# Further Membranolide Diterpenes from the Antarctic Sponge *Dendrilla membranosa*

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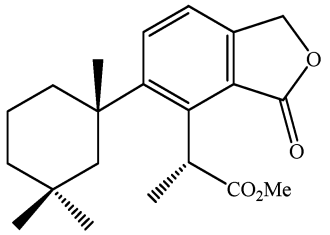
Chemical investigation of the Antarctic sponge *Dendrilla membranosa* collected from the vicinity of Palmer Station on Anvers Island, Antarctica, yielded three new diterpenes, membranolides B−D (**2**−**4**), as well as three previously reported sponge metabolites. Membranolides C and D (**3**, **4**), bearing carboxylic acid functional groups, display Gram-negative antibiotic and antifungal activities.

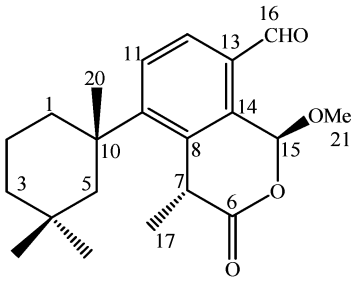
Sponges have proven a rich source of secondary metabolites among the Antarctic invertebrates. Many of these secondary metabolites are bioactive, with some causing feeding deterrence in ecologically relevant predators and some displaying potent antimicrobial or cytotoxicity properties.[1] The bright yellow Antarctic cactus sponge, *Dendrilla membranosa* Pallas (family Darwinellidae, order Dendroceratida), a common member of the benthos in the vicinity of Palmer Station, has a circumpolar distribution. Dayton[2] suggested that the sponge, which has neither apparent spicules nor mucus, is protected from predation by nudibranchs and the spongivorous sea-star *Perknaster fuscus* due to the presence of chemical defenses.

Diterpenes of the spongian-type have been isolated from sponges of the orders Dendroceratida and Dictyocertida as well as nudibranchs that feed upon them.[3] *D. membranosa* collected from McMurdo Sound, Antarctica, has been shown to elaborate highly oxidized diterpenes,[4−8] including gracillin derivatives[4−6] and the aromatized diterpene membranolide (**1**).[4] We report herein the isolation of three new membranolide-type diterpenes from *D. membranosa* collected from Palmer Station (64°46′ S, 64°03′ W) on Anvers Island, Antarctica.

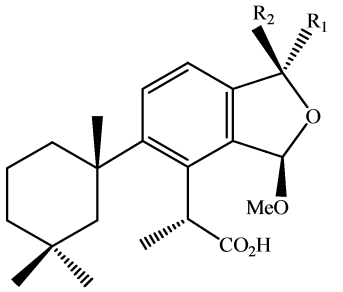
*D. membranosa* was collected using scuba between 30 and 40 m depth from several sites around Anvers Island. The $CH_2Cl_2$−$CH_3OH$ (1:1) extract of the freeze-dried sponge was subjected to step gradient flash chromatography on silica gel. The fraction eluting with 20% ethyl acetate in hexanes was further purified by silica gel HPLC to yield membranolide B (**2**). The fraction eluting with 25% ethyl acetate in hexanes was similarly further purified by HPLC to yield two additional compounds, membranolides C and D (**3**, **4**), along with membranolide[4] (**1**) and the known compounds aplysulfurin[9] and tetrahydroaplysulfurin.[10]




Membranolide (**1**)




Membranolide B (**2**)




Membranolide C (**3**): $R_1$ = OMe, $R_2$ = H
Membranolide D (**4**): $R_1$ = H, $R_2$ = OMe

Membranolide B (**2**) was isolated as a colorless, viscous oil. The $^1$H NMR spectrum showed an AB system of doublets at $\delta$ 7.75 and 7.72 ($J$ = 8.5 Hz) consistent with two ortho protons attached to a tetrasubstituted benzene ring, a partial structure also suggested by the UV spectrum ($\lambda_{max}$ 263 nm). Further functional groups on membranolide B were suggested by an aldehyde proton at $\delta$ 10.05 (s), an acetal proton at $\delta$ 6.80 (s), and a methoxyl methyl signal at $\delta$ 3.80 (s). The $^1$H NMR spectrum also contained signals corresponding to a *gem*-dimethyl group at $\delta$ 0.57 and 0.96 and a quaternary methyl at $\delta$ 1.25. The $^{13}$C NMR spectrum exhibited 21 signals, which included six aromatic carbons, an aldehyde, a lactone carbonyl, four each aliphatic methyl and methylene groups, an aliphatic methine, one acetal carbon, and a methoxyl signal.

The planar structure of membranolide B (**2**) was established by a series of 2D NMR techniques. Based on one-bond connectivities established by gHSQC and HMBC correlations (Table 1) of the high-field aromatic proton, H-12, with the aldehyde carbonyl (C-16) and with aromatic carbons C-9 and -14, and of the acetal proton, H-15, with aromatic carbons C-8, -13, and -14, secured the positions of

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**Table 1.** NMR ($CDCl_3$) Data of Membranolides B and C (**2**, **3**)

| | membranolide B (**2**) | | | membranolide C (**3**) | | |
|---|---|---|---|---|---|---|
| | $\delta_C$[a] | $\delta_H$ | HMBC | $\delta_C$[a] | $\delta_H$ | HMBC |
| 1 | 39.8 ($CH_2$) | 1.51 (m) | | 41.3 ($CH_2$) | 1.45 (m) | C-5, 10 |
| | | 2.23 (m) | | | 2.29 (br d) | |
| 2 | 19.9 ($CH_2$) | 1.67 (m) | | 20.0 ($CH_2$) | 1.65 (m) | |
| | | 1.72 (m) | C-1, 3 | | 1.80 (m) | |
| 3 | 39.8 ($CH_2$) | 1.33 (m) | C-1, 2, 18, 19 | 39.9 ($CH_2$) | 1.26 (m) | C-18 |
| | | | | | 1.32 (m) | C-4, 5, 18 |
| 4 | 31.7 (C) | | | 31.7 (C) | | |
| 5 | 50.4 ($CH_2$) | 1.58 (d, 14.5) | C-1, 3, 9, 10, 19 | 50.6 ($CH_2$) | 1.49 (d, 14) | C-1, 4, 9, 10, 18 |
| | | 2.05 (d, 14.5) | C-1, 3 | | 2.09 (d, 14) | C-1, 3, 4 |
| 6 | 174.7 (C) | | | 180.1 (C) | | |
| 7 | 39.5 (CH) | 4.53 (q, 7.5) | C-6, 8, 9, 14, 17 | 41.2 (CH) | 4.69 (q, 7) | C-6, 8, 9, 14, 17 |
| 8 | 138.1 (C) | | | 135.7 (C) | | |
| 9 | 154.0 (C) | | | 148.6 (C) | | |
| 10 | 40.4 (C) | | | 39.6 (C) | | |
| 11 | 128.6 (CH) | 7.75 (d, 8.5) | C-8, 9, 10, 13 | 128.9 (CH) | 7.57 (d, 8) | C-10, 13, 14 |
| 12 | 134.0 (CH) | 7.72 (d, 8.5) | C-9, 14, 16 | 121.6 (CH) | 7.25 (d, 8) | C-9, 14, 16 |
| 13 | 130.6 (C) | | | 137.3 (C) | | |
| 14 | 132.8 (C) | | | 139.4 (C) | | |
| 15 | 100.0 (CH) | 6.80 (s) | C-6, 8, 13, 14, 21 | 105.8 (CH) | 5.89 (s) | C-13, 21 |
| 16 | 191.8 (CH) | 10.05 (s) | C-14 | 104.8 (CH) | 5.86 (s) | C-14, 22 |
| 17 | 23.8 ($CH_3$) | 1.81 (d, 7.5) | C-6, 7, 8 | 16.8 ($CH_3$) | 1.59 (d, 7) | C-6, 7, 8 |
| 18 | 28.0 ($CH_3$) | 0.57 (s) | C-3, 5, 19 | 27.4 ($CH_3$) | 0.47 (s) | C-3, 4, 5, 19 |
| 19 | 32.2 ($CH_3$) | 0.96 (s) | C-3, 5 | 32.9 ($CH_3$) | 0.92 (s) | C-3, 4, 5, 18 |
| 20 | 32.4 ($CH_3$) | 1.25 (s) | C-5, 9 | 32.8 ($CH_3$) | 1.39 (s) | C-5, 9, 10 |
| 21 | 57.5 ($CH_3$) | 3.80 (s) | C-15 | 53.3 ($CH_3$) | 3.47 (s) | C-15 |
| 22 | | | | 55.1 ($CH_3$) | 3.50 (s) | C-16 |

[a] $\delta$ (multiplicity from DEPT).

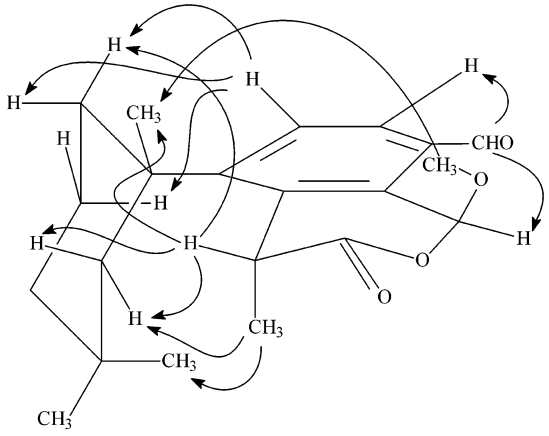


**Figure 1.** Key NOESY correlations for membranolide B (**2**).

aldehyde and acetal groups on the aromatic ring. Additional correlation of H-15 to the carbonyl at $\delta$ 174.7 (C-6) and that of H-7 to C-6 and -8 established the presence of a fused lactone ring. Further HMBC correlations of H-7 with C-9, -14, and -17, taken with the correlation of H-11 to C-8, -9, and -10, completed the assignment of the aromatic and lactone rings. The trimethylcyclohexane moiety was unambiguously assigned from HMBC correlations (Table 1) and connected to the aromatic ring at C-10 on the basis of correlations of the C-20 methyl protons ($\delta$ 1.25) with aromatic C-9. The anomalous high-field chemical shift ($\delta$ 0.57) of the C-4 axial methyl signal in the cyclohexane ring is characteristic of 1′,3′,3′-trimethylcyclohexylbenzenes due to the proximity of that methyl in the anisotropic shielding zone of the aromatic ring.[4]

Stereochemical analysis of membranolide B (**2**) was carried out via 2D NOE experiments. The most significant correlations (Figure 1) were observed from H-7 and H-11 to the equatorial protons on C-1 and -5; thus, H-7 showed spatial proximity to the equatorial hydrogen on position 5 ($H_e$-5) and to $H_3$-20, while H-11 showed analogous proximity to the other side of the cyclohexane ring, with correlations to $H_e$-1 and $H_a$-2. Taken with the anisotropic shielding of the axial C-4 methyl group (C-18), the cyclohexane ring adopts a chair conformation whereby the fused-bicycle substituent occupies an axial position, as previously demonstrated by X-ray crystallography for the related compound aplysulfurin.[9] Aplysulfurin was also isolated from our collections of *D. membranosa*. The lactone ring of membranolide B adopts a skewed chair conformation, constrained by three $sp^2$ carbons, that results in the C-7 methyl in a roughly equatorial position and the C-15 methoxyl in a roughly axial position. These assignments are supported by NOESY correlations of C-17 to the axial methyl (C-18) of the *gem*-dimethyl group on the cyclohexane ring and to the H-5 equatorial proton, as well as by the NOESY correlation of the methoxyl methyl with the methyl (C-20) group on C-10.

Membranolide C (**3**) was obtained as a viscous oil, and its $^1H$ NMR spectrum was reminiscent of membranolide B (**2**). Similar features included the *gem*-dimethyl group ($\delta$ 0.47 and 0.92), a quaternary methyl at $\delta$ 1.39, a methyl doublet at $\delta$ 1.59, and methine singlets at $\delta$ 5.86 and 5.89. Differing from membranolide B were additional methoxyl methyl and acetal methine signals. While the $^{13}C$ NMR spectrum showed a carbonyl resonance at $\delta$ 180.1, it could be discounted as a lactone on the basis of a broad band centered at 2925 $cm^{-1}$ in the IR spectrum, indicative of a carboxylic acid. The $^{13}C$ NMR spectrum additionally showed six aromatic, two acetal carbons, and two methoxyl signals. The positions of the acetal and methoxyl groups could be assigned on the basis of HMBC correlations (Table 1), particularly correlations of H-12 to C-14 and -16, H-15 to C-13 and -21, and H-16 to C-14 and -22, which also identified the fused furan ring. The stereochemistry about the furan ring was established by observation of a NOESY correlation from H-15 to $H_3$-22. The relative position of H-15 to the remaining stereocenters was suggested by a NOESY correlation to $H_3$-17.

Membranolide D (**4**) was also obtained as a viscous oil, and its spectral data were similar to that of membranolide

**Table 2.** Antibiotic Activity of Membranolides C and D (**3**, **4**) (200 $\mu$g/disk)

| compound | *S. aureus* | *E. coli* | *C. albicans* |
|---|---|---|---|
| **3** | 0 | 8 mm | 4 mm |
| **4** | 7 mm | 6 mm | 9 mm |

C (**3**) except for the C-15 and C-16 methine resonances appearing coincident as a singlet at $\delta$ 6.2 when acquired in $CDCl_3$. The spectral data of the compound in benzene-$d_6$ resolved the coincident signals, and the NOESY data confirmed the stereochemistry around C-16 in which the methoxy group is $\beta$. NOESY correlations were observed from H-15 to H-16 and -17 and from H-7 to $H_3$-22.

Membranolide B−D acetal functional groups did not prove prone to hydrolysis in our hands. Nonetheless, we were interested in establishing that the functional group was not an artifact of the isolation scheme. We conducted an extraction of freeze-dried sponge material with ethanol and found no metabolites bearing the ethyl acetal group. Terpenes, including sesquiterpenes,[11] diterpenes,[12] and sesterterpenes,[13] are well-precedented methyl acetal-bearing sponge metabolites, leading us to conclude that the new membranolides described here are natural products. Membranolides C and D (**3**, **4**) displayed modest yet broad spectrum antibacterial activity (Table 2).

## Experimental Section

**General Experimental Procedures.** Optical rotations were measured on an Autopol IV automatic polarimeter using a Na lamp at 25 °C. Ultraviolet spectra were recorded on a Hewlett-Packard 8452A diode array UV/vis spectrometer. Infrared spectra were recorded on a Nicolet Avatar 320 FT-IR. A Varian INOVA 500 instrument was used to record $^1$H (500 MHz) and $^{13}$C (125 MHz) NMR spectra at 25 °C; chemical shifts are reported in ppm with $CHCl_3$ (7.26 ppm for proton and 77.0 ppm for carbon) or benzene (7.15 ppm for proton, 128.02 ppm for carbon) as internal reference. Electron impact mass spectra were recorded on a Micromass 70-VSE spectrometer at the University of Illinois, Urbana−Champaign. HPLC was carried out with a Shimadzu LC-8A multisolvent delivery system connected to a Shimadzu SPD-10A UV−vis tunable absorbance detector using a YMC-Pack CN analytical column. EM Science silica gel 230−400 mesh was used in flash column chromatography. TLC was carried out on Whatman K6F silica gel 60A TLC plates with 0.25 mm thickness.

**Animal Material.** The sponge *Dendrilla membranosa* was collected at a depth of 30−40 m from Anvers Island, Palmer Station (64°46′ S, 64°03′ W), Antarctica, during November 2001.

**Extraction and Isolation.** The freeze-dried sponge (122 g) was extracted three times with dichloromethane−MeOH (1:1). The crude extract (3.2 g) was chromatographed on silica gel using hexanes−EtOAc step-gradient flash column chromatography of increasing polarity. Fraction 4 (60 mg) eluted with 20% EtOAc in hexanes was further subjected to silica gel column chromatography to yield membranolide B (**2**) (11.5 mg). The fraction eluting with 25% EtOAc in hexanes was further subjected to normal-phase CN analytical HPLC using 20% EtOAc in hexanes to yield membranolides C and D (**3**, **4**) (8.0 and 13.0 mg, respectively).

**Antibiotic Assays.** Bioassays were carried out by the disk diffusion method in which 200 $\mu$g of the compound was transferred in chloroform to a cotton disk. The cotton disk was placed on a Petri dish, to which the tester strains of bacteria or fungi had been swabbed. The zones of inhibition, measured from the edge of the disk to the edge of the clear zone, were observed after 24 h of incubation.

**Membranolide B (2):** viscous oil; $[\alpha]^{25}_D$ −121.0 (*c* 0.6, $CHCl_3$) IR $\nu_{max}$ 2927, 1749, 1699, 1593, 1467 cm$^{-1}$; UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 263 (3.95), 228 (3.90); $^1$H and $^{13}$C NMR data, see Table 1; EIMS *m*/*z* (%) 344 (100, [M$^+$]), 329 (20), 316 (35), 300 (70), 285 (50), 271 (80), 255 (35), 241 (25), 215 (55), 201 (40), 188 (100), 174 (55), 155 (30), 141 (45), 128 (70), 115 (60); HREIMS *m*/*z* 344.1991 (calcd for $C_{21}H_{28}O_4$, 344.1988).

**Membranolide C (3):** viscous liquid; $[\alpha]_D^{25}$ −100.8 (*c* 0.6, $CHCl_3$); IR $\nu_{max}$ 2925, 1733, 1690, 1565 cm$^{-1}$; UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 258 (3.38), 215 (3.93); $^1$H and $^{13}$C NMR data, see Table 1; EIMS *m*/*z* (%) 376 (1, [M$^+$]), 375 (5), 345 (20), 300 (100), 285 (25), 271 (15), 189 (20); HREIMS *m*/*z* 375.2181 ([M$^+$ − 1]) (calcd for $C_{22}H_{31}O_5$, 375.2171).

**Membranolide D (4):** viscous liquid; $[\alpha]_D^{25}$ +6.5 (*c* 0.6, $CHCl_3$); IR $\nu_{max}$ 2925, 1733, 1690, 1565 cm$^{-1}$; UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 258 (3.36), 215 (3.91); $^1$H NMR (500 MHz, $C_6D_6$) $\delta$ 7.38 (1H, d, 8, H-11), 7.08 (1H, d, 8, H-12), 6.26 (1H, s, H-15), 5.90 (1H, s, H-16), 4.6 (1H, q, 7, H-7), 3.5 (3H, s, $H_3$-21), 3.3 (3H, s, $H_3$-22), 1.85 (3H, d, 7, $H_3$-17) 1.5−2.3 (8H, m, H2-1, -2, -3, -5), 1.38 (3H, s, $H_3$-20), 0.85 (3H, s, $H_3$-19), 0.48 (3H, s, $H_3$-18); $^{13}$C NMR (125 MHz, $C_6D_6$) 179.9 (C-6), 148.5 (C-9), 140.3 (C-14), 138.1 (C-13), 136.4 (C-8), 128.8 (C-11), 121.8 (C-12), 105.6 (C-15), 105.2 (C-16), 54.4 (C-21), 53.7 (C-22), 50.6 (C-5), 41.3 (C-1), 39.9 (C-3), 39.5 (C-10), 33.0 (C-20), 32.8 (C-19), 31.7 (C-4), 26.8 (C-18), 20.0 (C-2), 17.0 (C-17); EIMS (70 eV) *m*/*z* (%) 376 (1, [M$^+$]), 375 (5), 345 (30), 317 (15), 303 (100), 300 (20), 285 (15), 271 (10), 189 (15); HREIMS *m*/*z* 375.2166 ([M$^+$ − 1]) (calcd for $C_{22}H_{32}O_5$, 375.2171).

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