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CHEMICAL AND ECOLOGICAL STUDIES OF THE ANTARCTIC SPONGE *DENDRILLA MEMBRANOSA*

BILL J. BAKER,* ROBERT W. KOPITZKE,

Department of Chemistry, Florida Institute of Technology, Melbourne, Florida 32901

WESLEY Y. YOSHIDA,

Department of Chemistry, University of Hawaii, Honolulu, Hawaii

and JAMES B. MCCLINTOCK

Department of Biology, University of Alabama at Birmingham, Birmingham, Alabama 35294

ABSTRACT.—The sponge *Dendrilla membranosa*, a conspicuous member of the Antarctic benthos that appears to be free of predation, produces several norditerpenes, including the previously unreported dendrillin [**1**], as well as amino acid and nucleotide derivatives. The norditerpenes, previously implicated as chemical defense agents, displayed no deterrent effect toward the chemosensory tube-feet of the major Antarctic sponge predator, the sea star *Perknaster fuscus*.

The benthos of McMurdo Sound, Antarctica, supports a stable and thriving community of sponges, corals, echinoderms, and other invertebrates that have adapted to the sub-freezing temperatures, low nutrient levels, and periodic low light levels (1). It has been suggested that polar latitudes lack sufficient predation pressure to drive sessile invertebrates to produce defensive metabolites (2). However, recent studies have documented that extracts from Antarctic marine invertebrates display a variety of bioactivities, including cytotoxic, behavioral, and antibiotic activities (3–5). Additionally, several laboratories have identified substances from Antarctic sponges that display pharmacological activity, including anticancer and antiviral activity (5–9).

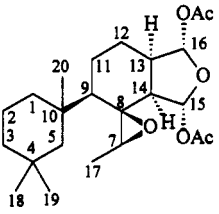
In a seminal paper characterizing the McMurdo Sound benthic ecosystem, Dayton *et al.* (1) noted that the sponge *Dendrilla membranosa* Pallas (family Aplysillidae, order Dendroceratida) lacked spiculation, yet was not heavily preyed upon, and suggested it must possess chemical defenses. Subsequent chemical investigation resulted in the isolation of two norditerpenes (8) and an amino acid derived pigment (9). We report here an additional norditerpene from *D. membranosa* and the evaluation of the deterrent effects (10) of the extracts of this sponge toward a major Antarctic sponge predator, the sea star *Perknaster fuscus*.

The bright yellow *D. membranosa* is highly conspicuous on the Antarctic benthos. Sponge samples were collected using scuba at between 30 and 40 m depth from Hut Point and Danger Slopes on Ross Island (77°51′S; 166°39′E) and between 10 and 40 m depth at New Harbor (77°34′S; 164°40′E) on the continental coast. Extraction of the freeze-dried sponge, on-site in Antarctica, provided a series of extracts of increasing polarity: hexane, $CHCl_3$, MeOH, and MeOH-H_2O (7:3). Each of these extracts was evaluated for its ability to elicit a behavioral response (see below) in the sea star *P. fuscus* that is indicative of avoidance (10); only the MeOH extract elicited such a response.

The hexane extract contained predominantly norditerpenes, in contrast to most McMurdo Sound sponges, which typically contain a significant proportion of sterols in their hexane extract. This extract was fractionated by gradient flash cc; the norditerpenes eluted with hexane-EtOAc (90:10).

9,11-Dihydrogracillin A (DHGA) and membranolide (8) were purified by normal-phase hplc using hexane-EtOAc (80:20) and their structures were estab-

lished based on comparison of their ^{1}H-nmr spectral data to those reported previously (8). A third norditerpene, dendrillin [**1**] was isolated from the same cc fraction by repeated hplc.



1

The ^{1}H-nmr spectrum of **1** was similar to that of DHGA. In particular, the diacetoxy diacetal moiety was suggested by the acetoxyl resonances (δ 2.08 and 2.03) and the corresponding acetal methines, H-15 and H-16, resonating at δ 6.57 and 5.90 (numbering system from Ref. 8). The terpenoid character of dendrillin was evidenced by the *gem*-dimethyl group (δ 0.86 and 0.96) and another quaternary methyl (δ 1.09). The olefinic proton signal of DHGA was missing in dendrillin and a new signal appeared at δ 3.04. Further comparison revealed the vinyl methyl doublet had shifted from δ 1.63 in DHGA to δ 1.36 in dendrillin. The ^{13}C-nmr spectrum showed analogous shifts; olefinic signals were absent in the spectrum of dendrillin, while new signals appeared at δ 61.9 and δ 63.0. These shifts and ms analysis suggested that dendrillin was an epoxide derivative of DHGA. This was confirmed by treatment of DHGA with *m*-CPBA, yielding a compound identical in spectral properties to the natural product.

The stereochemistry of **1** was secured by nOe difference spectroscopy. Irradiation of the epoxymethine H-7 proton at δ 3.04 resulted in a 15% enhancement of H-14 at δ 2.00, which is consistent with a β-orientation of the epoxide oxygen. While the α-epoxide might also be expected to show an nOe to H-14, H-7 in this orientation would also be expected to show an nOe to H-15 (δ 6.57). However, irradiation of H-15 resulted only in enhancement of the H_3-20 methyl singlet at δ 1.09, which, in the α-epoxide, is nearly as distant from H-15 as is H-7; the H-15/H_3-20 nOe secured the stereochemical relationship between C-9 and C-15 as being the same as found in DHGA. Further support for the β-orientation of the epoxide derives from consideration of the differences in chemical shifts in comparing the olefin to the epoxide; because H_3-20 in DHGA lies in the deshielding zone of the olefin, it might be expected to shift upfield in the epoxide. In fact, H_3-20 shifted downfield 0.9 ppm in the epoxide, which is supportive of an oxygen on the β-face serving to further deshield the methyl group; a similar argument could be used to explain the downfield shift (0.15 ppm) of H-15. A singlet is observed for H-16 due to a 90° dihedral angle with H-13. These results demonstrate **1** has the same relative stereochemistry as DHGA at C-9, C-10, C-13, C-14, C-15, and C-16 and that the epoxide, like the diacetoxy diacetal group, is oriented on the β-face of the cyclohexane ring (Figure 1).

The sequential MeOH extract from *D. membranosa*, having displayed significant activity in our ecological feeding deterrence tube-foot retraction assay, was also fractionated. Purification of a previously reported quinoline alkaloid, 4,5,8-trihydroxyquinoline-2-carboxylic acid, was achieved by LH-20 (MeOH) and reversed-phase hplc and was identified based on comparison of spectral data to

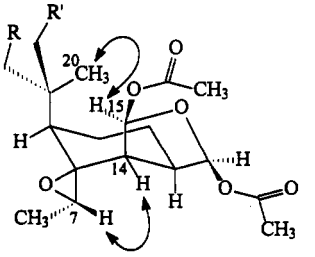


FIGURE 1. Key nOe enhancements observed in **1**.

those reported previously (9). Also isolated from this extract was 7-methyladenine, which has not been reported previously from marine invertebrates, and picolinic acid; 7-methyladenine was characterized based on comparison of ^{1}H- and ^{13}C-nmr data to those reported previously (11), while picolinic acid was compared to an authentic sample (Aldrich).

In terms of the ecological roles of *D. membranosa* chemistry in influencing sponge/sea star interactions, it has been suggested that DHGA and membranolide are involved in the chemical defense of *D. membranosa* (8). To evaluate the effectiveness of the norditerpenes in preventing predation by sea stars, the major predators of sponges on the Antarctic benthos (1), we utilized a behavioral assay based on the tendency of a sea star tube-foot to retract in response to various stimuli (12). Sea star tube-feet, in addition to providing locomotion, are chemosensory, allowing the animal to assess the suitability of prey as it moves along the benthos (13); *Perknaster fuscus* was chosen for the tube-foot retraction assays since it is spongivorous; *P. fuscus* feeds predominately on the sponge *Mycale acerata* but includes a variety of other sponges in its diet (1). The hexane extract, which was composed of an approximately 1:1 mixture of DHGA and fatty acids, had no significant activity toward *P. fuscus* in this assay at five times the natural concentration (12). Therefore, the suggestion (8) that the norditerpenes from the sponge might be responsible for the lack of predation on the sponge is not the case, at least for the sea star *P. fuscus*. The quinoline alkaloid pigment, 4,5,8-trihydroxyquinoline-2-carboxylic acid, isolated from the active MeOH extract, also failed to cause significant tube-foot retraction activity at concentrations greater than occur naturally.

Although we cannot yet definitively identify the sea star deterrent agent in *D. membranosa*, subsequent to leaving Antarctica, 7-methyladenine and picolinic acid were isolated from the active MeOH fraction of this sponge. 1-Methyl- and 3-methyladenine have been previously reported from marine invertebrates (14,15), the former of which is involved in induction of meiosis in sea stars. Given the known biological roles of nucleotides in marine invertebrates, especially in sea stars (14), as well as the known deterrent role of *N*-methylpicolinic acid (homarine) (16), it may well be that one of these two metabolites (7-methyladenine or picolinic acid) will play a role in sponge/sea star interactions.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All solvents were distilled from glass. A Bruker AMX-360 instrument was used to record ^{1}H- (360 MHz) and ^{13}C- (90 MHz) nmr spectra; chemical shifts are reported in ppm with the chemical shift of residual solvent nuclides used as internal standard. The ir spectrum was recorded on a Nicolet Magna-IR 550 instrument, the uv spectrum was recorded on a Hewlett-Packard 8452A diode-array spectrometer, and eims were recorded on a VG 70SE mass spectrometer.

ANIMAL MATERIAL.—Sponge samples were collected using scuba between 30 and 40 m depth from Hut Point and Danger Slopes on Ross Island (77°51′S; 166°39′E) and between 10 and 40 m depth at New Harbor (77°34′S; 164°40′E) on the continental coast. A voucher specimen is on hand at the Department of Chemistry, Florida Institute of Technology.

EXTRACTION AND ISOLATION.—A typical isolation began with 20 g of freeze-dried *Dendrilla membranosa*, extracted sequentially in hexane, $CHCl_3$, MeOH, and MeOH-H_2O (7:3). The hexane extract (4% of dry wt) was chromatographed on Si gel using step-gradient flash cc, collecting two-column volumes as two fractions for each step. The second column volume eluting with hexane-EtOAc (80:20) was further chromatographed using reversed- (MeOH-H_2O, 70:30) and normal- (hexane-EtOAc, 80:20) phase hplc to give 5.1 mg (0.026%) of **1**. Picolinic acid (7 mg, 0.035% of dry wt) was obtained by gradient reversed-phase hplc of the MeOH extract (20% of dry wt after removal of salts), eluting in the H_2O-MeOH (40:60) fraction. 7-Methyladenine eluted from a bed of C_{18} (1 g MeOH extract applied to a 5-cm diameter × 2-cm deep bed) with H_2O; 100 mg of this eluent was applied to LH-20 (2.5

cm×30 cm, MeOH), then the 7-methyladenine containing fraction, which eluted from 100 to 150 ml, was purified by reversed-phase hplc (H_2O-MeOH, 90:10) to yield 2.5 mg (0.65% dry wt) of the purine.

Dendrillin [**1**].—Colorless oil (5.1 mg) isolated from 20 g freeze-dried sponge; uv, end absorption with broad tailing centered at 280 nm; ir (film) ν max 2925, 2872, 1759, 1459, 1367, 1244 cm^{-1}; ^1H nmr ($CDCl_3$, 300 MHz) δ 6.57 (1H, d, J=6.5 Hz, H-15), 5.90 (1H, s, H-16), 3.04 (1H, q, J=5.7 Hz, H-7), 2.61 (1H, m, H-13), 2.08 (3H, s, OAc), 2.03 (3H, s, OAc), 2.00 (1H, br t, J=7.8 Hz, H-14), 1.90–1.85 (1H, m, H-12a), 1.75–1.68 (2H, m, H-12b, H-11a), 1.59–1.45 (4H, m, H_2-2, H-11b, H-1a), 1.4–1.3 (4H, m, H-1b, H-3a, H-5a, H-9), 1.36 (3H, d, J=5.8 Hz, H_3-17), 1.09 (3H, s, H_3-20), 1.0 (2H, m, H-5b, H-3b), 0.96 (3H, s, H_3-18), 0.86 (3H, s, H_3-19); ^{13}C nmr ($CDCl_3$, 90 MHz) δ 170.27 (s, $COCH_3$), 170.11 (s, $COCH_3$), 102.07 (d, C-16), 98.09 (d, C-15), 63.01 (d, C-7), 61.94 (s, C-8), 51.88 (t, C-5), 51.85 (d, C-14), 48.15 (d, C-9), 43.98 (d, C-13), 38.90 (t, C-3), 37.70 (s, C-10), 36.19 (t, C-1), 35.97 (q, C-19), 31.44 (s, C-4), 27.78 (q, C-18), 24.24 (q, C-20), 22.57 (t, C-12), 22.06 (t, C-11), 21.43 (q, $COCH_3$), 21.40 (q, $COCH_3$), 19.29 (t, C-2), 16.20 (q, C-17); eims (70 eV) m/z 348 (<5), 288 (5), 245 (10), 164 (40), 125 (100); hreims m/z 288.20825 ($M^+ - 2HOAc$), calcd for $C_{19}H_{28}O_2$, 288.20893 (Δmmu=0.7).

Conversion of 9,11-dihydrogracillin to **1**.—To a solution of 18 mg 9,11-dihydrogracillin A (46 mmol) in 5 ml of CH_2Cl_2 was added 15 mg 55% *m*-CPBA (48 mmol *m*-CPBA, remainder *m*-chlorobenzoic acid). After reflux overnight, the reaction was quenched with 10% NaOH and the aqueous layer re-extracted with CH_2Cl_2. Combined organic layers were purified by hplc (13% EtOAc/hexane) to yield 13 mg (32 mmol, 70%) of semi-synthetic **1**.

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