Sesquiterpene Metabolites of the Antarctic Gorgonian *Dasystenella acanthina*

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The ethereal extract of the Antarctic gorgonian *Dasystenella acanthina* was found to contain three main nonpolar and relatively transient sesquiterpene metabolites (**1**−**3**), which were isolated and submitted to spectral analysis. Two of them were identified as the previously reported compounds *trans*-*β*-farnesene (**1**) and isofuranodiene (**3**), whereas the third metabolite, the furanoeudesmane **2**, was unknown. The structure elucidation of this new sesquiterpene was solved mainly on the basis of its spectral properties and correlation with known compounds.

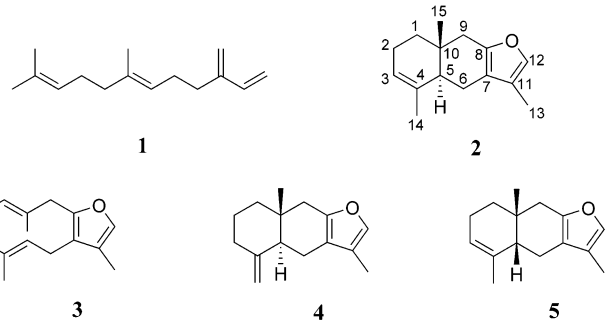
The subclass Octocorallia, which includes soft corals and gorgonians, has been studied extensively by marine natural product chemists and has yielded a large number of bioactive secondary metabolites, the majority of which are terpenes.[1,2] It has been suggested that such molecules could play a defensive role in the animals, which appear to be relatively free from predation.[3] Feeding deterrence properties of fatty acid derivatives as well as ichthyotoxic activities of terpenes from soft corals and gorgonians have been demonstrated.[4,5] Interesting cytotoxic activities have also been reported for several diterpenes from soft corals.[5]

The large majority of octocorals studied have been collected in the two main areas of the globe possessing coral reefs, those being the tropical Western Atlantic and the Indo-Pacific regions, whereas little is known on the chemistry of these animals from other geographical areas such as Antarctica.[6] Two reports of Antarctic octocoral chemistry have appeared in the literature so far. The first of them describes the presence in the stoloniferan coral *Clavularia frankliniana* of fatty glyceride esters, chimyl alcohol in particular, that were also found in its predator, the nudibranch *Tritoniella belli*,[7] whereas the more recent paper reports the finding of sesquiterpenes in the gorgonian coral *Ainigmaptilon antarcticus*.[8]

In the course of our investigations on the chemical ecology of Antarctic benthic invertebrates,[9,10] we have examined the gorgonian *Dasystenella acanthina*, Wright and Studer 1889 (subclass Octocorallia, order Gorgonacea, family Primnoidae), distributed in both Antarctic and subantarctic zones. Chemical analysis of the ethereal extract of the gorgonian has shown the presence of three main and unstable sesquiterpene metabolites, the known compounds **1** and **3** and the unprecedented furanoeudesmane **2**. We describe here the structure elucidation of this new sesquiterpene.

Specimens of *D. acanthina* (wet weight 30 g) were extracted exhaustively with acetone, and the extract was partitioned between water and Et_2O. The Et_2O-soluble fraction (520 mg) was analyzed by TLC, revealing the presence of three nonpolar compounds, **1**−**3**, at R_f 0.62, 0.44, and 0.36 (light petroleum ether), respectively, along with usual fatty acid and sterol components. The Et_2O extract was fractionated on a a Si gel column eluted with petroleum ether, giving, in order of increasing polarity, compounds **1** (50.1 mg), **2** (75.5 mg), and **3** (18.0 mg).

A preliminary NMR analysis revealed immediately that the three molecules were sesquiterpenes. Comparison of their spectral values with literature data led to the identification of *trans*-*β*-farnesene (**1**), a well-known alarm pheromone of many aphids and a component of essential oils,[11] and isofuranodiene (**3**),[12,13] whereas sesquiterpene **2** was a new compound, structurally related to atractylon (**4**), a furanoeudesmane previously isolated from the terrestrial plant *Atractylodes japonica*[14] and from Australian soft corals of the family Xeniidae.[12]



Compound **2**, which was isolated as an optically active solid ($[\alpha]^{25}_D$ 70.2°, *n*-hexane), had the molecular formula $C_{15}H_{20}O$, as deduced by HREIMS on the molecular ion at *m*/*z* 216, and gave a positive Ehrlich color reaction, suggesting the presence of a furan moiety, which was confirmed by both mass and NMR spectral data (Table 1). Because of its high instability in chloroform or methanol solution, NMR analysis of compound **2** was conducted in CD_2Cl_2. The mass spectrum of **2** showed a significant peak at *m*/*z* 108, due to a characteristic fragment formed by retro-Diels−Alder type ring cleavage in furanosequiterpenes with methylene groups in the α- and *β*-position to the furan ring. Analysis of both ^1H and ^{13}C NMR spectra confirmed the presence of a trisubstituted furan ring and also indicated an additional trisubstituted double bond. In fact, the proton spectrum displayed a broad singlet at *δ* 7.05 assigned to the furan α-proton H-12, a multiplet at *δ* 5.44 due to an olefinic proton (H-3), and two 3H doublets at *δ* 1.94 (J = 1.0 Hz) and 1.72 (J = 1.0 Hz), attributed to the furan *β*-methyl H_3-13 and the vinylic methyl H_3-14, respectively. In the carbon spectrum, where the two vinyl methyls resonated at *δ* 8.2 (C-13) and 21.4 (C-14), four signals due to sp^2 quaternary carbons were present in the downfield region at *δ* 150.6 (C-8), 134.9 (C-4), 120.1 (C-11), and 117.7 (C-7) along with two sp^2 methine carbons at *δ* 137.2 (C-12) and 122.2 (C-3). The ^{13}C NMR spectrum was completed by signals due to a methyl at *δ* 16.6 (C-15),

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Table 1. NMR Data[a] of Compound **2**

C	δ ^{13}C[b]	m[c]	δ ^{1}H[d]	m, *J* (Hz)	HMBC correlations[e]
1	37.5	CH_2	1.55	m	H-3, H_3-15
2	23.2	CH_2	2.01	m	H_2-1
			2.16	m	
3	122.2	CH	5.44	m	H_2-1, H_3-14
4	134.9	C			H_3-14
5	44.3	CH	2.18	m	H_2-1, H-3, H-6a, H_2-9, H_3-14, H_3-15
6	21.7	CH_2	1.97	m	
			2.61	dd, 15, 4	
7	117.7	C			H_2-6, H_2-9, H-12, H_3-13
8	150.6	C			H_2-6, H_2-9, H-12
9	39.0	CH_2	2.34	bs	H_2-1, H-5, H_3-15
10	33.5	C			H_2-1, H-6a, H_2-9, H_3-15
11	120.1	C			H-12, H_3-13
12	137.2	CH	7.05	bs	H_3-13
13	8.2	CH_3	1.94	d, 1	
14	21.4	CH_3	1.72	d, 1	H-3
15	16.6	CH_3	0.82	s	H_2-1, H_2-9

[a] Bruker 300, 400, and 500 MHz; CD_2Cl_2; chemical shifts (ppm) referred to CH_2Cl_2 (δ 5.32) for proton and to CD_2Cl_2 (δ 53.8) for carbon. [b] Assignments by HMQC and HMBC experiments. [c] By DEPT sequence. [d] Assignments by ^{1}H−^{1}H COSY experiment. [e] J = 10 Hz.

four methylenes at δ 39.0 (C-9), 37.5 (C-1), 23.2 (C-2), and 21.7 (C-6), a methine at δ 44.3 (C-5), and a quaternary carbon at δ 33.5 (C-10). The remaining resonances in the ^{1}H NMR spectrum were a 3H singlet at δ 0.82 attributed to a methyl linked to a quaternary carbon and multiplets integrating for seven protons between δ 1.55 and 2.61, which were assigned by ^{1}H−^{1}H COSY to the four methylene groups C-1, C-2, C-6, and C-9 and to methine C-5, as reported in Table 1. These data clearly suggested a furanoeudesmane carbon skeleton, which exhibits a furan with a β-methyl group, fused to a bicyclic moiety bearing an angular methyl group and a vinyl methyl biogenetically located at C-4, as in **2**. Analysis of the HMBC spectrum confirmed assignments as in Table 1.

The *trans*-junction between rings A and B was supported by the diagnostic upshifted value of angular methyl C-15 (δ 16.6) with respect to that reported for the corresponding carbon (δ 22.7) of the synthetic *cis*-fused isomer, 1,2-dihydrotubipofuran (**5**).[15] Comparison of ^{13}C NMR values of methylene groups in **2** (see Table 1) with those of the corresponding atoms in **5** (δ 23.2, 24.5, 29.8, and 35.1)[15] further confirmed the proposed relative stereochemistry. However, the absolute stereochemistry remains undetermined.

Compound **2** is structurally related to the known sesquiterpene atractylon (**4**), its exocyclic isomer, isolated from both terrestrial[14] and marine sources.[12] It is interesting to note that a compound with a presumed structure the same as **2** was reported to be produced together with atractylon (**4**) by acid-catalyzed cyclization of isofuranodiene (**3**).[12] With the aim of verifying the formation of compound **2** from isofuranodiene (**3**), a sample of **3** was dissolved in CD_2Cl_2 in a NMR tube at 20 °C, and traces of *p*-toluenesulfonic acid were added.[12] The reaction was monitored by recording the ^{1}H NMR spectrum of the mixture at 10 min intervals. The transformation of **3** into compounds **2** and **4** was followed by comparing the ^{1}H NMR spectrum of the reaction mixture with that of natural **2** and with literature data for compound **4**.[12] Complete conversion of isofuranodiene (**3**) into a mixture of racemic **2** and **4** (ratio ca. 1:1) occurred within 1 h. However, the possibility that furanoeudesmane **2** isolated from the gorgonian could be an artifact formed from isofuranodiene (**3**) during workup was excluded by the absence of atractylon (**4**) in the extract of the gorgonian and by the optical activity of natural **2**.

Biological activities of sesquiterpenes **1**−**3** were evaluated by assaying ichthyotoxicity and feeding deterrence, according to literature procedures.[16−18] Compounds **2** and **3** were toxic at 10 ppm in a *Gambusia affinis* ichthyotoxicity test. According to this result, an involvement of these molecules in the defensive mechanisms of the animal could be suggested.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Jasco DIP 370 digital polarimeter, and CD curves were recorded on a Jasco 710 spectropolarimeter. The IR spectra were taken on a Bio-Rad FTS 7 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on WM 500, Avance 400, and a DPX 300 MHz Bruker spectrometers in CD_2Cl_2; chemical shifts are reported in ppm referred to CH_2Cl_2 as internal standard (5.32 for proton and 53.8 for carbon). EIMS and HREIMS spectra were measured on a TRIO 2000 VG Carlo Erba and on a Kratos MS50 instrument, respectively. Si gel chromatography was performed using precoated Merck F_{254} plates and Merck Kieselgel 60 powder.

Animal Material. *D. acanthina* was collected off Terra Nova Bay, Antarctica, during the Austral summer 1999−2000, by dredging at a depth of 300 m, in the course of the 15th Italian Expedition. Intact gorgonian colonies were immediately frozen, then transferred to ICB in Naples, where they were kept at −80 °C until extraction. A voucher specimen is stored for inspection at ICB (sample BTN 71).

Extraction and Isolation. The frozen gorgonian material (wet weight 30 g) was immersed in acetone (100 mL) and extracted at room temperature by both using ultrasound vibration (5 min) and crumbling by a pestle. The treatment was repeated three times. After concentration, the aqueous residue was extracted with Et_2O (3 × 50 mL). The combined ether extracts were taken to dryness, yielding an oily residue (520 mg), which was chromatographed on a Si gel column using a petroleum ether−Et_2O gradient, as eluent. The fractions 7−12, 16−22, and 26−34, all eluted by petroleum ether, yielded compounds **1** (50.1 mg), **2** (75.5 mg), and **3** (18.0 mg), respectively. Compounds **1** and **3** were identified as *trans*-β-farnesene[11] and isofuranodiene,[12,13] respectively, by comparison of NMR and MS data with literature values.

Compound 2: amorphous powder; $[\alpha]_D$ 70.2° (*c* 0.5, *n*-hexane); CD $[\theta]_{204}$ (*n*-hexane) 3610; IR (liquid film) ν_{max} 2919, 2865, 1753, 1599 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 1; EIMS *m*/*z* (%) 216 (M^+, 100), 201 (17), 173 (10), 159 (12), 145 (15), 108 (50), 93 (20); HREIMS *m*/*z* 216.1522 (calcd for $C_{15}H_{20}O$, 216.1514).

Acid-Catalyzed Conversion of Isofuranodiene (3) into Compounds 2 and 4. A sample of **3** (2.3 mg) was dissolved in CD_2Cl_2 (0.5 mL), and the ^{1}H NMR spectrum was recorded at 20 °C (400 MHz): δ 7.06 (1H, bs, H-12), 4.95 (1H, m, H-1), 4.73 (1H, m, H-5), 3.47 (2H, AB quartet, J = 16 Hz, H2−9), 3.07 (2H, m, H2−6), 2.24 (1H, m, H-3a), 2.12 (2H, m, H_2-2), 1.91 (3H, d, J = 1 Hz, H_3-13), 1.78 (1H, m, H-3b), 1.60 (3H, bs, H_3-14), 1.25 (3H, s, H_3-15).[19] Then, traces of *p*-toluenesulfonic acid were added, and ^{1}H NMR spectra of the sample were recorded at 10 min intervals, at the same starting temperature. ^{1}H NMR of the final mixture (1 h, 400 MHz, selected values): δ 7.04 (bs, H-12 in **2** and **4**), 5.43 (m, H-3 in **2**), 4.86 (d, J = 2 Hz, H-14a in **4**), 4.70 (d, J = 2 Hz, H-14b in **4**), 2.60 (dd, J = 15.2 and 4.3 Hz, H-6a in **2**), 1.93 (bs, H_3-13 in **2** and **4**), 1.71 (bs, H_3-14 in **2**), 0.81 (s, H_3-15 in **2**), 0.74 (s, H_3-15 in **4**).

Biological Assays. Ichthyotoxicity (against the mosquito fish *Gambusia affinis*) and feeding deterrence (against the gold fish *Carassius auratus*) tests were conducted according to literature procedures.[16−18] Compounds **1**−**3** were assayed at 10 ppm in the ichthyotoxicity test and at 300 μg/cm^2 fish food[18] in the feeding deterrence test. Compound **1** was inactive.

Compounds **2** and **3** were ichthyotoxic at 10 ppm, whereas no effect was observed for both molecules in the feeding deterrence test.

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