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# GRACILIN A, AN UNIQUE NOR-DITERPENE METABOLITE FROM THE MARINE SPONGE SPONGIONELLA GRACILIS.

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Summary: From the marine sponge Spongionella gracilis a novel degraded $C_{19}$ terpene diacetate (2) has been isolated and its structure elucidated by spectral analysis, including 2D-NMR, and chemical evidence.

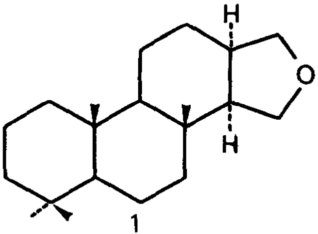
Marine sponges belonging to the order Dictyoceratida and Dendroceratida have provided a host of new terpenoid metabolites including a series of diterpenes based on the "spongian" ring system (1)[1-5].

In the course of our continuing studies for constituents of the Mediterranean marine organisms, we have been investigating the sponge Spongionella gracilis, order Dictyoceratida, family Dysideidae. We wish to report here the isolation and the structure elucidation of gracilin A (2), a novel $C_{19}$ terpene diacetate, which can be hypothetically related to the general skeleton 1.

The diethyl ether soluble material from the methanol-chloroform 1:1 extract of the sponge S.gracilis, collected in the Bay of Napoli, during the spring 1984, was chromatographed repeatedly over silica gel using dichloromethane as eluent to give gracilin A (2, 0.5% based on the dry weight of the sponge after extraction), oily; $[\alpha]_D$ -60.5° (c = 1.3, $CHCl_3$), $\lambda_{max}^{MeOH}$ 225 nm ($\epsilon$ = 7700). The molecular formula of compound 2 was determined as $C_{23}H_{34}O_5$ by elemental analysis and high resolution mass measurement of the parent ion which was hardly discernible in the mass spectrum of 2. Major fragment ions at m/z 330 ($M^+$-AcOH, 62%), 288 ($M^+$-AcOH-$CH_2CO$,100%) and 270 ($M^+$-AcOH, 87%) pointed to the presence of two acetoxy groups, confirmed by i.r. absorptions (film) at 1750 and 1235 $cm^{-1}$ and by $^{13}C$-NMR spectrum[6] of 2 which included two singlet resonances for the carbonyl atoms at $\delta$ 169.8 and 170.0. On the other hand, the appearance of two carbon doublets at $\delta$ 106.2 and 103.7 (no other signals attributable to $sp^3$ carbons linked to oxygen atoms are present in the $^{13}C$-NMR spectrum of 2), was consistent with AcO-CH-O groups. Since four of the five oxygen atoms in 2 could be accounted for as the two acetates, these groups must contain the remaining oxygen atom and the partial structure

AcO-CH-O-CH-OAc could be written. The $^{13}$C-NMR spectrum of 2 also showed signals for four olefinic carbons [δ 122.8 (d), 124.1 (d), 134.6 (s) and 155.1 (s)], while in the sp$^3$ region two singlets (δ 31.2 and 38.3), two methyne doublets (δ 46.4 and 57.7), five methylene triplets (δ 19.3, 25.9, 38.3, 39.0 and 52.7) and six methyl quartets (δ 17.7, 21.2, 21.2, 25.5, 27.4 and 35.7) were present. All the assignments reported in the Table were performed by 2D-NMR techniques (heteronuclear direct and long range $^{13}$C-H correlations). From the above data arises that 2, having seven degrees of unsaturation, must posses a tricyclic skeleton. In the $^1$H-NMR spectrum$^6$ of 2, the two acetoxymethyne protons at C-15 and C-16 appeared as doublets at δ 5.99 (J= 1.5 Hz) and 5.86 (J= 1.6 Hz), while the two olefinic protons at C-7 and C-11 resonated as a broad quartet at δ 5.34 (J= 6.9 Hz) and as a double doublet at δ 5.85 (J= 6.9 and 4.0 Hz), respectively. This spectrum also exhibited signals representative of two acetoxymethyls at δ 2.01 and 2.03, a secondary vinyl methyl at δ 1.59 (J= 6.9 Hz) and three Me groups linked to quaternary carbon (δ 1.22, 0.97 and 0.84). The two methyne protons at C-13 and C-14 were seen as a double double double doublet at δ 2.75 (J= 9.0, 6.5, 1.8 and 1.6 Hz) and a double doublet at δ 3.22 (J= 9.0 and 1.5 Hz), broadened by long range coupling with 7-H, respectively, whereas two one proton double double doublets at δ 1.76 (J= 14.8, 6.5 and 4.0 Hz) and 2.25 (J= 14.8, 6.9 and 1.8 Hz) were assigned to the methylene group at C-12. These attributions, which were confirmed by extensive spin decoupling experiments, allowed to establish the presence in the molecule of the partial structure A. The whole structure of 2, apart from the stereochemistry, was deduced on the basis of the following criteria. The remaining part of the molecule must comprise two quaternary carbon atoms, four methylene groups and three methyls, two of them linked to the same carbon. Since no allylic protons (apart from the above mentioned methylene protons at C-12) were present in the $^1$H-NMR spectrum of 2, C-9 must be adjacent to a quaternary carbon atom. Moreover a further analysis of a 500 MHz $^1$H-NMR spectrum of 2 allowed us to establish that a methylene group must be attached to the two quaternary carbon atoms, since its protons resonated as an AB system centered at δ 1.40 ($J_{AB}$ = 14.1 Hz). These requirements are only compatible with part structures B or C. Structure C could be easily ruled out on the basis of the chemical shifts of the methylene protons in the $^1$H-NMR spectrum, since cyclobutyl protons are generally observed at lower fields. Moreover, mass spectrum of 2 did not exhibit any of the fragment ion which could be expected by loss of $C_6H_{11}$ through an allylic fission if part structure C was present in the molecule, whereas an intense peak at m/z 125 ($C_9H_{17}$,68%) was present.

Additional evidence to confirm structure 2 was also obtained by LAH reduction to the expected diol 3, m.p. 133-134° (from hexane), $[\alpha]_D$ -189.9° (c= 0.8, $CHCl_3$), m/z 292 ($M^+$), $\lambda_{max}^{MeOH}$ 233 nm (ε =9470), $\nu_{max}$($CHCl_3$) 3500-3100 cm$^{-1}$ (OH). $^1$H-NMR: δ 5.67 (1H, dd, J = 4.9 and 3.0 Hz, 11-H), 5.37 (1H, bq, J = 7.1 Hz, 7-H), 3.57 (2H, AB part of an ABX system superimposed on 16-$H_2$ signal, $J_{AB}$ =11.2 Hz, 15 $H_2$), 3.55 (2H, d, J = 6.5 Hz, 16-$H_2$), 2.59 (1H, X part of an ABX system, 14-H), 2.20 (2H, m, 13-H and 12-$H_a$), 1.89 (1H, ddd, J= 16.5, 11.0 and 4.9 Hz, 12-$H_b$),



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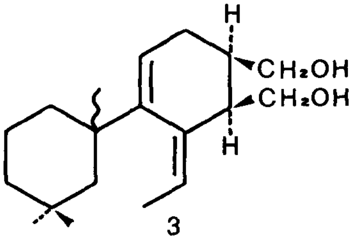

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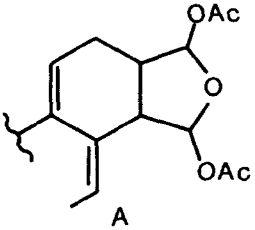

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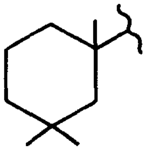

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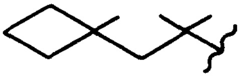
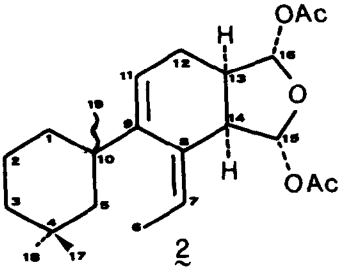


C

Table - Nuclear Magnetic Resonance Data for gracilin A

| $\delta\ ^{13}C$ | assignment | $\delta\ ^{1}H$ |
|---|---|---|
| 38.3$^{a}$ (t) | 1a | 1.35* m$^{c}$ |
| | 1b | 1.07 m |
| 19.3 (t) | 2 | 1.46* m |
| 39.0$^{a}$ (t) | 3a | 1.61 m$^{c}$ |
| | 3b | 1.32* m |
| 31.2 (s) | 4 | |
| 52.7 (t) | 5a | 1.31* |
| | 5b | 1.49* |
| 17.7 (q) | 6 | 1.59 (d) |
| 122.8 (d) | 7 | 5.34° (q) |
| 134.6 (s) | 8 | |
| 155.1 (s) | 9 | |
| 38.3 (s) | 10 | |
| 124.1 (d) | 11 | 5.85°° (dd) |
| 25.9 (t) | 12a | 2.25 (ddd) |
| | 12b | 1.76 (ddd) |
| 46.4 (d) | 13 | 2.75 (dddd) |
| 57.7 (d) | 14 | 3.22°° (dd) |
| 103.7 (d) | 15 | 5.99 (d) |
| 106.2 (d) | 16 | 5.86 (d) |
| 27.4$^{b}$ (q) | 17 | 0.84$^{d}$ (s) |
| 35.7$^{b}$ (q) | 18 | 0.97$^{d}$ (s) |
| 25.5 (q) | 19 | 1.22 (s) |
| 21.2 and 21.2 (q's) | $\underline{C}H_3CO-$ | 2.01 and 2.03 (s's) |
| 169.8 and 170.0 (s's) | $-\underline{C}OCH_3$ | |



2

J(Hz) 5a-5b =14.1; 6-7 =6.9;
11-12a =6.9; 11-12b = 4.0;
12a-12b =14.8; 12a-13 =1.8;
12b-13 =6.5; 13-14 =9.0;
13-16 =1.6; 14-15 =1.5.

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* superimposed on other signals.

a-d Values with identical superscript within each column may be interchanged.

° broadened by long range coupling with 14-H and 11-H.

°° broadened by long range coupling with 7-H.

1.68 (3H, d, J= 7.1 Hz, 7-Me), 1.39 (2H, AB system superimposed on other signals, $J_{AB}$ =12.5 Hz,5-$H_2$ ),1.24 (3H, s, 10-Me), 0.97 and 0.84 (3H each, s's, 4-Me's). $^{13}$C-NMR: δ 149.6 (s,C-9),139.4 (s, C-8), 125.1 (d, C-11), 120.7 (d, C-7), 65.3 and 61.7 (t's, $\underline{C}H_2$OH groups), 52.9 (t, C-5), 52.2 (d, C-14), 41.4 (d, C-13), 39.4 (t, C-1 or C-3), 38.5 (s, C-10), 37.9 (t, C-3 or C-1), 34.9 (q, C-18 or C-17), 31.4 (s, C-4), 28.2 (q, C-17 or C-18), 26.9 (t, C-12), 26.7 (q, C-19), 19.8 (t, C-2),and 18.4 (q, C-6).

Assignment of Z configuration to C-7 double bond of 2 was made by application of nuclear Overhauser effect; a NOE was registered for the 7-H signal while no detectable effect was observed for the 7-Me when the 14-H signal was saturated in a difference NOE experiment. The presence of NOE between 14-H and 13-H allowed us to establish the cis-relationship for these two protons, whereas the relative configuration of C-15 and C-16 was deduced from the values of the coupling constants between 14-H and 15-H (J= 1.5 Hz) and 13-H and 16-H (J= 1.6 Hz), respectively, through an examination of the molecular model (Dreiding).

Studies to establish the biogenesis of 2, as well as to assign the configuration to C-10 and the absolute stereochemistry to the molecule are currently in progress.

## References and Notes

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6. The $^1$H- and $^{13}$C-NMR spectra were recorded on a Bruker WM 250 and 500 MHz instruments. δ values ($CDCl_3$) are in ppm from TMS.

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