Luffariolides H and J, New Sesterterpenes from a Marine Sponge *Luffariella* Species

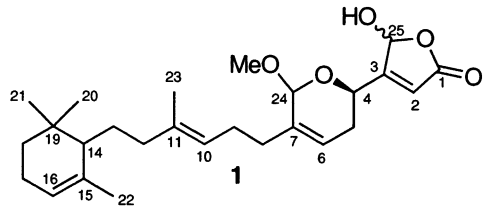
Masashi Tsuda,[†] Tadashi Endo,[†] Yuzuru Mikami,[‡] Jane Fromont,[§] and Jun'ichi Kobayashi*[,†]

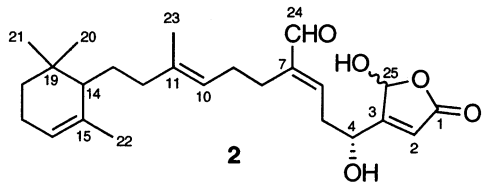
Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan, Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Chiba 260-0856, Japan, and Western Australian Museum, Perth, WA 6000, Australia

Received May 1, 2002

Two new sesterterpenoids, luffariolides H (**1**) and J (**2**), have been isolated from an Okinawan marine sponge *Luffariella* sp. The structures of **1** and **2** were elucidated on the basis of spectroscopic data.

Marine sponges of the genus *Luffariella* are a rich source of sesterterpenoids with biological activity.[1] In our search for bioactive substances from marine sponges,[2] we have investigated bioactive metabolites from marine sponges of the genus *Luffariella*.[3−6] Our recent study of extracts of an Okinawan *Luffariella* sponge (SS-996) resulted in the isolation of two new sesterterpenoids, luffariolides H (**1**) and J (**2**). Here we describe the isolation and structure elucidation of **1** and **2**.





The sponge *Luffariella* sp. (0.3 kg, wet weight) was extracted with MeOH, and the extracts were partitioned between EtOAc and water. The EtOAc-soluble materials were subjected to silica gel column chromatography ($CHCl_3$/MeOH) followed by reversed-phase HPLC (MeOH/H_2O/AcOH and then CH_3CN/NH_4Cl(aq)) to afford luffariolides H (**1**, 4.0 mg, 0.005%, wet weight) and J (**2**, 3.9 mg, 0.005%) together with known related sesterterpenoids, 24-*O*-methylmanoalide,[7,8] secomanoalide,[8] and luffariellins A[9] and B.[9]

Luffariolide H (**1**) showed a molecular ion peak at *m*/*z* 430 in the EIMS spectrum, and the molecular formula, $C_{26}H_{38}O_5$, was confirmed by HREIMS (*m*/*z* 430.2698 M^+, Δ −2.1 mmu). IR absorptions at 3375 and 1760 cm^{-1} of **1** were attributed to a hydroxyl group and an unsaturated γ-lactone moiety, respectively. ^1H and ^{13}C NMR data (Table 1) were reminiscent of those of manoalide,[7] except for the presence of a methoxy group. The signals for H-2, H-4, H-24, and H-25 were observed as doublets due to the presence of an epimeric hydroxyl group at C-25, to which a similar phenomenon was reported for manoalide.[3] Analysis of 2D NMR data (Figure 1) suggested that the C-1−C-13 part of **1** possessed the same structure as that of manoalide. The HMBC correlation for a methoxy signal to C-24 indicated that the methoxy group was attached to C-24. Comparing ^{13}C NMR data (Table 1) of **1** with those of manoalide, differences were observed for the chemical shifts of C-14, C-15, C-16, and C-19. The HMBC spectrum of **1** showed correlations for H_2-18, H_3-21, and H_3-22 to C-14, H-14, H-16, and H_3-22 to C-15, and H_2-19, H_3-20, and H_3-21 to C-19, indicating the presence of a $\Delta^{15(16)}$ cyclohexene ring. This assignment was supported by comparison of NMR data for the C-13−C-22 part of **1** with those for the corresponding moiety of cacospongins B and C[10] and luffarin P.[11] Thus, the structure of luffariolide H was elucidated to be **1**.

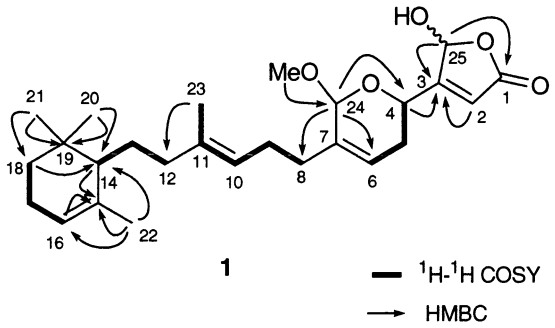


Figure 1. Selected 2D NMR correlations for luffariolide H (**1**).

HRFABMS data [*m*/*z* 439.2485 $(M + Na)^+$, Δ +2.4 mmu] of luffariolide J (**2**) suggested the molecular formula $C_{25}H_{36}O_5$. ^1H and ^{13}C NMR data (Table 1) of **2** were similar to those of secomanoalide[4] and luffariellin B,[5] except for those of C-14, C-15, C-16, and C-19. The presence of a $\Delta^{15(16)}$ cyclohexene ring in **2** was implied by 2D NMR data and comparison of the ^{13}C chemical shifts with those of **1**. Thus, the structure of luffariolide J was assigned as **2**.

Absolute configurations at C-4 of luffariolides H (**1**) and J (**2**) were considered to be *R* judging from co-isolation of 24-*O*-methylmanoalide[7] and secomanoalide[8] with the 4*R*-configuration from this sponge, while those of C-14 were not defined.

Several sesterterpenoids have been isolated from *Luffariella* sponges.[7−11] Luffariolides H (**1**) and J (**2**) exhibited cytotoxicity against murine lymphoma L1210 (IC_{50}, 3.2 and 4.5 μg/mL, respectively) and epidermoid carcinoma KB cells (IC_{50}, 7.5 and 8.2 μg/mL, respectively) in vitro. Luffariolides H (**1**) and J (**2**) showed antimicrobial

* To whom correspondence should be addressed. Tel: +81-11-706-4985. Fax: +81-11-706-4989. E-mail: jkobay@pharm.hokudai.ac.jp.
[†] Hokkaido University.
[‡] Chiba University.
[§] Western Australian Museum.

10.1021/np0202071 CCC: $22.00
Published on Web 08/22/2002

Table 1. ^{1}H and ^{13}C NMR Data of Luffariolides H (**1**) and J (**2**) in $CDCl_3$

	1							**2**						
positn	δ_H[a]				δ_C[b]			δ_H[a]				δ_C[b]		
1					171.8		s					170.4	171.2	s
2	6.05	s	6.13	s	117.5	118.4	d	6.03	s	6.11	s	118.3	118.4	d
3					167.2	167.5	s					169.3		s
4	4.76	m	4.82	m	62.2	63.1	d	4.76	m	4.83	m	66.4	66.9	d
5	2.28[a]	m			29.1		t	2.82[a]	m			24.4		t
6	5.68	brs			120.6	120.7	d	6.55	m			145.6	145.8	d
7					136.4		s					148.3	148.8	s
8	2.09[a]	m			32.5	32.6	t	2.03[a]	m			34.3	34.7	t
9	2.34[a]	dd, 5.8, 5.9			26.1		t	2.27[a]	m			26.8		t
10	5.11	t, 5.8			123.3		d	5.08	m			122.7		d
11					136.7		s					136.8		s
12	2.10[a]	m			40.5		t	1.95	m			40.5		t
13	1.35	m	1.52	m	29.9		t	1.32	m	1.47	m	29.8		t
14	1.42	t, 3.6			49.1		d	1.38	m			49.0		d
15					136.9		s					137.3		s
16	5.29	brs			120.6		d	5.26	m			120.0		d
17	1.95[a]	m			23.1		t	1.93[a]	m			23.0		t
18	1.14[a]	t, 4.5			31.8		d	1.11[a]	m			31.6		t
19					28.7		s					32.5		s
20	0.87[b]	s			27.4		q	0.84[b]	s			27.4		q
21	0.92[b]	s			27.5		q	0.95[b]	s			27.5		q
22	1.68[b]	s			23.5		q	1.65[b]	s			23.5		q
23	1.61[b]	s			16.1		q	1.56[b]	s			16.1		q
24	4.79	s	4.81	s	98.3	98.5	d	9.37	s			195.3		d
25	6.10	s	6.27	s	97.1	97.6	d	6.10	s	6.26	s	98.0	98.3	d
OCH_3	3.45[b]	s			55.8		q							

[a] 2H. [b] 3H.

activity against *Staphylococcus aureus* (MIC, 16.7 and 33.3 μg/mL, respectively), *Bacillus subtilis* (MIC, both 8.4 μg/mL), and *Mycrococcus luteus* (MIC, both 8.4 μg/mL).

Experimental Section

General Experimental Procedures. The IR and UV spectra were recorded on a JASCO FT/IR-5300 and a Shimadzu UV-1600PC spectrophotometer, respectively. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX-600 spectrometer. EI mass spectra were recorded on a JEOL FABmate spectrometer at 70 eV. FAB mass spectrum was obtained on a JEOL HX-110 spectrometer using nitrobenzyl alcohol as a matrix.

Animal Material. The sponge (order Dictyoceratida, family Thorectidae) was collected off Nakijin, Okinawa, and kept frozen until used. The sponge had a medium brown finely conulose surface skin with an orange-brown interior after preservation. The sponge was yellow-brown when alive. The sponge is very firm, moderately compressible, and springy. The thin conulose skin is 70 μm thick and consists of thickened mesohyl and a thin layer of sand grains. The primary fibers are thick, striated, and centrally cored with sand grains. They are 200 μm thick with mesh spaces of up to 1000 μm. They can be fasciculate toward the surface. The secondary fibers are striated, uncored, and 40−90 μm thick with mesh spaces up to 375 μm. The tertiary fibers are 10−13 μm thick with small mesh spaces.

Extraction and Isolation. The sponge (0.3 kg, wet weight) was extracted with MeOH (600 mL × 2), and the extract was partitioned between EtOAc (500 mL × 3) and H_2O (500 mL). Parts (1.1 g) of the EtOAc-soluble materials (1.67 g) were subjected to a silica gel column ($CHCl_3$/MeOH). The fraction eluted with $CHCL_3$/MeOH (98:2) was purified by reversed-phase HPLC [Luna Phenyl-hexyl, 5 μm, Phenomenex, 10 × 250 mm; eluent, MeOH/H_2O/AcOH, 85:15:0.1; flow rate, 2.5 mL/min; UV detection at 230 nm] and then C_{18} HPLC [Wakosil-II5C18 RS, Wako Pure Chemical Ind., Ltd., 10 × 250 mm; eluent, CH_3CN/5 mM NH_4Cl(aq), 75:25; flow rate, 2.5 mL/min; UV detection at 230 nm] to afford luffariolide H (**1**, 4.0 mg, 0.005%, wet weight), 24-*O*-methylmanoalide (0.002%), and luffariellin A (0.003%). The fraction eluted with $CHCl_3$/MeOH (95:5) in the first silica gel column was subjected to C_{18} HPLC [Wakosil-II5C18 RS, 10 × 250 mm; eluent, CH_3CN/5 mM NH_4-Cl(aq), 60:40; flow rate, 2.5 mL/min; UV detection at 230 nm] to give luffariolide J (**2**, 3.9 mg, 0.005%), secomanoalide (0.002%), and luffariellin B (0.003%).

Luffariolide H (1): colorless oil; UV (MeOH) λ_{max} 230 nm (sh); IR (KBr) ν_{max} 3375 and 1760 cm^{-1}; ^{1}H and ^{13}C NMR (see Table 1); EIMS *m*/*z* 398 [M − MeOH]$^+$ and 430 [M]$^+$; HREIMS *m*/*z* 430.2698 [M]$^+$ (calcd for $C_{26}H_{38}O_5$, 430.2719).

Luffariolide J (2): colorless oil; UV (MeOH) λ_{max} 230 nm (sh); IR (KBr) ν_{max} 3355, 1775, and 1680 cm^{-1}; ^{1}H and ^{13}C NMR (see Table 1); FABMS *m*/*z* 439 [M + Na]$^+$; HRFABMS *m*/*z* 439.2485 [M + Na]$^+$ (calcd for $C_{25}H_{36}O_5Na$, 439.2509).

Acknowledgment. We thank S. Oka and M. Kiuchi, Center for Instrumental Analysis, Hokkaido University, for EIMS and FABMS measurements and Z. Nagahama for his help with collection of the sponge. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

References and Notes

(1) Faulkner, D. J. *Nat. Prod. Rep.* **2002**, *19*, 1−48, and references therein.
(2) Tsuda, M.; Sakuma, Y.; Kobayashi, J. *J. Nat. Prod.* **2001**, *64*, 980−982, and references therein.
(3) Tsuda, M.; Shigemori, H.; Ishibashi, M.; Sasaki, T.; Kobayashi, J. *J. Org. Chem.* **1992**, *57*, 3503−3507.
(4) Kobayashi, J.; Zeng, C.-M.; Ishibashi, M. *J. Chem. Soc., Chem. Commun.* **1993**, 79−81.
(5) Kobayashi, J.; Zeng, C.-M.; Ishibashi, M.; Sasaki, T. *J. Nat. Prod.* **1993**, *56*, 436−439.
(6) Ishibashi, M.; Zeng, C.-M.; Kobayashi, J. *J. Nat. Prod.* **1993**, *56*, 1856−1860.
(7) de Silva, E. D.; Scheuer, P. J. *Tetrahedron Lett.* **1980**, *21*, 1611−1614.
(8) de Silva, E. D.; Scheuer, P. J. *Tetrahedron Lett.* **1981**, *22*, 3147−3150.
(9) Kernan, M. R.; Faulkner, D. J. *J. Org. Chem.* **1987**, *52*, 3081−3083.
(10) Tasdemir, D.; Concepción, G. P.; Mangalindan, G. C.; Harper, M. K.; Hajdu, E.; Ireland, C. M. *Tetrahedron* **2000**, *56*, 9025−9030.
(11) Butler, M. S.; Capon, R. J. *Aust. J. Chem.* **1992**, *45*, 1705−1743.

NP0202071