with the relative 15*S**,16*S**,17*R**,19*S**,20*S** was proposed, but this relative stereochemistry could not be related to the axial geometry at H-5 and H-23 or equatorial geometry at H-9.

Fijianolide B (**2**),[12] $C_{30}H_{42}O_7$, was recognized as a structural isomer of A by the many $^{13}C-^1H$ and $^1H-^1H$ correlations that were visible in the COSY NMR spectra. The COSY NMR data along with the $^{13}C/^1H$ chemical shift comparisons between **2** and **1** showed that the structure and stereochemistry of fijianolide B was the same as A except within the region of C-15 to C-20. The ^1H shift of δ 5.25 (C_6D_6) at H-19 in **2** was nearly identical with that in **1**, indicating that the ester linkage was still at C-19. Acetylation of **2** yielded **5** in which downfield shifts of $\approx$1 ppm were observed for the protons at H-20 and H-15. The most important of the long range $^{13}C-^1H$ COSY NMR correlations for fijianolide B acetate (**5**) were those from C-9 to H-5, from C-23 to H-27, and from C-1 to H-3. The trans epoxide ring at C-16/17 was identified by the characteristic $^{13}C/^1H$ NMR (C_6D_6) shifts of δ 61.1/2.84 dd = 2.1, 2.1 Hz [C/H-16] and 52.5/3.10 ddd = 11.1, 1.9, 1.9 Hz [C/H-17] and the $J_{16-17} \approx 2$ Hz.[13] If fijianolides A and B are biogenetically related by an obvious S_n2 transposition which involves an inversion of stereochemistry at C-17 in going from **1** to **2** or vice versa, then it can be assumed that the relative stereochemistry at C-15/16/19/20 in **2** is the same as in **1**, thus the five chiral centers in **2** are proposed as 15*S**,16*S**,17*S**,19*S**,20*S**.

The separate crude extract of the nudibranch *Chromodoris lochi*, often found attached to *S. mycofijiensis*, contained fijianolide B and mycothiazole. These metabolites may be products of symbionts which pass them along to the sponge where they are stored in such a manner as to be transmitted to the nudibranch. Moderate in vitro cytotoxicity was shown by the fijianolides, IC_{50}'s (μg/mL) for **1** and **5** were respectively 11 and 0.5 against HT-29 (human colon tumor cells) and respectively 9 and 6 against P388 (murine lymphoma cells).[14] The fijianolides can now be added to a short list of fascinating bioactive macrocyclic polyketides from marine organisms, and some of these have either been implied or have been shown as being produced by symbiotic microbiota.[15] We are now attempting to identify the symbiont(s) that may be responsible for the production of the fijianolides in order to understand their biosynthetic origin.

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Registry No. 1, 114995-72-1; 2, 114995-73-2.

Supplementary Material Available: NMR data for **1**–**5** (2 pages). Ordering information is given on any current masthead page.

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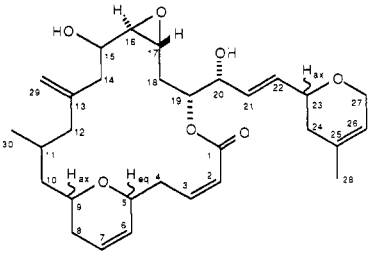
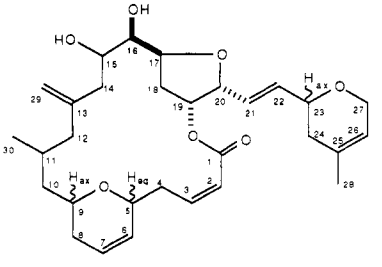
Laulimalides: New Potent Cytotoxic Macrolides from a Marine Sponge and a Nudibranch Predator[†]

Summary: Two new macrolides, laulimalide and isolaulimalide, were isolated from an Indonesian sponge, *Hyattella* sp., and a nudibranch predator, *Chromodoris lochi*. Laulimalide displays potent cytotoxicity, IC_{50} = 15 ng/mL, against the KB cell line.

Sir: While screening marine invertebrates suspected of having symbiotic microalgae for cytotoxicity, we found that the lipophilic extract of an Indonesian sponge, *Hyattella* sp.,[1] had an IC_{50} of 50 ng/mL against the KB cell line. Chromatography of 50-mg batches, first on silica Bond Elut (dichloromethane/acetone), then C-18 Bond Elut (methanol/water, 7:3), and finally reversed phase HPLC



1



2

(12) **2**: NMR (C_6D_6) shifts in ppm from TMS, assignments based on results of $^1H-^1H$ and $^{13}C-^1H$ COSY NMR data; [atom number], ^{13}C δ's at 75 MHz, ^1H δ's and J's at 300 MHz [1], 166.3; [2] 120.9, 5.80 (d = 12.3); [3] 150.4, 6.12 (ddd = 12.0, 10.8, 4.5); [4] 34.0, 3.90 (m), 2.08 (m); [5] 73.3, 4.12 (m); [6] 129.3, 5.48 (bd = 10.2); [7] 125.3, 5.67 (bd = 10.2); [8] 32.1, 1.75 (m); [9] 68.1, 3.71 (m); [10] 43.9, 1.50 (m), 1.20 (bd = 12.0); [11] 29.9, 1.75 (m); [12] 46.2, 2.55 (dd = 10.8, 2.4), 1.85 (dd = 10.8, 5.4); [13] 145.9; [14] 37.7, 2.14 (bs), 2.13 (bs); [15] 67.3, 4.04 (m); [16] 61.1, 2.84 (dd = 2.1, 2.1); [17] 52.5, 3.10 (ddd = 11.1, 1.9, 1.9); [18] 33.4, 2.36 (ddd = 10.8, 1.5, <1), 1.55 (m); [19] 72.7, 5.25 (ddd = 10.5, 3.9, 1.8); [20] 72.9, 4.18 (dd = 9.3, 4.5); [21] 129.2, 5.81 (dd = 16.2, 5.4); [22] 133.4, 5.94 (dd = 16.2, 5.4); [23] 73.6, 3.90 (m); [24] 36.0, 2.03 (m), 1.60 (m); [25] 131.3; [26] 120.2, 5.16 (bs); [27] 65.8, 4.09 (bs), 3.95 (bs); [28] 21.1, 0.86 (d = 6.0, 3 H); [29] 112.4, 4.94 (bs), 4.89 (bs); [30] 23.0, 1.50 (s, 3 H). $CDCl_3$ ^{13}C NMR data in Table 1S, supplementary material. MS: LREI m/z (relative intensity) 514 [M^+ (13)], 496 [$M^+ - H_2O$ (5)], 478 [$M^+ - 2H_2O$ (3)], 361 [$M^+ - C_9H_{13}O_2$ (10)], 53 [(100)]. IR: (neat) cm^{-1} 3600–3200, 2900, 1720, 1270. UV: (MeOH) 208 (10 500). **5** $[\alpha]^{20}_D$ –8.0° (*c* 0.04, $CHCl_3$). MS: FAB (thioglycerol/glycerol) 705 [M^+ + thioglycerol], 597 [M^+ – H].

(13) Lyle, G. G.; Keefer, L. K. *J. Org. Chem.* **1966**, *31*, 3921.

(14) Additional cytotoxicity IC_{50}'s (μg/mL) for **1** and **5** were respectively 12 and 2 against A549 (human lung tumor cells) and respectively 14 and 5 against HL-60 (human promyelocytic leukemia). We warmly thank Dr. N. Burres of the Harbor Branch SeaPharm Project for these results.

(15) For examples of parallel chemistry comparable to **1** or **2** being ascribed to microorganisms, see: (a) Schmitz, F., J.; Gunasekara, S. P.; Yalamanchili, G.; Hossain, M. B.; van der Helm, D. *J. Am. Chem. Soc.* **1984**, *106*, 7251. (b) Ishibashi, M.; Moore, R. E.; Patterson, G. M. L.; Xu, C.; Clardy, J. *J. Am. Chem. Soc.* **1986**, *51*, 5300. (c) Kobayashi, J.; Ishibashi, M.; Wälchili, M. R.; Nakamura; H.; Hirata, Y.; Sasaki, S.; Ohizumi; Y.; *J. Am. Chem. Soc.* **1988**, *110*, 490.

[†] A preliminary account of this work was made at the Gordon Research Conference on Marine Natural Products, Oxnard, CA, Feb 29–Mar 4, 1988.

0022-3263/88/1953-3644$01.50/0

Table I. ^{13}C (75 MHz) and ^{1}H (300 MHz) NMR Spectral Data for Laulimalide and Isolaulimalide in Dimethyl Sulfoxide-d_6

Laulimalide, chemical shift, ppm				Isolaulimalide, chemical shift, ppm			
atom	^{13}C (mult)	^{1}H (mult, J(Hz), intgrtn)	NOE[1]	atom	^{13}C (mult)	^{1}H (mult, J(Hz), intgrtn)	NOE
1	165.5 (s)			1	165.3 (s)		
2	121.3 (d)	5.88 (dd, 11, 0.5, 1H)	3	2	123.0 (d)	5.80 (d, 10.1, 1H)	
3	146.1 (d)	6.37 (td, 11, 4, 1H)	2	3	143.3 (d)	6.23 (td, 10.1, 7.4, 1H)	2
4a	42.9 (t)[2]	3.57 (dt, -16, 11, 1H)	4b	4a	43.1 (t)[2]	2.96 (td, 10.8, 4.8, 1H)	4b, 5
4b		2.13 (dddd, -16, 8, 4, 0.5, 1H)	4a	4b		2.56 (td, 10.8, 7.4, 1H)	3, 4a, 9
5	71.9 (d)	4.16 (br dd, 8, 4, 1H)	3,6	5	77.4 (d)	4.17 (m, 1H)	4a, 6
6	129.4 (d)	5.76 (d, 10.5, 1H)		6	126.2 (d)	5.76 (d, 10.3, 1H)	
7	124.9 (d)	5.79 (br dm, 10.5, 5, 1, 1H)		7	125.6 (d)	5.85 (br dd, 10.3, 5, 1H)	
8a	45.9 (t)[2]	2.05 (br dt, -17.4, 5, 1H)		8a	45.1 (t)[2]	1.97 (dt, -17, 5, 1H)	
8b		1.78 (br dd, -17.4, 8.2,1, 1H)		8b		1.83 (br dd, -17, 10, 2, 1H)	
9	67.6 (d)	3.67 (m, 8.2, 6.3, 5, 3.4,1H)		9	66.5 (d)	3.58 (m, 1H)	4b, 8a, 11, 30
10a	31.5 (t)[2]	1.40 (ddd, -14, 7.7, 6.3, 1H)		10a	31.7 (t)[2]	1.38 (br dd, -13.7, 9.9, 1.5, 1H)	10b
10b		1.15 (ddd, -14, 5.3, 3.4, 1H)		10b		1.10 (br ddd, -13.7, 8.0, 2.9, 1H)	9, 10a
11	28.8 (d)	1.58 (m, 1H)		11	27.1 (d)	1.80 (m, 1H)	
12a	33.6 (t)[2]	2.21 (dd, -12.2, 4.1, 1H)		12a	35.7 (t)[2]	1.75-2.05 (m, 1H)	
12b		1.70 (m, 1H)		12b		1.75-2.05 (m, 1H)	
13	148.6 (s)			13	146.4 (s)		
14a	37.6 (t)[2]	1.93 (d, -14.8, 1H)	14b	14a	35.7 (t)[2]	1.75-2.05 (m, 1H)	
14b		1.79 (dd, -14.8, 8.4, 1H)	14a	14b		1.75-2.05 (m, 1H)	
15	66.2 (d)	3.82 (ddd, 8.4, 4.9, 1.9, 1H)		15	73.0 (d)	3.73 (dt, 5.7, 5.2, 1H)	16, 29a
16	61.3 (d)	2.71 (t, 1.9)	15	16	77.0 (d)	3.61 (m, 1H)	17, 29a
17	51.8 (d)	2.86 (ddd, 8.7, 4.9, 1.9, 1H)	19, 18a	17	78.6 (d)	4.09 (dt, 10.8, 5.9, 1H)	16, 18b
18a	32.9 (t)[2]	2.12 (dt, -14, 4.9, 1, 1H)	18b	18a	35.8 (t)[2]	2.24 (ddd, -14.1, 10.8, 4.0, 1H)	18b, 19
18b		1.33 (ddd, -14, 11, 8.7, 1H)	18a	18b		1.96 (ddd, -14.1, 5.9, 0.5, 1H)	
19	72.7 (d)	4.98 (ddd, 11, 4.9, 1, 1H)		19	80.9 (d)	5.44 (br t, 4.0, 0.5, 1H)	18a, 20
20	71.5 (d)	4.07 (br m, 5.2, 4.9, 1H)	19, 21	20	72.6 (d)	4.49 (dd, 5.2, 4.0, 1H)	19, 21, 22
21	129.7 (d)	5.59 (dd, 15.9, 4.9, 1H)		21	128.8 (d)	5.44 (dd, 15.5, 5.2, 1H)	20, 23
22	132.4 (d)	5.71 (dd, 15.9, 5.3, 1H)		22	133.3 (d)	5.76 (dd, 15.5, 4.9, 1H)	
23	73.1 (d)	3.93 (dt, 8.2, 5.3, 1H)		23	70.7 (d)	3.87 (dt, 8.5, 4.9, 1H)	21, 22, 24, 27
24a	35.8 (t)[2]	1.83 (m, 1H)	28	24a	35.6 (t)[2]	1.75-1.85 (m, 1H)	23
24b		1.83 (m, 1H)		24b		1.75-1.85 (m, 1H)	
25	131.2 (s)			25	131.1 (s)		
26	120.3 (d)	5.45 (br s, 1H)	27, 28	26	120.3 (s)	5.39 (br s, 1H)	27, 28
27a	65.0 (t)	4.04 (br s, 1H)	26	27a	65.0 (t)	3.99 (br s, 1H)	26
27b		4.04 (br s, 1H)		27b		3.99 (br s, 1H)	
28	23.0 (q)	1.63 (br s, 3H)	24, 26	28	23.0 (q)	1.61 (br s, 3H)	24, 26
29a	112.1 (t)	4.74 (br s, 1H)		29a	113.0 (t)	4.77 (br s, 1H)	16
29b		4.68 (br s, 1H)		29b		4.70 (br s, 1H)	30
30	20.8 (q)	0.74 (d, 6.1, 3H)	11	30	19.9 (q)	0.78 (d, 5.6, 3H)	11
15 OH		4.52 (d, 4.9, 1H)		15 OH		4.40 (d, 5.7, 1H)	
20 OH		5.22 (d, 5.2, 1H)		16 OH		4.80 (d, 4.0, 1H)	

[a] Superscript 1 denotes NOE's for laulimalide were observed in acetone-d_6. [b] Superscript 2 denotes tentative assignments.

(acetonitrile/water, 6:4) yielded latrunculin A[2-4] (70 mg, 15%, IC_{50} = 150 ng/mL), laulimalide[5] (1, 7 mg, 1.5%, IC_{50} = 15 ng/mL), and isolaulimalide (2, 5 mg, 1.0%, IC_{50} >200 ng/mL). The same compounds were also isolated from the acetone extract of a nudibranch, *Chromodoris lochi*, that was found grazing on the sponge.[6] We report here the structures of 1 and 2.

FAB-MS indicated a molecular weight of 514 for 1 and 2 (MH^+, m/z 515; MK^+, m/z 553; MCs^+, m/z 647) and high resolution EIMS revealed the formula $C_{30}H_{42}O_7$ (m/z 514.293, 0 mmu error) for both compounds.

The ^{13}C NMR spectra of 1 and 2 (Table I) confirmed the presence of 30 carbons. The nature of the carbons was revealed by a DEPT experiment,[7] but only 40 protons were accounted for, which suggested two exchangeable protons subsequently observed by ^{1}H NMR.

The IR spectrum (film, NaCl) showed the presence of OH (3425 cm^{-1}) and α,β-unsaturated ester groups (1710–1720 cm^{-1}), the latter confirmed by the UV spectrum [MeCN, λ_{max} 195 nm (log ϵ 4.5), 215 nm sh].

The gross structure of 1 was deduced from ^{1}H NMR spectra, particularly from 2D-COSY,[8] 2D-Relay,[9] 2D-Double Relay,[9] and difference 1D-NOE spectra in benzene-d_6, acetone-d_6, and dimethyl sulfoxide-d_6. The Δ^2 double bond is cis on the basis of $J_{2,3}$ (11 Hz) and a strong NOE between H-2 and H-3. A significant NOE between H-9 and one of the C-4 protons indicated that C-5, -6, -7, -8, and -9 comprised a dihydropyran, where H-9 and C-4 were axial. Coupling could not be observed between H-5 and H-6 (except weakly in acetone-d_6), but homoallylic coupling between H-5 and one of the protons on C-8 (2.4 Hz in acetone-d_6), an appreciable NOE between H-5 and H-6, and the magnitudes of the couplings between H-6 and

(1) The sponge was collected offshore from Manado, northern Sulawesi, Indonesia, at a depth of 20 m and identified by Professor P. Bergquist. Blending twice with dichloromethane/methanol (1:1) yielded 3.7 g of extract residue from 64.8 g (dry wt) of sponge (5.7%).

(2) Kashman, Y.; Groweiss, A.; Shmueli, U. *Tetrahedron Lett.* **1980**, *21*, 3629–3632.

(3) Groweiss, A.; Shmueli, U.; Kashman, Y. *J. Org. Chem.* **1983**, *48*, 3512–3516.

(4) Kashman, Y.; Groweiss, A.; Lidor, R.; Blasberger, D.; Carmely, S. *Tetrahedron* **1985**, *41*, 1905–1914.

(5) *Laulima*, Hawaiian for *people working together* seems to be an appropriate name for this research in which three research groups cooperated.

(6) Twenty animals (1.83 g of dry wt) steeped twice in acetone yielded 0.33 g (18%) of dark brown tarry residue. Gradient silica chromatography (hexane/ethyl acetate, 5:1, to ethyl acetate) followed by HPLC (Lichrosorb RP-18, methanol/water, 78:22; μBondapak RP-18, methanol/water, 73:27) yielded latrunculin A (6.25 mg, 1.9% of first extract), laulimalide (1, 1.27 mg, 0.39%), and isolaulimalide (2, 1.47 mg, 0.45%). Other nudibranch predators were found grazing on the sponge, viz. *Chromodoris elizabethina*, *C. willani*, and an unidentified *Chromodoris* sp. Latrunculin A was the major metabolite in all of these nudibranchs, which were identified by Clayton Carlson and Patty Jo Hoff.

(7) Doddrell, D. M.; Pegg, D. T.; Bendall, M. R. *J. Magn. Reson.* **1982**, *48*, 323.

(8) Bax, A.; Freeman, R.; Morris, G. *J. Magn. Resn.* **1981**, *42*, 164.

(9) Bax, A.; Drobny, G. *J. Magn. Reson.* **1985**, *61*, 306–320.

H-7 (10.3 Hz), H-9 and pseudoaxial H-8 (8.2 Hz), and H-9 and pseudoequatorial H-8 (5 Hz) further supported the presence of this dihydropyran.

COSY cross peaks for allylic coupling between the C-29 protons and the protons on C-12 and C-14 and for long-range coupling between the C-30 methyl protons and the C-12 protons, relay cross peaks from the H_3-30 signal to the H-29 (*E* to C-12) and H_2-10 signals and from the H-15 signal to the H-29 (*E* to C-14) signal, and double relay cross peaks from the H_3-30 signal to the H-9 signal and from the H-16 signal to the H-29 (E to C-14) signal indicated clearly the positions of the secondary methyl and terminal methylene group relative to C-9 and C-16.

Proton signals at 2.71 and 2.86 ppm, which coupled weakly (1.9 Hz) and correlated with methine carbon signals at 61.3 and 51.8 ppm, respectively, suggested the presence of a trans-1,2-disubstituted epoxide at C-16 and C-17. A chemical shift of 4.98 ppm for H-19 strongly suggested that the lactone oxygen was linked to C-19.

Doublet signals could be seen in dimethyl sulfoxide-d_6 for the two OH protons and COSY cross peaks indicated that these were attached to C-15 and C-20. A coupling constant for 15.9 Hz indicated that the Δ^{21} double bond was trans. COSY cross peaks from the H-26 signal to the H_2-24, H_2-27, and H_3-28 signals and significant NOEs between H-26 and H_2-27, H-26 and H_3-28, and H_2-24 and H_3-28 showed that a dihydropyran ring with a methyl substituent at C-25 was present at the terminus of the macrolide sidechain.

Proton NMR analysis of **2** showed that C-1 to C-15 and C-21 to C-30 were identical with those in **1**. Further analysis established that four of the remaining five carbons had formed a tetrahydrofuran by S_N2 attack of the C-20 hydroxyl in **1** on C-17 of the epoxide. Cross peaks in the COSY spectrum in dimethyl sulfoxide-d_6 indicated attachment of the OH groups to C-15 and C-16. No coupling but a small NOE could be observed between H-15 and H-16. Difference NOE data (Table I) suggested that C-16 to C-20 in isolaulimalide had the relative stereochemistry depicted in **2**.

Upon treatment with 0.01 N HCl in acetone (4 h, room temperature), **1** isomerized completely to **2**. If the tetrahydrofuran in **2** is formed by an S_N2 attack of the C-20 hydroxyl group on C-17 of the epoxide, C-16 to C-20 in laulimalide should have the relative stereochemistry shown in **1**.

It is worthwhile to note that the structures of **1** and **2** do not fully conform to polyketide biogenetic principles.

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