Table I. Magnetic Susceptibility, χ_M, of $Cp_2Co_2(\mu\text{-}NO)_2$[6]

T (K)	χ_M (emu mol^{-1})	μ_{eff} (in μ_B)[a]	H_0 (kG)
9	12.9×10^{-4}	0.30	5
9	8.45×10^{-4}	0.26	40
281	-2.35×10^{-6}		5
281	3.32×10^{-6}	0.085	40

[a] This value is calculated by using the equation, $\mu_{eff} = 2.823 (\chi_M T)^{1/2}$.

The conclusion is inescapable, $Cp_2Co_2(\mu\text{-}NO)_2$ has a diamagnetic ground state.

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Registry No. $Cp_2Co_2(\mu\text{-}NO)_2$, 51862-20-5.

Mycalamide A, an Antiviral Compound from a New Zealand Sponge of the Genus *Mycale*

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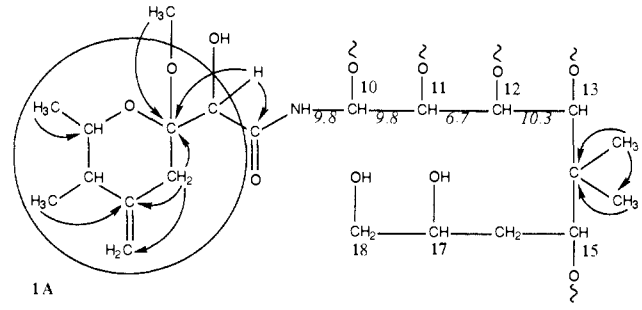
Many sponge metabolites with in vitro biological activities have been identified,[1] but very few have been reported with in vivo antitumor or antiviral activity.[2,3] We now report the bioactivity-directed isolation and structure determination of mycalamide A (**1**), from a sponge extract with in vivo antiviral properties.

In our screening of New Zealand marine invertebrates, an extract of a sponge of the genus *Mycale*[4] from the Otago Harbour showed promising in vitro antiviral activity.[5] Reverse phase flash chromatography[6] on a larger scale extract (11.0 g from 200 g of sponge) concentrated the bioactivity into a brown oil (307 mg) with significant in vivo antiviral activity.[7] Gel permeation and silica gel chromatography on a subsample of this material (140 mg) gave mycalamide A (**1**, 1.7 mg),[8] a new compound with strong in vitro antiviral activity.[9]



1A

Figure 1. Connectivities from NMR experiments with long-range HETCOR linkages and selected proton-proton coupling constants (in Hz) shown.

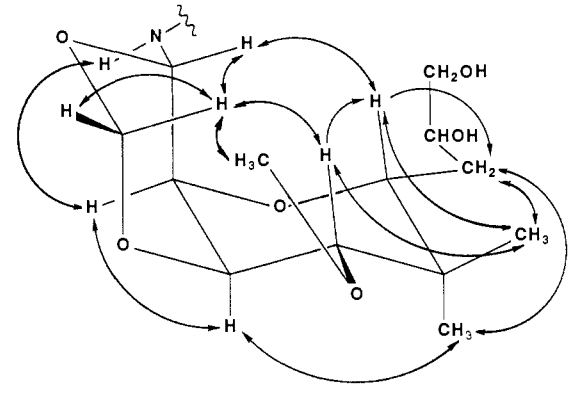
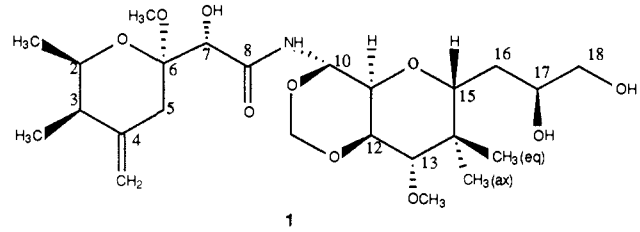


Figure 2. Configuration of the central region of mycalamide A (**1**) showing NOE interactions.



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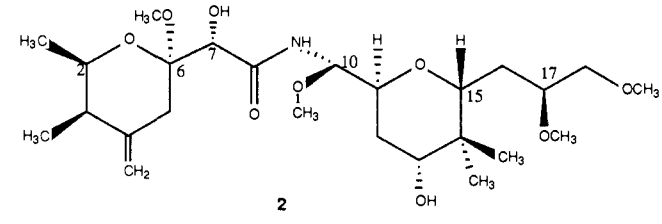

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(7) This material, ca. 2% mycalamide A (**1**), was tested in mice infected with A59 coronavirus: four mice dosed with virus, and the extract at 0.1 mg/kg survived 14 days; eight mice dosed with virus only all died within 8 days.

(8) Mycalamide A (**1**), an oil, $[\alpha]_{365}$ +110° (*c* 0.2, $CHCl_3$): IR (film) 3700-3100, 2960, 1740, 1700, 1540, 1470, 1390, 1100, 1080, 1040 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.49 (NH9, d, 9.8), 5.87 (H10, t, 9.8), 5.13 (10-O-CH_2, d, 6.9), 4.87 (10-O-CH_2, d, 6.9), 4.84 (4=CH_2, m), 4.73 (4=CH_2, m), 4.30 (H7, s), 4.22 (H12, dd, 6.7, 10.3), 3.98 (H2, dq, 2.7, 6.6), 3.86 (H11, dd, 6.7, 9.8), 3.74 (H17, m), 3.60 (H15, dd, 4.0, 5.5), 3.55 (13-O-CH_3, s), 3.55 (H18, m, hidden), 3.46 (H13, d, 10.3), 3.38 (H18, dd, 6.2, 11.2), 3.29 (6-O-CH_3, s), 2.36 ($H_2$5, m), 2.24 (H3, dq, 2.7, 7.0), 1.54 ($H_2$16, m), 1.19 (2-CH_3, d, 6.6), 0.99 (3-CH_3, d, 7.0), 0.98 (14-CH_3(eq), s), 0.87 (14-CH_3(ax), s) ppm (couplings in Hz); ^{13}C NMR ($CDCl_3$) δ 171.52 (C8), 145.40 (C4), 110.41 (4=CH_2), 99.66 (C6), 86.71 (10-O-CH_2), 79.01 (C13), 78.91 (C15), 74.30 (C12), 73.62 (C10), 72.77 (C7), 71.51 (C17), 71.16 (C11), 69.70 (C2), 66.41 (C18), 61.75 (13-O-CH_3), 48.88 (6-O-CH_3), 41.61 (C14), 41.31 (C3), 33.70 (C5), 31.95 (C16), 23.10 (14-CH_3(eq)), 17.89 (2-CH_3), 13.51 (14-CH_3(ax)), 12.03 (3-CH_3).

(9) The minimum dose of mycalamide A (**1**) that inhibited the cytopathic effect of either test virus[5] over a whole (17 mm) well was 5 ng/disk. No in vivo antiviral results on pure mycalamide A (**1**) have yet been obtained, but in vitro assays showed that it was responsible for the in vitro activity of the crude extract and thus probably the in vivo activity as well.[7]

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HREIMS on mycalamide A (**1**) showed a weak molecular ion at 503.27220 daltons corresponding to a molecular formula of $C_{24}H_{41}NO_{10}$ (calculated 503.27305, −1.7 ppm), consistent with the ^1H and ^{13}C NMR data.[8] A DEPT NMR experiment showed 37 protons attached to carbon atoms, while CIMS using ND_3 as the reagent gas[10] confirmed the presence of four exchangeable protons. A one-proton doublet at δ_H 7.49 ppm, which exchanged slowly with D_2O, together with an IR absorption at 1700 cm^{-1} and a quaternary carbon at δ_c 171.52 ppm, indicated a secondary amide. The other three exchangeable protons were therefore present in hydroxyl groups. The NMR spectra showed only one other double bond, a 1,1'-disubstituted carbon–carbon double bond (δ_c 110.41, 145.40 ppm). The remaining unsaturation required by the molecular formula had to be satisfied by three rings.

A recollection of this active *Mycale* species allowed the isolation of enough mycalamide A (**1**, 10 mg) to solve its structure by a combination of HETCOR, COSY, long-range HETCOR (Figure 1) and difference NOE experiments.[11] These results, and consideration of chemical shifts,[8] led to the connectivities shown in Figure 1, with only a methoxyl group and a dioxymethylene group remaining unconnected. A search[12] on the substructure 1A (Figure 1) retrieved pederin (**2**)[13] and related compounds. The ^1H NMR shifts of the region of pederin (**2**) from C2 to C7[14] matched closely those for the corresponding protons in mycalamide A (**1**),[8] thus establishing the structure and relative stereochemistry of this region.[15] Comparison of the rest of the substructure in Figure 1 with pederin (**2**) showed that the same length carbon chain was present but with a different substitution pattern. The different vicinal substituents at C17 and C18 (methoxyl groups in pederin (**2**), hydroxyl groups in mycalamide A (**1**)) were shown by the sharpening of the H17 and H18 NMR signals on D_2O-exchange and confirmed by the chemical shifts of C17 and C18.[16]

The central section of mycalamide A (**1**) had to contain two rings, a methoxyl, a dioxymethylene group in a six-membered or larger ring,[17] and no hydroxyls. These constraints allowed a number of trial structures, but only that shown in Figure 2 satisfied the geometric requirements of the coupling constants (Figure 1) and the NOE results. This structure contained C11 to C15 in a tetrahydropyran ring as in pederin (**2**), with the dioxymethylene group attached to C12 and C10 forming an unusual 2,4,7-trioxadecalin.[18] Further work is under way to establish the absolute stereochemistries of C2 to C7, C10 to C15, and C17 (drawn as for pederin (**2**)[13] for convenience).

It is quite remarkable that pederin (**2**) and related compounds, isolated from the terrestrial beetle *Paederus fuscipes*,[13,19] are the only previously known compounds with structures similar to mycalamide A (**1**), isolated from a marine sponge. However, within weeks of the structural assignment described here, the closely related structure of a Japanese sponge component onnamide A was established independently.[20] It is not yet known whether mycalamide A (**1**) is a sponge metabolite, produced by a symbiotic organism or accumulated from a dietary source.[21] Experiments to explore this point are under way.

Acknowledgment. Research support from the Harbor Branch Oceanographic Institution, Inc. (HBOI) and SeaPharm, Inc. is gratefully acknowledged. We thank Professor J. Jillett and his staff at the Portobello Marine Laboratory, University of Otago, for assistance with collections; Professor P. R. Bergquist, University of Auckland, and Dr. C. N. Battershill for sponge identifications; V. L. Calder, G. Barns, Dr. M. Lui (HBOI), and Dr. S. Cross (HBOI) for bioassays; Dr. S. Bloor, D.S.I.R., Wellington, for rotation measurements; and the Canterbury Medical Research Foundation and the New Zealand University Grants Committee for equipment.

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Isolation and Structure Elucidation of Onnamide A, a New Bioactive Metabolite of a Marine Sponge, *Theonella* sp.

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Marine sponges of the genus *Theonella* have been shown to elaborate diverse chemical structures with interesting biological activities.[1] We have recently described the isolation of misakinolide A, a dimeric 40-membered lactone having antitumor activity from a species of *Theonella*.[1a] In our screening for bioactivity in marine organisms occurring in Okinawan waters, another species of *Theonella* gave an extract showing antiviral activity. Bioassay-guided separation led to the isolation of an active constituent, onnamide A (**1**)[2] which belonged to a class of metabolites new to *Theonella* species. We herein report the isolation and structure elucidation of onnamide A (**1**).

A sample (7.5 kg) of *Theonella* sp.[3] was extracted by steeping in methanol. Evaporation gave an aqueous suspension which was

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