Pergamon

Tetrahedron Letters 40 (1999) 3977–3980

TETRAHEDRON
LETTERS

Hoffmanniolide: a Novel Macrolide from *Prorocentrum hoffmannianum*

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Received 14 January 1999; revised 2 March 1999; accepted 3 March 1999

Abstract: Hoffmaniolide, a novel macrolide, was isolated and identified from the marine dinoflagellate *Prorocentrum hoffmannianum.* Crown copyright © 1999 Published by Elsevier Science Ltd. All rights reserved.

Key words: macrolide, dinoflagellate, *Prorocentrum hoffmannianum.*

Some species of marine dinoflagellates produce a variety of complex bioactive polyethers of the linear, macrocyclic, or ladder-frame type.[1] The linear polyethers, represented by the diarrhetic shellfish poisoning (DSP) toxins, are potent phosphatase PP1 and PP2A inhibitors[2-3] produced by species of the genus *Prorocentrum.* The macrocyclic polyethers, represented by the amphidinolides, are another group of polyketides obtained from an *Amphidinium* species, and have potent cytotoxicity against tumor cells.[4-6] The ladder-frame polyethers possess a fused polyether structure, and include the brevetoxins[7,8] and ciguatoxins[7,9] which are ion channel modulators.[7-9] This paper reports the isolation and structural elucidation of hoffmanniolide **1**, a new macrocyclic polyether similar to the amphidinolides, from the DSP toxin-producing dinoflagellate *Prorocentrum hoffmannianum,*[10] a species that is phylogenetically distinct from *Amphidinium* spp.

During an investigation of DSP toxin distribution in various *Prorocentrum* species, gel permeation chromatography of a butanol-soluble fraction (381.4 mg) of *P. hoffmannianum* yielded a fraction that eluted after the water-soluble sulfated DSP toxins.[11] This non-toxic fraction contained an UV-active spot that gave a distinctive purple-red stain with vanillin/sulfuric acid. Further purification of this fraction by normal phase (Si gel; CH_2Cl_2/MeOH 6:4) and then reversed phase HPLC (C8; 25% MeCN, 75% H_2O, UV det., 210 nm), yielded hoffmanniolide **1** (630 μg).

The molecular formula of **1**, $C_{42}H_{70}O_{17}S$ (M-H 877.4266 ± 0.0011, (n = 7) calc. 877.4255), derived from high resolution liquid secondary ion mass spectrometry (LSIMS) experiments, was supported by the observation of 42 resonances in the ^1H-decoupled ^{13}C NMR spectrum. The 42 carbons were further characterized by ^{13}C-DEPT NMR as 3 quaternary carbons, 21 methines, 16 methylenes and 2 methyl groups, and resonances of directly-bonded protons were identified by 2D $^1H/^{13}C$ HMQC NMR spectra (Table 1). The presence of ten ^{13}C resonances between 100 ppm and 150 ppm suggested five double bonds, and the resonance at 168.2 ppm and IR band at 1716 cm^{-1} indicated the presence of a C=O group. The UV data (λ 235 nm weak shoulder) suggested the carbonyl was an α,β-unsaturated ketone. Together, the double bonds and the carbonyl account for 6 of the 8 degrees of unsaturation in **1** required by the molecular formula, implying the existence of two rings. The presence of a sulfate group was indicated by strong absorption at 1264 cm^{-1} in the IR spectrum,[12] and by the loss of 80 mu ($-SO_3$) and 120 mu ($-NaHSO_4$) from the $[M+Na]^+$ ion of the sodium salt (m/z 923) in LSIMS-MS and –MS/MS spectra. This was confirmed by smooth desulfation of **1** to yield a less polar product[13] (MS: 797 $[M-H]^-$; no IR stretch at 1264 cm^{-1}).

Two major partial structures of **1** were identified by analysis of TOCSY, COSY and HMBC spectral data (Figure 1). Partial structure **a** corresponds to C1 to C23, in which protons at positions 2 – 23 are all in the same TOCSY-defined ^1H spin system. The characteristic low field proton chemical shift of H3 (δ 6.97) and an HMBC relationship between H2 (δ 6.06) and C1 (δ 168.2) suggest the presence of an α,β -unsaturated carbonyl moiety. An E configuration was established based on the NOESY correlations from H2 to H4 and the coupling constant $^3J_{2,3}$ (15.8Hz). Five carbons in **a** exhibited resonances in the range δ_C 60 to 90 ppm, and were ascribed to carbons bearing

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PII: S0040-4039(99)00513-4

oxygen. Four of these (C4, C5, C10 and C22), displayed deuterium-induced upfield ^{13}C chemical shifts (0.09 to 0.15 ppm) upon changing the NMR solvent from CD_3OH to CD_3OD indicating they were linked to hydroxyl groups.[14, 15] The remaining oxygen-bearing carbon C18 was only shifted *ca* 0.02 ppm, and the low-field ^1H NMR chemical shift of H18 (δ 5.10) suggested an acyl link with the sole carbonyl group at C1 to form a macrocyclic ester.

Table 1. ^1H and ^{13}C Assignments for Hoffmaniolide (**1**)

C #	δ ^{13}C (mult.)*	δ ^1H (mult.)†	C#	δ ^{13}C (mult.)*	δ ^1H (mult.)†	C#	δ ^{13}C (mult.)*	δ ^1H (mult.)†
1	168.2 (s)		15	132.5 (d)	5.33 (m)	29	71.7 (d)	3.73 (ddd)
2	122.1 (d)	6.06 (dd)	16	29.4 (t)	2.03 (m), 2.11 (m)	30	36.1 (t)	1.56 (ddd), 1.85(ddd)
3	150.0 (d)	6.97 (dd)	17	35.4 (t)	1.58 (m), 1.73 (m)	31	72.6 (d)	3.79 (ddd)
4	75.7 (d)	4.34 (ddd)	18	73.2 (d)	5.10 (m)	32	73.2 (d)	3.25 (dd)
5	74.0 (d)	3.63 (ddd)	19	43.5 (t)	1.26 (m), 1.72 (m)	33	70.7 (d)	4.43 (dd)
6	37.5 (t)	1.42 (m), 1.54 (m)	20	26.9 (d)	1.63 (m)	34	79.5 (d)	3.94 (dd)
7	29.1 (t)	2.08 (m), 2.24 (m)	21	46.5 (t)	1.21 (m), 1.53 (m)	35	70.6 (d)	3.92 (ddd)
8	131.9 (d)	5.66 (dddd)	22	67.0 (d)	4.41 (ddd)	36	40.8 (t)	1.46 (m), 1.98 (m)
9	134.7 (d)	5.39 (ddt)	23	132.1 (d)	5.21 (m)	37	66.3 (d)	3.96 (m)
10	73.4 (d)	3.96 (m)	24	136.0 (s)		38	41.5 (t)	1.68 (m)
11	31.8 (t)	1.40 (m), 1.53 (m)	25	47.8 (t)	2.77 (bs)	39	60.4 (t)	3.69 (t)
12	25.9 (t)	1.21 (m), 1.36 (m)	26	145.6 (s)		40	20.1 (q)	0.96 (d)
13	33.0 (t)	1.88 (m), 1.97 (m)	27	39.6 (t)	2.04 (dd), 2.33 (dd)	41	16.3 (q)	1.64 (d)
14	130.7 (d)	5.25 (m)	28	74.3 (d)	3.64 (ddd)	42	114.5 (t)	4.87 (bd), 4.92 (bd)

* s = C, d = CH, t = CH_2, q = CH_3; †s = singlet, d = doublet, t = triplet, m = multiplet, b = broad. Spectra were recorded at 500.13 MHz (^1H) and 125.77 MHz (^{13}C) using CD_3OD as solvent. Chemical shifts δ_H and δ_C (ppm) were referred to CHD_2OD = 3.30 ppm (^1H), CD_3OD = 49.0 ppm (^{13}C) respectively.

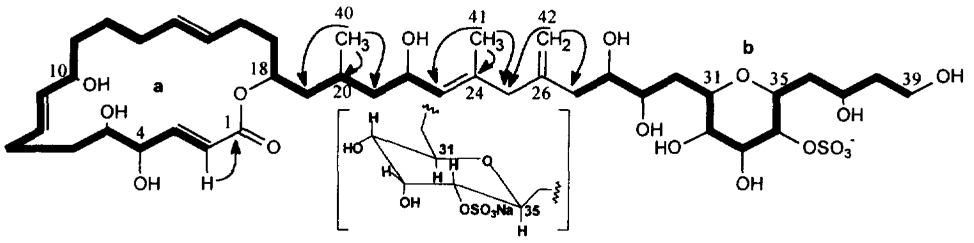

Figure 1. Structure of Hoffmaniolide **1**. Arrows denote HMBC correlations from H to C, bold lines denote spin systems.
Inset: relative stereochemistry of six-membered ring C31-C35 determined from ^1H-^1H spin-spin couplings: ^3J(H31,H32) 9.9 Hz, ^3J(H32,H33) 2.9 Hz, ^3J(H33,H34) 2.6 Hz, ^3J(H34,H35) 9.8 Hz (Error ± 0.3 Hz).

The ester linkage was confirmed by treatment of **1** with methoxide which yielded the expected hydroxy methyl ester ($[M-H]^-$ 909).[16] The COSY correlations H4 (δ 4.34)/H3 (δ 6.97) and H5 (δ3.63)/H4 (δ4.34) extended this structure to C5, adding two secondary alcohols. The connectivity from C5 to C23 was determined in similar fashion and in particular the C8,9 double bond and C10 hydroxy group were determined by HMBC's H7/C5, C6, C8, C-9; H8/C10. The NOESY correlations H8/H10 and H7/H9 together with the ^3J(H8,H9) coupling constant (15.6 Hz) established the double bond configuration as E. The same configuration was found for the C14,15 double bond (NOESY correlation H13/H15: ^3J(H14,H15) 15.5 Hz). The position of this bond was clear from COSY and TOCSY results, which showed three consecutive CH_2 groups between C10 and C14, and two between C15 and C18. The methyl group at C20 was located by HMBC of H40/C19, C20 and C21.

Partial structure **b** corresponds to C27 to C39, in which all protons are in the same TOCSY spin system. From the terminal primary alcohol group at C39 (δ_C 60.4, δ_H 3.69), eight methines and four methylenes are linked in a long chain according to the COSY data. Among the nine oxygen-bearing carbons with δ_C 60 to 90 ppm in **b**, isotopically induced upfield shifts ($\Delta\delta_C$ 0.09 to 0.15 ppm) were found for C28, C29, C32, C33, C37 and C39, indicating they are hydroxyl-bearing carbons. Of the three remaining oxygen-bearing carbons C31, C34 and C35, the sulfate group was placed at C34 based on the low-field resonance (δ_C 79.5 ppm) for this position, and was confirmed by preparation of the desulfated product of **1**. In the ^{13}C and ^{1}H NMR data of desulfated product, the only resonances affected, when compared to the corresponding resonances in **1**, were those for C33, C34, C35 (-2.2, +5.7, -1.9 ppm respectively) and H33, H34, H35 (+0.47, +0.78, +0.24 ppm), in accord with a sulfate group at position 34. Thus C31 and C35 are linked to form a pyran ring, which accounts for the remaining degree of unsaturation in the molecular formula. Of the five remaining carbons unaccounted for, a methyl group (C41) and a terminal double bond (C42) were found to be located on C24 and C26 respectively. The critical HMBC's H41/C23, C24, C25 and H42/C25, C26, C27 clearly linked the methyl group, vinyl group, and partial structures **a** and **b**, and established the planar structure of hoffmanniolide as **1** (Figure 1). The NOESY correlations between H23/H25 and H22/H41 indicated this double bond also possessed an E configuration.

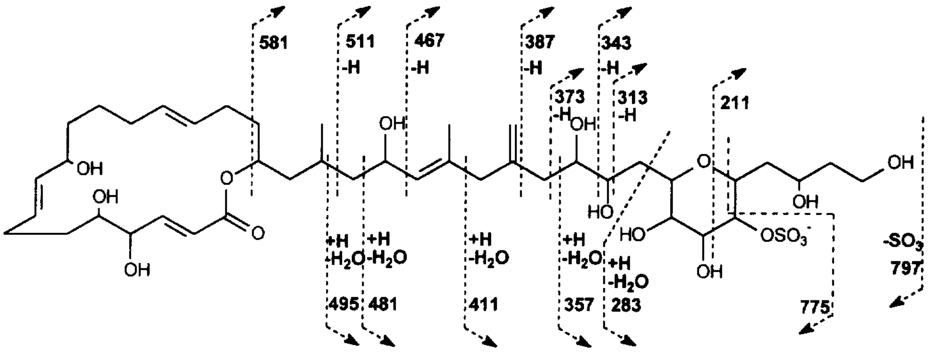

Figure 2. LSIMS-MS/MS spectrum of the [M-H]- ion (m/z 877) of **1**, collision energy 800 eV using Xe as the collision gas.

LSIMS-MS/MS experiments on the $[M-H]^-$ ion (see Figure 2), the $[M+Na]^+$ ion of the sodiated form (m/z 923), as well as the $[M+Na]^+$ ions of the desulfated product (m/z 821) and of the sodiated form of the methanolysis product, (m/z 955), gave supporting evidence for the structure **1**, although little fragmentation through the macrocyclic ester ring was observed.

Dinoflagellates belonging to the prorocentroid and gymnodinoid groups have been found to biosynthesize a variety of polyketide polyethers.[1] These compounds are characterized by a number of novel biosynthetic features including a carbon deletion step and addition of pendant methyl groups by an Aldol mechanism.[17] Until biosynthetic data are available for **1**, any comments are speculative, but the polyketide chain contains an odd number of carbons suggesting a deletion step, and the primary alcohol group at C39 suggests a glycolate starter unit as found in the co-metabolites DTX-5a and 5b. Indeed, the identification of hoffmanniolide as a macrocyclic polyether lactone **1** from *P. hoffmannianum* illustrates the biosynthetic capability of this genus to produce either linear or macrocyclic polyethers. Interestingly, **1** is structurally similar to the amphidinolide macrocylic lactones from *Amphidinium* species,[4-6] a phylogenetically distinct group of dinoflagellates. Thus both families of organisms have acquired very similar biosynthetic capabilities during their evolutionary development.

In contrast to the structurally-similar amphidinolides,[4-6] hoffmaniolide **1** showed no evidence of cytotoxicity up to 100 μg/ml, but in view of the structural diversity of these compounds it is not possible at present to attribute this difference to any particular combination of structural features.

Acknowledgements

We thank Mr. Ian Burton of IMB, NRC for recording and discussing NMR data, Dr Mark Ragan of IMB, NRC for helpful discussions, Dr Don Tindall of the Department of Plant Biology, Southern Illinois University, Carbondale IL, for providing the biomass of *Prorocentrum hoffmannianum*, and Dr. Jonathan Blay of Department of Pharmacology, Dalhousie University, Halifax NS for conducting cytotoxicity studies. Issued as NRCC publication number 42288.

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13. Desulfation reaction: Hoffmanniolide **1**, (400 μg) was refluxed at 120°C in dioxane/pyridine for 1.0 h. The reaction mixture was dried in a stream of nitrogen, the residue re-dissolved in methanol, and purified by HPLC (Zorbax, RX-C8 column eluting with MeCN/H_2O 35:65, UV det., 210nm) to give the desulfated product of **1**: IR $\upsilon max/cm^{-1}$ 3275, 2921, 1651, 1577, 1416, 1010: LSIMS-MS: 797 $[M-H]^-$, LSIMS-MS/MS 779, 713, 695, 648, 501, 389; ^{13}C NMR (CD_3OD, 125 MHz) all δ_C within 0.2 ppm of corresponding shifts for **1** except 73.5 (C32, d), 72.8 (C33, d), 73.6 (C34, d), 72.6 (C35, d), 41.4 (C36, t); ^1H NMR (CD_3OD, 500 MHz): all δ_H within 0.01 ppm of **1** except 3.74 (1H, H31), 3.22 (1H, H32), 4.00 (1H, H33), 3.20 (1H, H34), 3.74 (1H, H35), 1.42, 1.90 (2H, H36).
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16. Methoxide reaction: Compound **1** (180 μg) was added in CH_3ONa solution (250 μl, 1.1 M in absolute methanol). After 2 h, the mixture was neutralized with HOAc, evaporated to dryness, and the products separated by reversed phase HPLC (Zorbax RX-C8, MeCN/H_2O 25:75). Negative LSIMS of the main reaction product gave a peak at $[M-H]^-$ 909, 32 Da more than that of **1**, consistent with ring-opening and formation of the hydroxy methyl ester product.
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