A New Sesquiterpene as an Antifouling Substance from a Palauan Marine Sponge, *Dysidea herbacea*

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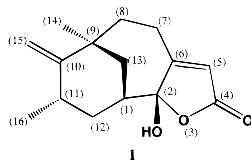
Bioassay-guided fractionation of an extract from a marine sponge, *Dysidea herbacea*, led to the isolation and identification of the new sesquiterpene **1**. This compound showed repellent activity against the blue mussel, *Mytilus edulis galloprovincialis*.

An unusually diverse array of metabolites has been isolated from various specimens of *Dysidea herbacea*.[1] One reason for this high diversity could be that some of the metabolites are produced by symbiotic microorganisms known to coexist with *D. herbacea*.[2] Alternatively, this species, as presently defined, may not be homogeneous.[3] The metabolites reported to date include polybrominated biphenyl ethers,[4,5] chlorinated nitrogenous metabolites[6−9] that are reminiscent of certain blue-green algal metabolites,[3] and sesquiterpenes[10] and sterols[3] that are almost certainly true sponge metabolites.[11] As part of our research aimed at the discovery of new active metabolites, including antifouling substances,[5] we have examined a sample of *D. herbacea* collected from a marine lake in Palau. Our specimen contained a new furanosesquiterpene (**1**) that is quite similar to the previously isolated compounds nakafuran-9 and a derivative, isolated from the congeneric sponge, *Dysidea fragilis,* and its predators, the nudibranchs *Hypselodoris godeffroyana* and *Chromodoris maridadilus.*[12−14]



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The EtOAc-soluble fraction of the MeOH extract of *D. herbacea* was chromatographed on a Si gel column using eluents of increasing polarity composed of $CHCl_3$ and MeOH to yield a mixture of compounds. The fractions containing the bioactive sesquiterpene **1** were further purified by a Si gel column, an ODS column, and ODS HPLC. The fractionations were monitored by an assay system involving the repellent activity using blue mussels.[15] Compound **1** was obtained as a white solid. The mass spectral data for both **1** (FABMS *m*/*z* 249 $[M + H]^+$) and nakafuran-9-hydroxy butenolide, 2-hydroxy-9,10,11-trimethyl-3-oxatricyclo[7.3.1.$0^{2,6}$]trideca-5,10-dien-4-one reported by Aiello et al.[12] indicated these two compounds have the same molecular formula, $C_{15}H_{20}O_3$. In the ^{1}H and ^{13}C NMR spectra of **1**, one proton signal δ 5.78 (1H, s) and four carbon signals [(δ 171.2, C-4, 116.9, C-5, 172.0, C-6; and 108.9, C-2) were observed, which suggested the presence of a γ-hydroxy-α,β-unsaturated-γ-lactone with additional substituents at the β and γ positions. The ^{1}H COSY and HMBC spectra were used for further structure determination. Although these ^{1}H NMR spectral data appeared close to those of nakafuran-9 hydroxybutenolide, the signals for the C-15 methylene and the C-16 methyl protons were different, suggesting that the carbon skeleton of **1** was not identical with that of nakafuran-9. The presence of an exomethylene group was supported by two proton signals, [δ 4.70 (1H, s) and 4.78 (1H, s)] and two carbon signals (δ 158.8, C-10 and 104.1, C-15) in the ^{1}H and ^{13}C NMR spectra of **1**. NMR signals for the C-16 methyl group appeared at δ 0.98 (3H, d, $J = 6.4$ Hz) and 19.7, respectively. The C-11 signal appeared upfield at δ 30.9. These data suggested the presence of a double bond between C-10 and C-15. The structure and stereochemistry of **1** were confirmed by 2D NOE studies. The α-configuration of Me-11 was shown by the NOE correlation between the Me-11 protons and the H-8α proton. Thus, the structure 2-hydroxy-9,11-dimethyl-10-methylene-3-oxatricyclo [7.3.1.$0^{2,6}$]tridec-5-en-4-one was proposed for **1**. Antifouling activity of this compound was tested against blue mussels using a foot-stimulant method.[15] A 100% antifouling activity was observed at a concentration of 100 ppm. However, test mussels showed no reactivity at a concentration less than 10 ppm.

Experimental Section

General Experimental Procedures. Optical rotations were determined on a Horiba SEPA-300 high-sensitivity polarimeter. IR spectra were measured on a JASCO FT/IR-7000 spectrophotometer.^{1}H NMR and ^{13}C NMR spectra were recorded on Varian Unity 500 NMR spectrometer using $CDCl_3$. Mass spectra were measured on a JEOL JMS−SX102 mass spectrometer.

Animal Material. *D. herbacea* was collected in the Milky Way marine lake of Palau in July 1996, frozen on site, and stored at −20 °C before extraction. A voucher specimen is deposited at our laboratory. The sponge was identified by Professor P. R. Bergquist of the University of Auckland, New Zealand.

Extraction and Isolation. Frozen samples of *D. herbacea* (1.6 kg) were homogenized and extracted with MeOH (2 L × 3) at room temperature. The combined extracts were evaporated under reduced pressure to give a residue (23.2 g). This was partitioned between EtOAc and H_2O and subsequently between *n*-BuOH and H_2O. The EtOAc layer was concentrated to dryness (15.3 g) and chromatographed on a Si gel column using a $CHCl_3$−MeOH gradient (from 0 to 5% MeOH in $CHCl_3$), and 15 fractions (fractions 1−15) were obtained. The active fraction 5 (552.4 mg) was separated on a Si column with a mixed EtOAc−hexane gradient solvent system (from 50% to 100% EtOAc in hexane) and fraction 5-4 (137.4 mg) was obtained as impure **1**. The active fraction 5-4 was further purified by ODS column and ODS HPLC (C_{18} column 10 × 250 mm; detector UV at 215 nm; flow rate 2 mL /min) with a

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mixed solvent system: H_2O–MeOH gradient (from 40% to 100% MeOH) to give pure compound **1** (38 mg).

Sesquiterpene 1: white solid; $[\alpha]^{24}_{D}$ + 0.23° (*c* 0.35, MeOH); IR ($CHCl_3$)ν_{max} 942, 1122, 1230, 1458, 1647, 1734, 2964, 3288, 3447 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.44 (1H, br t, J = 6.1 Hz, H-1), 5.78 (1H, s, H-5), 2.68 (2H, t, J = 7.0 Hz, H-7), 1.58 (1H, m, H-8β), 1.89 (1H, m, H-8α), 2.14 (1H, m, H-11), 1.22 (1H, m, H-12α), 1.57 (1H, m, H-12β), 1.52 (1H, dd, J = 14.6, 6.1 Hz, H-13β), 2.18 (1H, br d, J = 14.6 Hz, H-13α), 1.20 (3H, s, H-14), 4.70 (1H, s, H-15), 4.82 (1H, s, H-+15), 0.98 (3H, d, J = 6.4 Hz, H-16); ^{13}C NMR (500 MHz, $CDCl_3$) δ 40.1 (C-1, d), 108.9 (C-2, s), 171.2 (C-4, s), 116.9 (C-5, d), 172.0 (C-6, s), 25.1 (C-7, t), 38.3 (C-8, t), 38.8 (C-9, s), 158.8 (C-10, s), 30.9 (C-11, d), 34.9 (C-12, t), 37.7 (C-13, t), 31.5 (C-14, q), 104.1 (C-15, t), 19.7 (C-16, q); FABMS *m*/*z* 249 $[M + H]^+$.

Bioassay. Candidate antifouling extracts, fractions, and pure isolates were dripped onto the foot of the blue mussel (*Mytilus edulis galloprovincialis*), and the animal's reactions were observed. When a solution containing an active compound, such as $CuSO_4$, was dripped on the foot, the mussel contracted its foot immediately. After being treated with an inactive compound, the foot of the mussel either showed no reaction or contracted after a few seconds. The number of responding mussels was counted. The foot was immediately washed with 1 mL of artificial seawater after each observation and allowed to rest for 15 min. The concentration of the test solution was raised, and the experiment was repeated from the point of dripping artificial seawater as control until all the mussels showed a reaction. The activities of the test compounds were indicated by the percentage of mussels showing a reaction at each concentration of test solution and calculated as follows: number of reactive mussels/(number of reactive mussels + unreactive mussels) × 100 (%). Ten mussels were used for each test sample.

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