Dendrinolide, a New Degraded Diterpenoid from the Antarctic Sponge *Dendrilla membranosa*

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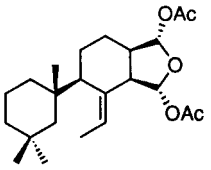
A new glacian diterpenoid, named dendrinolide (**5**), was isolated, together with the 9,11-dihydrogracilin A (**1**), from the Me_2CO extract of the Antarctic sponge *Dendrilla membranosa*. Its structure has been elucidated by interpretation of spectral data and comparison with the similar product **6**, previously found in the Mediterranean sponge *Spongionella gracilis*.

Benthic organisms from the Antarctic Sea have received a great deal of interest because of their ability to adapt themselves to the extreme conditions offered by polar regions. In recent years the Antarctic sponge *Dendrilla membranosa* Pallas (Dendroceratida, Aplysillidae), an organism that has not been observed to be preyed upon,[1] has been investigated in order to demonstrate the presence of chemical defenses,[1−3] and three degraded diterpenoids **1**−**3**[1,3] were isolated from it. All three compounds are closely related to gracilin A (**4**),[4] the main metabolite of the Mediterranean *Spongionella gracilis* (Dictyoceratida, Disideidae). Recently, the relative stereochemistry of 9,11-dihydrogracilin A (**1**) was completely solved by X-ray analysis of a keto derivative obtained by ozonolysis of the natural norditerpenoid.[5]



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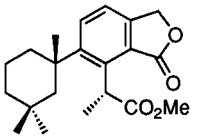

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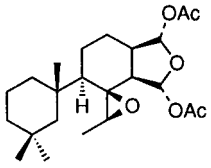

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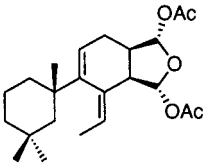

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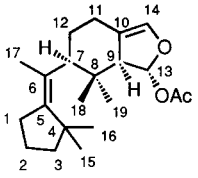

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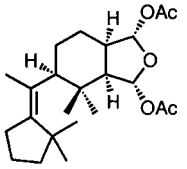

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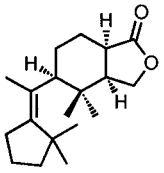

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In this study, we report the structure elucidation of the rearranged norditerpene **5** isolated from *D. membranosa*. Dendrinolide (**5**) is structurally similar to the oxygenated terpene **6**, found in *S. gracilis*,[6] and to glacionolide (**7**) isolated from the extracts of the nudibranch *Cadlina luteomarginata* and of its prey *Aplysilla glacialis*.[7]

Dendrinolide (**5**) was obtained as an optically active oil {$[\alpha]_D$ + 89.0° (*c* 0.7, $CHCl_3$)} from *D. membranosa*. HREIMS measurements gave a [M^+] ion at *m*/*z* 332.2369 consistent with the molecular formula $C_{21}H_{32}O_3$. The ^{13}C-NMR data (Table 1) showed some diagnostic downshifted signals due to an ester carbonyl function (170.1 ppm), together with an acetal carbon (99.6 ppm) and tetrasubstituted (128.4 and 145.1 ppm) and trisubstituted (114.6 and 134.7 ppm) double bonds.

The presence of an acetoxy group, suggested by an intense EIMS fragment at *m*/*z* 272 (M^+ − 60), was confirmed either by a sharp singlet at δ 2.10 in the ^{1}H-NMR spectrum and by a strong IR absorption at 1740 cm^{-1}. The resonances at δ 6.36 (H-13) and 6.06 (H-14), together with the positive Ehrlich reaction, suggested the presence of a substituted 5-acetoxy-4,5-dihydrofuran ring. The ^{1}H-NMR spectrum (see Table 1) also indicated the presence of four tertiary methyl groups and a vinyl methyl. Homodecoupling experiments and ^{1}H−^{1}H COSY revealed the presence of a spin system located between two double bonds, attributable to the sequence of protons of the partial structure $CHCH_2CH_2$. The signals assigned to the two protons at C-12 (δ 1.74 and 1.46) were coupled both with the resonance at δ 2.62 (H-7) and with those at δ 2.46 and 2.10 (H_2-11). On the other hand, irradiation at δ 2.46 (H-11) showed an allylic coupling with the vinyl proton at δ 6.06 (H-14) and a weak effect on the broad singlet at δ 2.41, whereas irradiation at δ 2.41 (H-9) simplified the sharp doublet at 6.35 ppm (H-13, J = 2 Hz), suggesting that the angle between H-9 and H-13 is close to 90°. The multiplicity of H-7 and H-9 suggested that these two protons were connected by a quaternary carbon (C-8) that also bears two methyl groups. A series of NOE experiments confirmed this hypothesis. The remaining resonances were attributable to a five-membered ring connected to the major fragment of **1** through the tetrasubstituted double bond. This hypothesis was supported by the observations of two strong fragments in the mass spectrum at *m*/*z* 149 (M^+ − 60−123) and 123 deriving

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Table 1. NMR (500 MHz, CDC_{l3}) Data of Dendrinolide (**5**)[a]

position	1H-NMR δ (m,*J*)	13C-NMR δ (m)
1	2.25 (m)	34.3 (t)
2	1.54 (m)	22.7 (t)
3	1.54 (m)	46.9 (t)
4		41.4 (s)
5		145.1 (s)
6		128.4 (s)
7	2.62 (dd, 12.5, 2.7 Hz)	48.4 (d)
8		36.7 (s)
9	2.41 (br s)	63.8 (d)
10		114.6 (s)
11	2.46 (dd, 13.6 and 4.0 Hz) 2.10 (m)	22.6 (t)
12	1.74 (dddd, 12.7, 12.7, 12.7, 5.0 Hz 1.46 (m)	27.9 (t)
13	6.36 (t, 2.0 Hz)	99.6 (d)
14	6.06 (d, 1.9 Hz)	134.7 (d)
15	1.25[b]	29.6 (q)
16	1.17[b]	29.0 (q)
17	1.49	18.3 (q)
18	0.89	17.3 (q)
19	0.95	26.3 (q)
CH_{3CO}	2.10	21.3 (q)
CH_3CO		170.1 (s)

[a] Numbering is according to that reported for the glaciane carbon skeleton.[7] [b] Values, that are designated with the same letter are interchangeable.

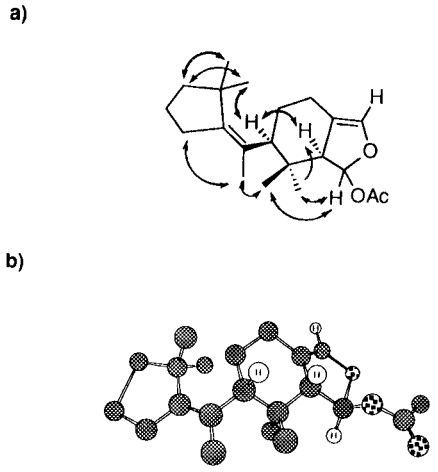

Figure 1. (**a**) Selected NOEs of dendrinolide (**5**) in $CDCl_3$. (**b**) Stereochemical picture of dendrinolide (**5**) was drawn on the basis of coupling constants and NOEs by CS CHEM3D PRO software (CambridgeSoft Corporation).

from the molecular peak, after elimination of HOAc, by cleavage between the carbons 6 and 7.

The *Z* configuration of the tetrasustituted double bond, suggested by comparing the ^{13}C-NMR values of C-1 (34.3), C-6 (145.1), C-5 (128.4), and C-17 (18.3) with those reported in the literature,[6,7] was supported by NOE measurements that showed the through-space interaction between CH_3-17 and the protons at C-1 (δ 2.25) of the cyclopentane ring (Figure 1). NOE experiments also revealed a *cis* relationship of protons H-7 and H-9 that was diagnostic to assign the relative stereochemistry of C-9. Finally, the relative stereochemistry at C-13 is suggested both by analogy with the co-occurring terpenoids and by the small coupling constant between H-9 and H-13.

The workup of *D. membranosa* also revealed the presence of large amounts of free fatty acids, of which the abundant polyunsaturated component was easily purified after methylation with CH_2N_2. Details of the lipid analysis will be reported elsewhere. The isolation of dendrinolide (**5**) from the extract of *D. membranosa* is further evidence for the metabolic relationship between the Antarctic sponge and the Mediterranean *S. gracilis*. Although the organisms belong to different taxonomic orders, they contain diterpenoid compounds whose structures are closely related.

Experimental Section

General Experimental Procedures. Merck Kieselgel 60 (70−230 mesh) was used for Si gel chromatography, and precoated Kieselgel 60 F_{254} plates (Merck 0.25-mm precoated plates) were used for analytical TLC. NMR spectra were recorded by a Bruker WM-500 (500 MHz) and AMX 400 (400 MHz) spectrometers. Chemical shifts are reported in parts per million with $CHCl_3$ (7.26 ppm for proton and 77.0 ppm for carbon) as reference. Mass spectra were measured on a Carlo Erba TRIO 2000 VG instrument. IR spectra were recorded by a Bio-Rad FTS-7 spectrophotometer. Optical rotations were measured on a JASCO DIP-370 polarimeter. HREIMS measurements were performed on a Kratos MS 50.

Animal Material. The sponge, *D. membranosa*, was collected at a depth of 60 m near Gulache Inlet Station, Terranova Bay, New Zealand, in December 1990, and was stored at −80 °C until the analysis. In life, the sponge was amorphous and yellowish green. A voucher specimen is deposited at Istituto di Zoologia, Università di Genova, Genova, Italy.

Fractionation of the Lipophilic Extract of *D. membranosa*. The frozen sponge (450 g frozen wt) was extracted three times with Me_2CO (3 × 300 mL). The concentrated extract was then partitioned between Et_2O (3 × 200 mL) and H_2O (150 mL). The Et_2O-soluble fraction (1.7 g) was chromatographed on Si gel (Kieselgel 60, 100 g) using a petroleum ether−Et_2O step gradient of increasing polarity. Fractions 2 and 3 (8 and 156 mg), eluted with light petroleum−Et_2O (9:1), was further fractionated by the same technique (7 g Si gel, eluent light petroleum−Et_2O, 95:5) to yield pure dendrinolide (**5**, 18 mg). Fraction 5, eluted with light petroleum−Et_2O (8:2), contained pure 9,11-dihydrogracilin A (**1**, 310 mg).

Dendrinolide (1): yellow oil (18 mg, 0.004% of frozen sponge); $[\alpha]^{25}_D$ + 89° (*c* 0.7, $CHCl_3$); IR (hexane solution) ν_{max} 2866 (CH, aliphatic), 1740 (C=O, ester), 1223, 1009 (C−O, ester); EIMS (70 eV) *m*/*z* [M^+] 332 (5), 272 (85), 257 (27), 176 (30) 161 (20), 149 (60), 123 (55), 43 (100); HREIMS (*m*/*z*) 332.2369 (required 332.2351); ^{1}H- and ^{13}C-NMR values, see Table 1.

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Supporting Information Available: Copies of ^{1}H- and ^{13}C-NMR spectra of dendrinolide (**5**) (2 pages). Ordering information is given on any current masthead page.

References and Notes

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