Clavosolides A and B, Dimeric Macrolides from the Philippines Sponge *Myriastra clavosa*

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A specimen of the sponge *Myriastra clavosa* from the Philippines contained two dimeric macrolides, clavosolides A and B. Clavosolide A is a symmetrical dimer, while clavosolide B is rendered unsymmetrical by the replacement of one of the methoxyl groups by a hydroxyl group. The structures of the clavosolides were elucidated by interpretation of spectroscopic data.

Marine sponges have provided a seemingly inexhaustible supply of bioactive metabolites.[1] In our continuing search for cytotoxic agents from marine sponges, we have screened many hundreds of sponges from the Philippines in a cell-line panel that is used to assign a priority to crude extracts based on efficacy and selectivity. Although the crude extracts of *Myriastra clavosa* had been assigned a high priority in the cell-line panel, we were initially hesitant to pursue this lead due to the report by Fu et al.[2] that *M. clavosa* contained the potent cytotoxins clavosines A−C, which are related to the calyculins. However, it was obvious from the ¹H NMR spectrum that the crude extract contained a suite of unusual metabolites. In this paper we report the isolation and structural elucidation of two interesting, albeit noncytotoxic, dimeric macrolides, clavosolides A (**1**) and B (**2**).
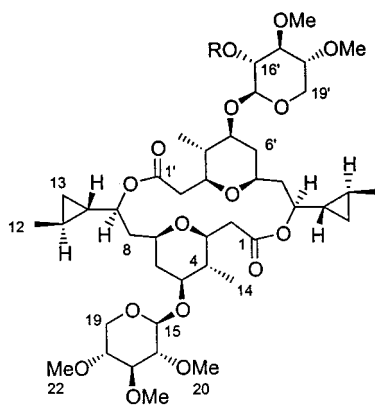
1 R = Me
2 R = H

The sponge *Myriastra clavosa* Ridley 1884 (order Astrophorida, family Ancorinidae) was collected by hand using scuba at a depth of 10−15 m near Boracay Island in the Philippines and was kept frozen until it was extracted with methanol. The ethyl acetate soluble material from the methanol extract was chromatographed on Sephadex LH-20 and silica gel to obtain two fractions containing ¹H NMR signals in the cyclopropyl region of the spectrum. These fractions were further purified by HPLC using hexane−EtOAc mixtures as eluants to obtain clavosolides A (**1**, 4.3 × 10⁻³ % wet wt) and B (**2**, 1.4 × 10⁻³ % wet wt).

Table 1. ¹³C and ¹H NMR Data for Clavosolide A (**1**)

C#	δ_C	δ_H	mult, *J* (Hz)	HMBC	ROESY
1,1′	170.7				
2,2′	39.3	2.55	dd, 17.5, 3	C-1, C-3, C-4	H-14, H-4
		2.41	dd, 17.5, 7	C-1, C-3, C-4	
3,3′	77.0ᵃ	3.42	m	C-1	
4,4′	42.6	1.38	m	C-3, C-5, C-6	H-2
5,5′	83.1	3.25	t, 11.5	C-14, C-15	H-3, H-7, H-15
6,6′	40.8	2.05	dd, 11.5, 5	C-4, C-5	H-5, H-7, H-8 (1.88)
		1.37	q, 11.5	C-5, C-7	
7,7′	74.8	3.42	m		
8,8′	41.1	1.87	dt, 15, 9	C-7, C-9	
		1.66	br d, 15		H-6 (2.05)
9,9′	77.1ᵃ	4.41	br t, 9	C-1′, C-8, C-10	H-7, H-11, H-13 (0.33)
10,10′	24.8	0.72	tt, 9, 5	C-11, C-12	
11,11′	12.0	0.83	m		H-9
12,12′	18.6	0.96	d, 3 H, 6.5	C-9, C-10, C-11	H-13 (0.22)
13,13′	11.0	0.22	dt, 8, 5	C-9, C-11, C-12	H-12
		0.33	dt, 8, 5	C-9, C-10, C-12	H-9
14,14′	12.7	0.96	d, 3 H, 6.5	C-4, C-5	H-3, H-5, H-2 (2.55), H-20
15,15′	105.4	4.27	d, 8	C-16	H-17
16,16′	83.8	2.96	t, 8	C-15, C-17, C-20	H-19 (3.10)
17,17′	85.6	3.12	t, 8	C-16, C-18, C-21	H-15
18,18′	79.4	3.25	td, 8, 5	C-17, C-19, C-22	H-19 (3.10)
19,19′	63.2	3.96	dd, 11, 5	C-15, C-17, C-18	
		3.10	dd, 11, 8	C-15, C-18	H-16, H-18
20,20′	60.7	3.57	s, 3 H	C-16	H-14
21,21′	60.8	3.62	s, 3 H	C-17	
22,22′	58.5	3.47	s, 3 H	C-18	

ᵃ Assignment may be reversed.

Clavosolide A (**1**), [α]$_D$ −48.5° (*c* 1, CHCl₃), was isolated as a viscous oil that was slightly greenish in color due to the high chlorophyll content of the extract. The molecular formula of clavosolide A (**1**), C₄₄H₇₂O₁₆, was established from a high-resolution FABMS measurement of the [M + Na]⁺ peak at *m/z* 879.4709. Since the ¹³C NMR spectrum (Table 1) contained only 22 signals, it was apparent that **1** was a symmetrical dimer. The only significant band in the IR spectrum was at 1730 cm⁻¹ (ester). The lack of a hydroxyl band implied that all 16 oxygen atoms were involved in ester or ether linkages. The COSY spectrum was extremely helpful in interpreting the ¹H NMR spectrum (Table 1), despite the fact that the Me-12 and Me-14 signals overlapped exactly and the H-3 and H-7 signals were at the same chemical shift. Nonetheless, two contiguous sequences of signals from H₂-2 to Me-12, H₂-13, and Me-14 and from H-15 to H₂-19 could easily be distinguished. The geminal coupling constant between the H-2 signals at δ 2.41 and 2.55 was 17.5 Hz, indicating that the methylene group was adjacent to the ester carbonyl, which

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was confirmed by the HMBC correlation between H-2 and C-1 (δ 170.7). The H-2 signals showed HMBC correlations to a signal at δ 77.0, which was assigned to C-3, and a methine signal at 42.6 (C-4). The H-4 signal at δ 1.38 was coupled to the H-3 signal at 3.42, the Me-14 signal at 0.96, and the H-5 signal at 3.25, which was in turn coupled to the H-6 signals at 1.37 and 2.05. The second signal at δ 3.42 was assigned to H-7 and was coupled to the H_2-6 and H_2-8 signals. Linking C-3 and C-7 with an ether oxygen and placing the substituents at C-3, C-4, C-5, and C-7 in the equatorial conformation creates an almost symmetrical tetrahydropyran ring system and helps explain the overlap of both the H-3 and H-7 signals at δ 3.42 and the H-4$_{ax}$ and H-6$_{ax}$ signals at 1.38/1.37. Further support for this assignment was provided by the ROESY correlations from the Me-14 signal to H-3, H-5, and the H-2 signal at δ 2.55, from the H-5 signal to the overlapping H-3 and H-7 signals, and from the H-6$_{eq}$ signal to H-5, H-7, and the H-8 signal at 1.66. The H_2-8 signals were coupled to the H-9 signal at δ 4.41, the chemical shift of which suggested attachment of the ester oxygen at that position: this was confirmed by the HMBC correlation from H-9 to the C-1' carbonyl signal. The H-9 signal was coupled to the H-10 cyclopropyl signal at δ 0.72, which was in turn coupled to signals at 0.22 (H-13), 0.33 (H-13), and 0.83 (H-11), which was coupled to the methyl signal at 0.96. Interpretation of the coupling constant and ROESY data indicated that the stereochemistry about the cyclopropyl group was *trans*. The H-9 signal showed ROESY correlations to H-7, the H-8 signal at δ 1.66, H-11, and the H-13 signal at 0.33. Since $J_{9,10} = 8$ Hz and there are no ROESY correlations between H_2-8 and the cyclopropyl protons, we propose that the cyclopropyl group is oriented such that H-7 and H-9 are on the same side of the macrolide ring and the cyclopropyl methylene is directed toward the ester group with the H-9/H-10 dihedral angle >160°. A molecular model of clavosolide A (**1**) supports this analysis.[3]

The substituent attached at C-5 was shown to be a 2,3,4-trimethoxy-β-xylose moiety. The site of attachment was defined by the HMBC correlation from H-5 to the anomeric carbon at δ 105.5 (C-15) and a ROESY correlation from H-5 to H-15. The COSY experiment revealed a contiguous series of signals from H-15 to H_2-19, and the HMBC correlations from H_2-19 to C-15 defined the pyranose ring system. The locations of the three methoxyl groups were also assigned using the HMBC correlations. The stereochemistry was determined by interpretation of vicinal coupling constants and ROESY correlations. The appearance of the H-16 and H-17 signals as triplets ($J = 8$ Hz) requires the axial conformation for H-15, H-16, H-17, and H-18. This conformation was confirmed by the presence of ROESY correlations from H-15 to H-17 and H-19$_{ax}$ and from H-16 to H-18. Molecular modeling was again useful in assigning the relative stereochemistry between the macrolide and sugar rings. The axial methyl group at C-4 prevents rotation about the C(5)−O bond and strongly favors a conformation in which the O−C(15) bond points away from the C-4 methyl group. Similarly, the potential interaction between H-6$_{eq}$ and the methoxyl group at C-16 results in a preference for the conformation in which the sugar ring oxygen is situated closest to H-6$_{eq}$. Since there are NOEs between H-15 and H-5 (it cannot be to H-18, which occurs at the same chemical shift value) and between H-14 and H-20, the relative configuration must be 5S,15S or 5R,15R and the absolute configuration of the dimer could in principle be determined by knowing the absolute configuration of the sugar moiety. Attempts to obtain the sugar

Table 2. ^{13}C and ^{1}H NMR Data for Clavosolide B (**2**)

C#	δ_C	δ_H	mult, J (Hz)
1,1'	170.5		
2,2'	39.4	2.55	dd, 17.5, 3
		2.41	dd, 17.5, 7
3,3'	76.9	3.42	m
4	42.7	1.38	m
4'	42.5	1.38	m
5	83.1	3.23	m
5'	82.9	3.29	m
6	40.8	2.05	dd, 11.5, 5
		1.37	q, 11.5
6'	40.7	2.05	dd, 11.5, 5
		1.37	q, 11.5
7,7'	74.9	3.42	m
8,8'	41.4	1.87	dt, 15, 9
		1.66	br d, 15
9,9'	77.2	4.41	br t, 8
10,10'	25.0	0.72	tt, 10, 5
11,11'	12.2	0.83	m
12,12'	18.8	0.96	d, 3 H, 6.5
13,13'	11.2	0.22	dt, 8, 5
		0.33	dt, 8, 5
14	12.9	0.96	d, 3 H, 6.5
14'	13.2	0.95	d, 3 H, 6.5
15	105.3	4.26	d, 8
15'	104.5	4.36	d, 8
16	83.8	2.96	t, 8
16'	72.9	3.40	t, 8
17	85.6	3.11	t, 8
17'	83.3	3.20	t, 8
18,18'	79.4	3.25	td, 8, 5
19	63.2	3.96	dd, 11, 5
		3.10	dd, 11, 8
19'	62.5	4.04	dd, 11, 5
		3.25	dd, 11, 8
20	60.8	3.58	s, 3 H
21	60.2	3.62	s, 3 H
21'	60.8	3.61	s, 3 H
22	58.5	3.47	s, 3 H
22'	58.8	3.47	s, 3 H

moiety as its methyl acetal by treatment of **1** with methanol containing a trace of *p*-toluenesulfonic acid gave no reaction at room temperature, while on heating an unidentified rearrangement product containing only two methoxyl groups was obtained. By assuming the normal D-configuration of xylose, the stereochemistry of clavosolide A (**1**) may be proposed as 3S,3'S,4R,4'R,5S,5'S,7S,7'S,9S,9'S, 10S,10'S,11S,11'S.[3]

Clavosolide B (**2**), [α]$_D$ −41.0° (c 0.5, CHCl$_3$), was isolated as a very pale green viscous oil. The molecular formula, $C_{43}H_{70}O_{16}$, was established from a high-resolution FABMS measurement of the [M + Na]$^+$ peak at m/z 865.4521. In addition to the ester band at 1735 cm^{-1}, the IR spectrum contained a hydroxyl band at 3460 cm^{-1}, which, together with the difference in the molecular formulas, suggested that one of the methoxyl groups in clavosolide A (**1**) was replaced by a hydroxyl group in clavosolide B (**2**). This small difference caused clavosolide B to be an unsymmetrical dimer, which was reflected in the increased complexity of the ^{1}H and ^{13}C NMR spectra, which were assigned to the best of our ability as shown in Table 2. The location of the hydroxyl group at C-16' was unambiguous. The C-15 to C-19 and H-15 to H-19 signals were assigned by analogy with the corresponding signals for **1**. The second acetal proton signal at δ 4.36 (H-15') was coupled to the H-16' proton signal at 3.40 (cf. 2.96 for H-16), which is directly coupled to the ^{13}C signal at 72.9 (cf. 83.8 for C-16). These differences in the chemical shifts clearly indicated that the hydroxyl group was at C-16'.

The clavosolides are not related to any known sponge metabolites. Microscopic examination of *M. clavosa* re-

vealed the presence of a high concentration of cyanobacterial cells, which gave rise to the chlorophyll observed in the crude extract. This raises the possibility that the clavosolides might be of cyanobacterial origin.

Experimental Section

General Experimental Procedures. All solvents were redistilled prior to use. Infrared spectra were recorded on a Perkin-Elmer 1600 spectrometer. ^{1}H NMR, COSY, ROESY, gHMQC, and gHMBC spectra were recorded on a Varian Inova 300 MHz spectrometer, and ^{13}C NMR and DEPT spectra were recorded on a Varian Gemini 400 spectrometer. High-resolution FAB mass spectra were run on a VG-ZAB mass spectrometer at the UC Riverside Regional Facility.

Biological Material. The sponge *Myriastra clavosa* Ridley 1884 (Collection # NCI-2879) was collected by hand using scuba (-15 m) at Boracay Island, Philippines, in 1998 and was immediately frozen. The sponge was initially identified by Mary Kay Harper, and the identification was confirmed by Dr. John Hooper, Queensland Centre for Biodiversity, Brisbane. Voucher specimens are available on request.

Extraction and Purification. The sponge (350 g wet wt) was extracted with methanol (6 × 400 mL), and the extracts were concentrated to obtain a dark green gum (ca 10 g), which was partitioned between EtOAc and H_2O to obtain organic (1.87 g) and aqueous (7.81 g) extracts. Both extracts inhibited the growth of the HCT-116 cell line, with the aqueous extract being the more active. The organic extract was purified by chromatography on Sephadex LH-20 using MeOH as eluent followed by chromatography on a silica gel column using a gradient from hexane to EtOAc to MeOH as eluant. Fractions containing the cyclopropyl group, as determined by ^{1}H NMR spectroscopy, were further purified by HPLC on Porasil using mixtures of hexane and EtOAc to obtain clavosolides A (**1**, 4.3 × 10^{-3} % wet wt) and B (**2**, 1.4 × 10^{-3} % wet wt).

Clavosolide A (1): slightly greenish viscous oil; $[\alpha]_D$ $-48.5°$ (*c* 1, CHCl$_3$); IR (film) 1730 cm^{-1}; ^{1}H NMR (CDCl$_3$), see Table 1; ^{13}C NMR (CDCl$_3$), see Table 1; HRFABMS obsd *m/z* 879.4709 (M + Na)$^+$, C$_{44}$H$_{72}$O$_{16}$Na requires 879.4718.

Clavosolide B (2): slightly greenish viscous oil; $[\alpha]_D$ $-41.0°$ (*c* 0.5, CHCl$_3$); IR (film) 3460, 1735 cm^{-1}; ^{1}H NMR (CDCl$_3$), see Table 2; ^{13}C NMR (CDCl$_3$), see Table 2; HRFABMS obsd *m/z* 865.4521 (M + Na)$^+$, C$_{43}$H$_{70}$O$_{16}$Na requires 865.4562.

Supporting Information Available: Copies of the ^{1}H and ^{13}C NMR spectra of clavosides A and B and the COSY, gHMQC, gHMBC, and ROESY spectra of clavosolide A. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Faulkner, D. J. *Nat. Prod. Rep.* **2001**, *18*, 1−49.
(2) Fu, X.; Schmitz, F. J.; Kelly-Borges, M.; McCready, T. L.; Holmes, C. F. B. *J. Org. Chem.* **1998**, *63*, 7957−7963.
(3) Molecular modeling using PCModel for Windows (Serena Software) revealed that the macrolide ring was relatively flat and quite rigid. We examined four possible symmetrical isomers by keeping the absolute configuration of the macrolide constant and changing the absolute configurations of the cyclopropyl and sugar pendant groups. It is interesting to note that none of the conformations generated were exactly symmetrical, but the small differences in bond angles and H−H distances were insufficient to cast any doubt on the interpretation of the NOE data, which clearly required that clavosolide A (**1**) possessed the stereochemistry shown.

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