

APPLICATION OF 2D-NMR SPECTROSCOPY IN THE STRUCTURAL DETERMINATION OF GRACILIN B,
A BIS-NOR-DITERPENE FROM THE SPONGE SPONGIONELLA GRACILIS.¹

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Summary. The structure of gracilin B, an unusual metabolite based on a bis-nor-diterpene skeleton isolated from the sponge Spongionella gracilis, has been determined mainly by using two dimensional heteronuclear ¹³C - ¹H shift correlation spectroscopies.

Nor-diterpenes and bis-nor-diterpenes are very rare metabolites from marine origin since only a few examples can be found in the literature²⁻⁵. In the course of our survey on the constituents of mediterranean invertebrates, we recently isolated gracilin A (1), a nor-diterpene diacetate from the sponge Spongionella gracilis⁶; we wish to report here that this organism also produces gracilin B (2), a highly oxygenated metabolite which, to the best of our knowledge, represents the first bis-nor-diterpene observed from a marine sponge. The new compound (2), [α]_D +191.0 (c = 1.0, CHCl₃), m.p. 167-168°, $\lambda_{\max}^{\text{MeOH}}$ = 296 (ε = 20150), was obtained in a 2.6% yield from the methanol-chloroform 1:1 extract of the sponge S. gracilis, collected in the Bay of Napoli, by repeated chromatographies over silica gel using dichloromethane and increasing amounts of diethyl ether in n-hexane as eluent. Its molecular formula C₂₂H₂₈O₈ was deduced by HRMS⁷. The mass spectrum showed a weak molecular ion at m/z 420 (8%) with strong peaks at m/z 360 (M⁺-AcOH, 28%), 318 (M⁺-AcOH-CH₂CO, 100%) and 300 (M⁺-2AcOH, 14%), indicating a facile loss of two acetate groups. The infrared spectrum (CS₂) contained bands at 1760, 1745 and 1630 cm⁻¹. ¹H-NMR spectrum of 2 (250 MHz, CDCl₃) was particularly detailed and by spin decoupling studies the protons at C-1, C-2 and C-3 [δ 1.34 (2H, bt, J = 5.8 Hz, 3-H₂), 1.55 (2H, m, 2-H₂) and 2.19 and 2.40 (1H each, m's, 1-H₂)] and those at C-15, C-10, C-11 and C-16 [δ 3.12 (1H, dd, J = 12.4 and 6.0 Hz, 11-H), 3.95 (1H, dd, J = 12.4 and 6.8 Hz, 10-H), 5.95 (1H, d, J = 6.8 Hz, 15-H) and 6.07 (1H, d, J = 6.0 Hz, 16-H)] were readily inter-related. The protons 7-H and 8-H were seen as an AB system at δ 7.17 (J_{AB} = 12.1 Hz) while 12-H and 13-H resonated as two apparent singlets at δ 5.06 and 6.09, respectively. Signals due to the methylene protons 5-H₂ (δ 2.01, 2H, s), two acetates (δ 1.99 and 2.02, 3H each, s's) and two Me groups linked to quaternary carbon atoms (δ 0.80 and 0.84, 3H each, s's) were also present. Owing to the presence of quaternary

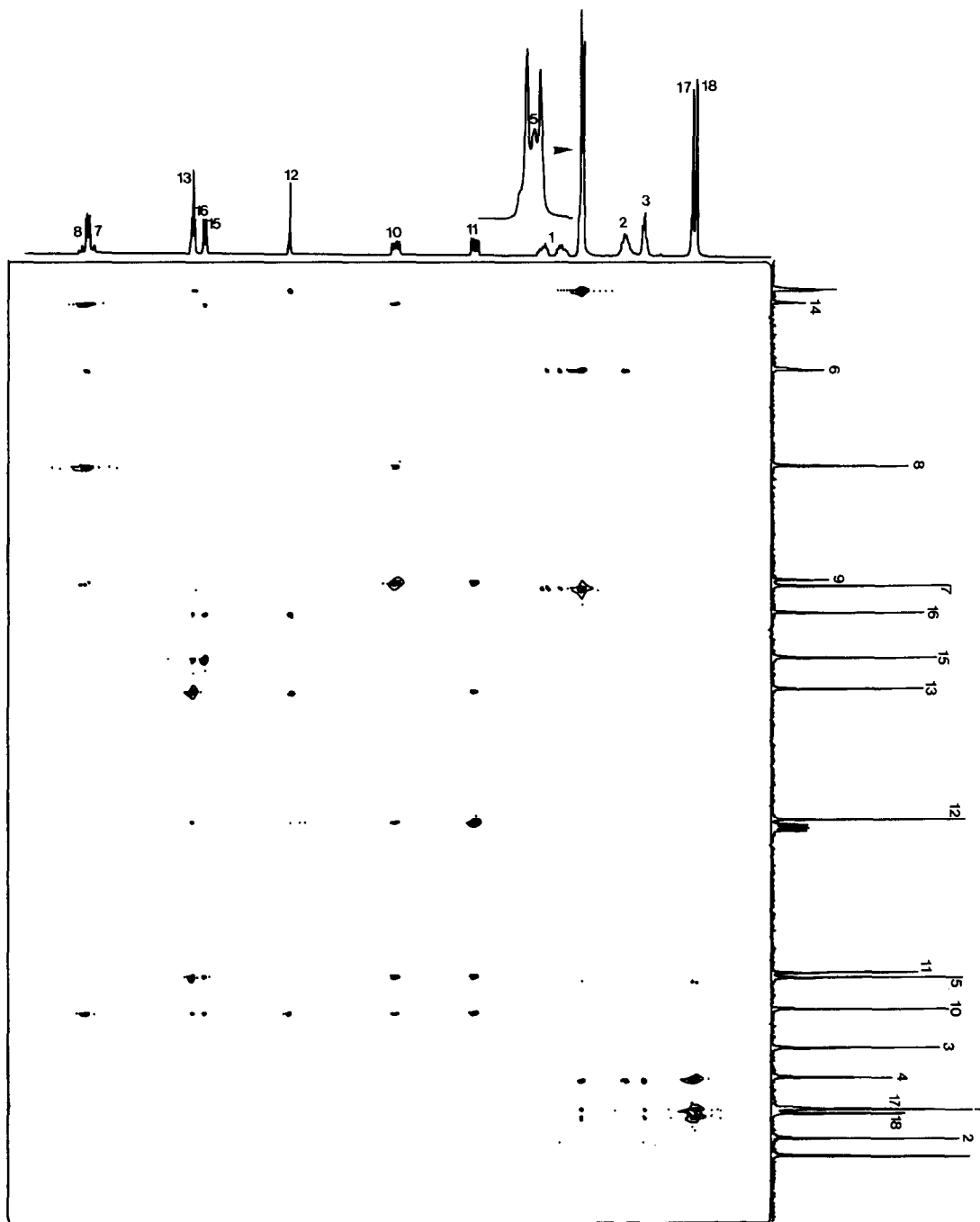
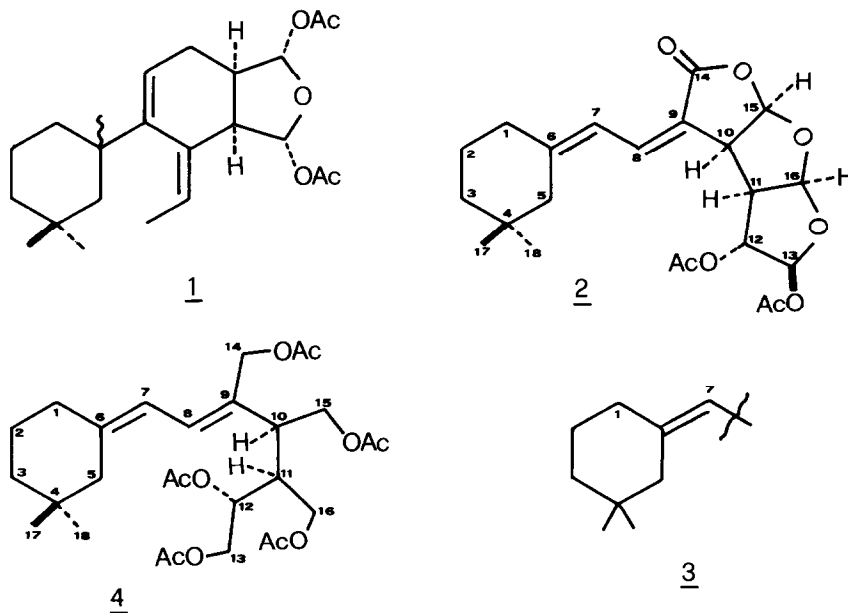


Figure. 62.9 MHz ^{13}C - ^1H long range shift correlated 2D-NMR (WM-250) of 2 in CDCl_3 . The shift correlation with polarization transfer via J-coupling experiment has been carried out with the aid of a Bruker micro-program; fixed delays D_3 and D_4 were adjusted to give maximum polarization for $J_{\text{CH}} = 5.0$ Hz. In the contour plot some peaks due to J^1 correlation are present, indicating a not complete nulling of direct correlations.



carbon atoms in 2 and to the very small, if any, coupling constants of 12-H with 11-H and 13-H, further structural information could not be obtained by $^1\text{H-NMR}$ spectral analysis. Two dimensional $^{13}\text{C}-^1\text{H}$ shift correlated spectrum of 2 allowed the assignment of all the signals due to protonated carbons in the $^{13}\text{C-NMR}$ spectrum of 2⁸ which also comprised resonances attributable to three >C=O groups and three fully substituted carbons, two of them in the sp^2 region.

Of the numerous structural possibilities which incorporated the above data, structure 2 was confidently assigned based upon 2D-long range $^{13}\text{C}-^1\text{H}$ shift correlated spectrum (Figure) which allowed to observe almost all two and three bond $^{13}\text{C}-^1\text{H}$ couplings in the molecule. The basic carbon skeleton was established as follows. The only sp^3 quaternary carbon atom (C-4) was seen to correlate with the protons of the two Me groups, with 5-H₂, 3-H₂ and 2-H₂; on the other hand, the carbons resonating at δ 155.7 (C-6) and at 118.7 (C-7) were shown to be long range coupled with 5-H₂, 1-H₂, 2-H₂ and 7-H and with 5-H₂ and 1-H₂, respectively, thus unequivocally establishing the presence of the substructure 3. The extension of this part structure up to C-14 through C8-C9 was straightforward taking into account UV data and $^1\text{H-NMR}$ spectrum; a confirm came from the presence of a correlation between C-14 and 8-H. This substructure was combined with the above mentioned fragment C15-C10-C11-C16 through the C9-C10 linkage, observing a series of correlation peaks in the 2D-NMR spectrum (Figure), particularly those regarding C-14 with 10-H, C-9 with 10-H and 11-H, C-8 with 10-H and C-10 with 8-H. Ultimately, the positioning of C-12 and C-13 was also made on the basis of long range couplings of C-10 with 12-H, C-12 with 10-H, 11-H and 13-H, C-16 with 12H, and C-13 with 12-H and 11-H.

That the two acetoxy groups were linked to C-12 and C-13 was clearly indicated by long range couplings of the two >C=O groups resonating at δ 169.5 and 169.3, with 12-H and 13-H, respectively. What remained to establish to complete structure 2, was the location of the

last three oxygen atoms, which must be comprised by C-14 and C-15, C-15 and C-16 and C-16 and C-13 to form three five-membered rings, since C-14 was seen to correlate with 15-H, C-15 with 16-H, C-16 with 15-H and 13-H, and C-13 with 16-H.

The correct assignment of structure 2 to gracilin B was secured by its reduction with LAH at room temperature for 2h followed by acetylation which gave the expected pentaacetate 4 as the major product; $[\alpha]_D +3.5$ (c = 0.4, CHCl₃), $\nu_{\max}^{\text{CCl}_4} 1750$ and 1230 cm^{-1} , $\lambda_{\max}^{\text{MeOH}} = 249$ ($\epsilon = 16800$), ¹H NMR spectrum (CDCl₃): δ 6.34 (1H, d, J = 11.7 Hz, 7-H), 5.90 (1H, d, broadened by long range coupling with 14-H₂ and 10-H, J = 11.7 Hz, 8-H), 5.29 (1H, ddd, J = 6.7, 5.0 and 2.6 Hz, 12-H), 4.67 (2H, AB system, broadened by long range coupling with 8-H, J_{AB} = 12.4 Hz, 14-H₂), 4.43 (1H, dd, J = 12.4 and 2.6 Hz, 13-H_a), 4.26 (2H, d, J = 6.2 Hz, 15-H₂), 4.10 (2H, AB part of an ABX system, superimposed to 13-H_b signal, J_{AB} = 11.7 Hz, 16-H₂), 4.09 (1H, dd, superimposed to 16-H₂ signal, J = 12.4 and 6.7 Hz, 13-H_b), 2.73 (1H, dt, broadened by long range coupling with 8-H, J = 9.9 and 6.2 Hz, 10-H), 2.30 (1H, m, 11-H), 2.16 and 2.14 (1H each, m's, 1-H₂), 2.05 (2H, s, 5-H₂), 2.07, 2.04, 2.03, 2.03, and 2.02 (3H each, s's, acetates), 1.54 (2H, m, 2-H₂), 1.34 (2H, t, J = 5.8 Hz, 3-H₂) and 0.87 (6H, s, 17-H₃ and 18-H₃). In the mass spectrum of 4 the parent peak is lacking; diagnostically important peaks at m/z 478 (M⁺-AcOH, 2%), 418 (M⁺-2AcOH, 4%), 376 (M⁺-2AcOH-CH₂CO, 6%), 358 (M⁺-3AcOH, 11%), 316 (M⁺-3AcOH-CH₂CO, 18%), 298 (M⁺-4AcOH, 48%), 256 (M⁺-4AcOH-CH₂CO, 100%) and 238 (M⁺-5AcOH, 100%) are present, indicating the loss of five acetate groups.

The assignment of the overall relative stereochemistry of 2 was accomplished by nOe difference experiments and observing J values in the ¹H-NMR spectrum. The Z configuration of the two double bonds was deduced by the positive nOe registered between 7-H and 1-H₂ and between 8-H and 12-H. The cis relationship among 15-H, 10-H, 11-H and 16-H was also established on the basis of nOe studies. The relative stereochemistry at C-12 and C-13 was based upon an examination of the molecular model taking into account the very small (if any) coupling constants of 12-H with 13-H and 11-H which indicated that the dihedral angles of 12-H with the adjacent protons must be in the range 80-100°.

REFERENCES and NOTES

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7. Mass spectral data were provided by "Servizio di Spettrometria di Massa del C.N.R. e della Università di Napoli".
8. ¹³C-NMR spectrum (CDCl₃): δ 169.5 and 169.3 (2 -COCH₃), 167.0 (C-14), 155.7 (C-6), 139.2 (C-8), 119.8 (C-9), 118.7 (C-7), 113.9 (C-16), 106.2 (C-15), 100.8 (C-13), 78.5 (C-12), 52.0 (C-11), 51.1 (C-5), 45.8 (C-10), 39.2 (C-3), 34.2 (C-4), 28.5 (C-1), 28.7 and 27.8 (C-17 and C-18), 23.5 (C-2), 20.4 and 20.4 (2CH₃CO-).

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