.42 (1H, m)	2, 8, 9, 14
2	35.7	1.25 (1H, q, 11.8)	1, 3, 4, 9, 14	36.4	1.35 (1H, q, 11.8)	1, 3, 4, 9, 14
		1.65 (1H, m)	1, 3, 4, 9, 14		1.68 (1H, m)	1, 3, 4, 9, 14
3	72.2	5.34 (1H, dt, 11.7, 6.4)	2, 4, 10, 16	71.4	5.33 (1H, dt, 11.7, 6.4)	2, 4, 10, 16
4	22.6	1.65 (1H, m)	2, 5, 6, 10, 11	25.5	2.03 (1H, d, 6.1)	2, 3, 5, 6, 9, 10, 11
5	41.9			40.2		
6	147.3			178.0		
7	123.2	5.60 (1H, m)	5, 6, 8, 9, 15	129.7	5.94 (1H, m)	5, 6, 8, 9, 15
8	88.6	4.09 (1H, d, 0.6)	1, 5, 6, 7, 18	208.5		
9	48.4	1.48 (1H, dd, 1.5, 9)	1, 2, 5, 8, 10, 14	49.0	1.89 (1H, d, 10.9)	1, 2, 5, 6, 8, 10, 14
10	31.5	1.78 (1H, br s)	3, 4, 5, 6, 9	33.1	2.06 (1H, br s)	4, 5, 6, 9, 11, 12
11	140.6			139.4		
12	115.9	5.05 (1H, q, 1.6)	10, 13	116.9	5.11 (1H, q, 1.6)	10, 13
		5.20 (1H, br s)	10, 11,13		5.20 (1H, br s)	10, 11, 13
13	24.1	1.77 (3H, s)	10, 11, 12	24.1	1.76 (3H, s)	10, 11,12
14	19.5	1.01 (3H, d, 6.4)	1, 2, 3, 9	19.3	1.23 (3H, d, 6.1)	1, 2, 3, 9
15	11.6	1.49 (3H, t, 1.2)	5, 6, 7	13.2	1.84 (3H, d, 1.2)	5, 6, 7
16	170.9			170.8		
17	21.6	2.09 (3H, s)	3, 16	21.5	2.11 (3H, s)	3, 16
18	55.6	3.34 (3H, s)	8			

[a] ^{1}H NMR experiments at 500 MHz, recorded in ppm (integration, multiplicity, *J*-coupling in Hz); [b] ^{13}C NMR experiments at 125 MHz, recorded in ppm.

J774.A1 macrophages.[20] The selectivity index (SI) of shagene A (**1**) is therefore greater than 70 as compared to the lower SI of the control drug miltefosine. Miltefosine shows an IC_{50} of 3 μM against the axenic amastigote form, 1.4 μM for the infected macrophage stage, but greater than 50 μM cytotoxicity against mammalian cells. Shagene B (**2**) showed no activity (>20 μg/mL) alluding to the importance of having the methoxy substituent at C-8.

The bright yellow and highly branched octocoral was collected during austral spring 2011 at depths of approximately 180 m via trawling. The location sampled was the sesquiterpenes namesake, Shag Rocks, which are a series of small islands along the Scotia Arc (S53° 26′ 16.10″, W42° 02′ 15.52″). Specimens were frozen immediately due to noticeable oxidation of the yellow pigment. Once freeze-dried, the coral was processed with a Soxhlet extractor with refluxing CH_2Cl_2. The crude extract was mounted on silica gel and subsequently fractionated by normal-phase MPLC. Further purification of the nonpolar portion eluting in EtOAc-*n*-hexane (1:1) was accomplished by HPLC equipped with UV and ELSD detection. The more lipophilic colorless oil shagene A (**1**) (27 mg, 0.009% dry wt) was collected as the major component of the mixture with shagene B (**2**) (2 mg colorless oil, 0.0007% dry wt) considerably less abundant.

The ^{1}H NMR spectra of shagenes A (**1**), $[\alpha]^{26}_{D}$ −0.9, and B (**2**), $[\alpha]^{26}_{D}$ +0.2, displayed characteristic peaks indicative of smaller cyclic terpenes with 16 proton signals largely composed of single proton integrations, multiple downfield olefinic protons, and methyl substituents. The ^{13}C NMR spectrum of **1** showed 18 carbon signals including five downfield signals of four olefinic carbons and the carbonyl carbon of the acetyl group (δ_C 170.9). As represented in Table 1, the spectra display functionality indicative of one acetate methyl (δ_H 2.09), one methoxy group (δ_H 3.34), one olefinic methylene (δ_H 5.05, 5.20), one trisubstituted olefin (δ_H 5.60), three vinyl or aliphatic methyl groups, six sp^3 methines, one sp^3 methylene, and two olefinic quaternary carbons. HREIMS of **1** provided the molecular formula $C_{18}H_{26}O_3$ (*m*/*z* calcd 290.1882, found *m*/*z* 290.1878) demonstrating six degrees of unsaturation and providing validation for the hypothesized 15 carbon skeleton of a tricyclic sesquiterpene bearing a quaternary carbon at the junction of all three rings. Further verification of this scaffold and relative stereochemistry were accomplished via 2D NMR experiments (COSY, gHMQC, gHMBCAD, and ROESY).

One-bond correlations from the HMQC experiment for **1** confirmed the olefinic methylene protons at C-12 (δ_C 115.9) and the quaternary nature of carbons C-5 and C-11 (δ_C 41.9, 140.6). Shared HMBC correlations (Figure 1) of the two

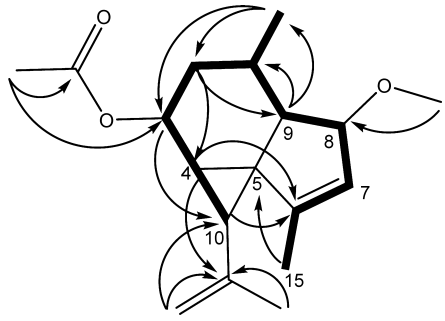

Figure 1. Key COSY and HMBC correlations of **1**.

protons at C-12 and H_3-13 (δ_H 5.05, 5.20, and 1.77) with C-10 (δ_C 31.5) and C-11 provided proof of the isopropenyl attachment to the cyclopropane at C-10. Placement of C-5 as a bridgehead carbon for this cyclopropane, the cyclohexane and cyclopentene fused rings, is verified by HMBC correlations with H-4, H-7, H-8, H-9, H-10, and H_3-15. Chemical shifts for C-4 (δ_C 22.6), C-5, and C-10 of shagene A are similar to those reported for the cyclopropane ring of the tricyclic skeleton comprising tamariscene (δ_C 30.1, δ_C 34.2, δ_C 33.6)[15] with the

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added functionality of shagene A accounting for shift variance. Connectivity of the cyclopentene moiety can be completed on the basis of COSY coupling of the olefinic proton H-7 (δ_H 5.60) with vinyl methyl protons H_3-15 (δ_H 1.49) and the oxygen-bearing methine H-8 (δ_H 4.09). HMBC coupling of the methoxy protons with the oxygen bearing methine at C-8 verifies their connectivity. Further analysis of the 2D COSY spectrum shows H-1 (δ_H 1.19) to be vicinal to H-9 (δ_H 1.48), the methyl protons at H_3-14 (δ_H 1.01), and the diastereotopic methylene protons at H-2 (δ_H 1.25, δ_H 1.65). The acetate methyl protons provided HMBC correlations to the carbonyl C-16 (δ_C 170.9) and C-3 (δ_C 72.2) of the cyclohexane ring. COSY coupling of H-3 (δ_H 5.34) with neighboring methylene protons H-2 and methine H-4 (δ_H 1.65) confirms a large portion of the cyclohexane ring. HMBC correlations of H-4 with C-2, C-5, C-6, C-10, and C-11 provide the evidence of a third bridgehead carbon, this time between the cyclohexane and cyclopropane rings to complete the connectivity of the overall scaffold.

Relative stereochemistry of shagene A (**1**) was deducible upon analysis of strong, unanticipated ROESY correlations (Figure 2) between the exocyclic methylene protons on C-12

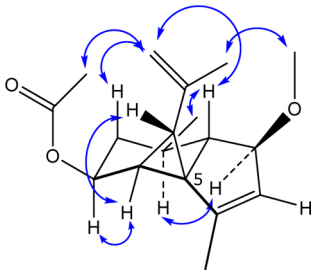


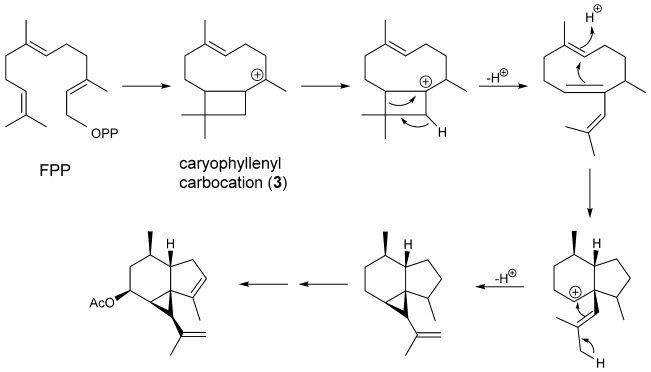
Figure 2. Key ROESY correlations for shagene A (**1**).

with the cyclopentane and cyclohexane bridgehead H-9, a methylene proton at C-2, and the methyl protons of the acetate. This places the isopropenyl group in the periphery of the acetate and methyl substituents of the cyclohexane ring essentially blocking that face of the molecule. Spatial placement of the methyl substituent at C-1 is a result of correlations between H_3-14 with H-9, and ROESY correlations between H-1 and H-8 place the methoxy group on the same face as H-9. Additional support for the β-methoxy is provided by conformational analysis of the H-9 coupling constants. Energy-minimized conformation of the α-methoxy configuration requires a large $^3J_{HH}$ coupling between H-8 and H-9 due to a nearly eclipsed conformation. On the other hand, the β-methoxy displays a roughly 118° dihedral angle resulting in a small $J_{H8,H9}$. The observed $J_{H8,H9}$ of 1.5 Hz indicates a β-methoxy substituent at C-8. H_3-18 and H_3-13 show weak ROESY correlation.

The obvious difference in the ^{1}H NMR spectra between compounds **1** and **2** was the absence of the proton signals associated with the methoxy functionality at δ_H 3.34 ppm and its oxymethine at δ_H 4.09 ppm as well as the downfield shift of multiple proton signals surrounding C-8. A carbon shift in **2**, but absent in **1** was observed at δ_C 208.5, establishing the oxidized α,β-unsaturated ketone moiety of **2**. The presence of a carbonyl at C-8 was further verified by the absence of H-8 COSY correlations with H-9 and H-7. Evidence of a conjugated ketone was indicated by absorbance at a wavelength of 257 nm in the UV spectrum and the extra carbonyl stretch on IR (1696 cm^{-1}). HREIMS of **2** revealed an expected mass similar to **1** save one carbon and four protons (m/z calcd 274.1569, found 274.1569) providing the molecular formula $C_{17}H_{22}O_3$. Validation of **2** having the same connectivity and stereochemistry as **1** is shown in the 2D NMR experiments (COSY, HSQC, HMBC, and ROESY).

The proposed biosynthetic pathway to **1** and **2** is shown in Scheme 1. Structural similarities to biosynthetic intermediates

Scheme 1. Proposed Biosynthetic Pathway for Sesquiterpenes 1 and 2



displayed in the biosynthesis of valerenic acid makes these molecules likely to undergo cyclization through a caryophyllene intermediate (**3**) via copalyl diphosphate synthase (CPS).[21] Alternative pathways have been suggested with a concerted three-ring cyclization step; however, this would produce an undesirable stereochemical outcome.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H, ^{13}C, COSY, HSQC, HMQC, HMBC, and ROESY NMR spectra for structure elucidation of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(13) Shagene A (**1**): UV (CH_2Cl_2) λ_{max} (ε) 240 (5945) nm; IR (thin film) 2959, 3050, 2933, 2877, 1734, 1655, 1458, 1369, 1246, 1089, 1026 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; 70 eV HREIMS *m*/*z* 290.1878 ([M^+]) ($C_{18}H_{26}O_3$ calcd, 290.1882). Shagene B (**2**): UV (CH_2Cl_2) λ_{max} (ε) 257 (5425), 240 (6357) nm; IR (thin film) 3080, 2960, 2929, 2858, 1734, 1696, 1607, 1462, 1380, 1242, 1186, 1030 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; 70 eV HREIMS *m*/*z* 274.1569 ([M^+]) ($C_{17}H_{22}O_3$ calcd, 274.1569).

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