Jaspisamides A-C, New Cytotoxic Macrolides from the Okinawan Sponge Jaspis Sp.

Jun'ichi Kobayashi, Osamu Murata, Hideyuki Shigemori, and Takuma Sasaki

J. Nat. Prod., **1993**, 56 (5), 787-791• DOI:
10.1021/np50095a021 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 56, No. 5, pp. 787-791, May 1993

JASPISAMIDES A–C, NEW CYTOTOXIC MACROLIDES FROM THE OKINAWAN SPONGE *JASPIS* SP.

JUN'ICHI KOBAYASHI,* OSAMU MURATA, HIDEYUKI SHIGEMORI,

Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan

and TAKUMA SASAKI

Cancer Research Institute, Kanazawa University, Kanazawa 920, Japan

ABSTRACT.—Three new macrolides, jaspisamides A [**1**], B [**2**], and C [**3**], with cytotoxic activities have been isolated from the Okinawan marine sponge *Jaspis* sp. and their structures determined by the spectroscopic data. This is the first isolation of macrolides from a sponge of the genus *Jaspis*.

Several cytotoxic and antifungal macrolides containing oxazole rings have been isolated from nudibranch egg masses (1–4) and sponges of the genera *Halichondria* (4,5) and *Mycale* (6). During our search for bioactive metabolites from Okinawan marine organisms (7–11), we examined extracts of the sponge *Jaspis* sp. and obtained three new cytotoxic macrolides, jaspisamides A [**1**], B [**2**], and C [**3**], together with known related compounds halichondramide [**4**] (5), dihydrohalichondramide [**5**] (4), and isohalichondramide [**6**] (4). In this paper we describe the isolation and structural elucidation of **1–3**.

The sponge *Jaspis* sp. was collected off Ishigaki Island, Okinawa and kept frozen until required. The MeOH extract of the sponge was partitioned between EtOAc and H_2O. The EtOAc-soluble fraction was subjected to Si gel cc followed by Si gel and reversed-phase hplc to yield jaspisamides A [**1**] (0.00054%, wet wt), B [**2**] (0.00008%), and C [**3**] (0.0002%) together with halichondramide [**4**], dihydrohalichondramide [**5**], and isohalichondramide [**6**].

Jaspisamide A [**1**], a colorless solid, was shown to have molecular formula $C_{44}H_{62}N_4O_{13}$ by the hrfabms, *m/z* 855.4382 $[M+H]^+$, Δ −1.0 mmu. The uv spectrum exhibited a broad absorption at 244 nm (ϵ 24000). The ir absorption at 3350 cm^{-1} indicated the presence of OH and/or NH groups, while the presence of ester and amide groups was implied by bands at 1700 and 1650 cm^{-1}, respectively. The ^{1}H-nmr spectrum of **1** (Table 1) was quite similar to that of halichondramide [**4**], previously isolated from a Palauan sponge *Halichondria* sp. (5). In the ^{1}H nmr of **1** a pair of doublet signals was observed in a ratio of 2:1 for H-34 (δ 5.06 and 5.10), H-35 (δ 6.52 and 7.18), and an *N*-methyl formamide group (δ 3.03 and 3.07, NMe; δ 8.06 and 8.28, NCHO), respectively. The magnitude of chemical shift differences of the doublets was proportional to the distance from the *N*-methyl formamide group, suggesting that each pair of doublet signals was due to restricted rotation around the C-N bond of the *N*-methyl formamide group. Such a phenomenon was previously observed for halichondramide [**4**] (5) and the kabiramides (2). In the ^{1}H-nmr spectrum of **1** (Table 1), three adjoining oxazole rings gave rise to proton signals at δ 8.08 (1H, s, H-14), 8.04 (1H, s, H-17), and 7.57 (1H, s, H-11). The H-9 at δ 4.98 (1H, d, J = 4.4 Hz) was coupled to H-8 at δ 3.27 (1H, dq, J = 6.8 and 4.4 Hz) that was in turn coupled to 8-Me at δ 0.97 (3H, d, J = 6.8 Hz). The olefinic proton at δ 6.95 (1H, dd, J = 16.0 and 7.3 Hz, H-20) was coupled to a trans-olefinic proton at δ 6.44 (1H, d, J = 16.0 Hz, H-19) and to methylene protons at δ 2.22 and 2.5 (H_2-21). An ester oxygen on C-1 was connected to a methine (C-24), which was implied by the ^{1}H chem-

2 R_1=OH, R_2=H
3 R_1=H, R_2=Me
4 R_1=H, R_2=H

6

1

5

TABLE 1. ^{1}H-nmr Data of Jaspisamides A [**1**], B [**2**], and C [**3**].

Proton	Compound					
	1	*J*(Hz)	*2*	*J*(Hz)	**3**	*J*(Hz)
H-2	2.61 dd	15,11	2.64 dd	15,11	2.61 dd	15,11
H-2′	2.50 m		2.50 m		2.50 m	
H-3	4.73 m		4.42 m		4.44 m	
H-4	2.50 m		2.50 m		2.50 m	
H-4′	1.80 m		2.50 m		2.50 m	
H-5′	4.77 m		7.33 dd	16,10	7.29 m	
H-6	3.08 m		6.23 d	16	6.22 d	16
H-8	3.27 dq	6.8,4.4	4.03 m		4.02 m	
H-9	4.98 d	4.4	4.35 d	8.8	4.36 d	8.8
H-11	7.57 s		7.67 s		7.66 s	
H-14	8.08 s		8.10 s		8.10 s	
H-17	8.04 s		8.04 s		8.05 s	
H-19	6.44 d	16	6.34 d	16	6.29 d	16
H-20	6.95 dt	16,7.3	7.11 ddd	16,9.3,4.9	7.15 dt	16,7.3
H-21	2.50 m		2.50 m		2.50 m	
H-21′	2.22 m		2.27 m		2.25 m	
H-22	1.72 m		4.03 m		1.67 m	
H-22′	1.32 m				1.40 m	
H-23	1.78 m		1.78 m		1.91 m	
H-24	5.17 m		5.10 m		5.12 m	
H-25	1.58 m		1.77 m		1.55 m	
H-25′	1.58 m		1.56 m		1.55 m	
H-26	2.98 brd		3.09 brd		2.99 brd	
H-27	1.70 m		1.78 m		1.70 m	
H-28	1.70 m		1.80 m		1.75 m	
H-28′	1.36 m		1.28 m		1.36 m	
H-29	2.53 m		2.50 m		2.50 m	
H-29′	2.50 m		2.50 m		2.50 m	
H-31	2.74 m		2.74 m		2.67 m	
H-32	3.44 m		3.46 m		3.31 m	
H-33	2.50 m		2.46 m		2.40 m	
H-33′	2.15 m		2.14 m			
H-34	5.10 m		5.10 m		5.12 m	
	5.06 m		5.08 m		5.10 m	
H-35	6.52 d	14	6.52 d	14	6.46 d	14
	7.18 d	14	7.18 d	14	7.13 d	14
8-Me	0.97 d	6.8	0.89 d	6.8	0.92 d	6.8
23-Me	0.90 d	6.8	0.95 d	6.8	0.91 d	6.8
27-Me	0.84 d	6.8	0.86 d	6.8	0.85 d	6.8
31-Me	0.99 d	6.8	0.99 d	6.8	0.97 d	6.8
NCHO	8.28 s		8.29 s		8.29 s	
	8.06 s		8.06 s		8.04 s	
NMe	3.03 s		3.04 s		3.04 s	
	3.07 s		3.07 s		3.08 s	
9-MeO	3.47 s		3.19 s		3.18 s	
32-MeO	3.29 s		3.30 s		3.32 s	
26-MeO	3.33 s		3.30 s		3.35 s	
33-Me					1.16 d	6.8

ical shift (δ 5.17, H-24). The ^{1}H-^{1}H COSY spectrum of **1** revealed the presence of four segments, C-2 to C-4, C-8 to C-9, C-19 to C-29, and C-31 to C-35, which were also found in halichondramide [**4**]. The only structural difference between compounds **1** and **4** was found for a segment from C-5 to C-6 as

follows. An oxymethine proton at δ 4.77 (H-5) was coupled to methylene protons (δ 3.08, H_2-6) in **1**, while two olefinic protons (δ 7.27, H-5 and δ 6.28, H-6) were observed for **4**. Thus the structure of jaspisamide A was concluded to be **1**.

Jaspisamide B [**2**] showed uv, ir, and ^{1}H-nmr (Table 1) spectra closely related to those of halichondramide [**4**]. The molecular formula, $C_{44}H_{60}N_4O_{13}$, of **2** was established by the hrfabms *m/z* 853.4282 $[M+H]^+$, Δ +4.7 mmu. The mol wt of **2** was larger than that of **4** by 16 daltons, suggesting the presence of an extra hydroxy group in **2**. This was supported from connectivities between the oxymethine proton (1H, δ 4.03, m, H-22) and methylene protons (δ 2.27 and 2.50, H_2-21) and between one of H_2-21 and an olefinic proton (δ 7.11, H-20) observed in the ^{1}H-^{1}H COSY spectrum of **2**. Thus the structure of jaspisamide B was assigned as **2**.

Jaspisamide C [**3**] also showed uv and ir spectra similar to those of halichondramide [**4**] and had a molecular formula of $C_{45}H_{62}N_4O_{12}$, which was established by the hrfabms *m/z* 851.4451 $[M+H]^+$, Δ +0.9 mmu. The ^{1}H-nmr (Table 1) spectrum of **3** resembled that of **4**, except for one methyl signal at δ 1.16 (3H, d, $J=6.8$ Hz) in **3**. The methyl protons were coupled to a methine proton at δ 2.40 (H-33), which showed a cross peak to an oxymethine proton at δ 3.31 (H-32) in the ^{1}H-^{1}H COSY spectrum. These spectral data led us to assign the structure of jaspisamide C as **3**.

Jaspisamides A [**1**], B [**2**], and C [**3**] are new congeners of halichondramide [**4**], a unique 28-membered macrolide including a three contiguous oxazole ring system, which may biosynthetically involve introduction of nitrogens into a polyketide intermediate (12). This is the first isolation of macrolides (6) from a sponge belonging to the genus *Jaspis*, although this genus has been shown to contain triterpenes (13–16) and cyclic peptides (17,18). Compounds **1–3** appear to be also biogenetically related to mycalides (6) obtained from a sponge *Mycale* sp. collected in Gokasho Bay, Japan. It is interesting from a chemotaxonomic point of view that structurally related macrolides have been found among sponges of the genera *Halichondria*, *Mycale*, and *Jaspis*, which belong to different orders. Compounds **1–3** exhibited cytotoxicities against L1210 murine leukemia cells in vitro, with IC_{50} values of <0.001, <0.001, and <0.001 μg/ml, and against KB human epidermoid carcinoma cells in vitro with IC_{50} values of 0.015, 0.006, and 0.013 μg/ml, respectively.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Optical rotations were determined on a JASCO DIP-370 polarimeter. Uv and ir spectra were obtained on a Shimadzu UV-220 spectrometer and a JASCO IR Report-100 spectrometer, respectively. ^{1}H-nmr spectra were recorded on a JEOL EX-400 spectrometer. Fabms spectra were obtained on a JEOL HX-110 spectrometer. Wako C-300 Si gel was used for glass cc. Tlc was carried out on Merck Si gel GF_{254}.

SPONGE MATERIAL.—The sponge *Jaspis* sp. (order Astrophorida; family Epipolasidae) was collected by scuba off Ishigaki Island, Okinawa and kept frozen until used. Preserved sponge has a blue-black exterior, which is smooth but wrinkled, and a yellow-fawn interior. The specimen is firm, compressible, and spongy. Oxeas occur in parallel bands at right angles to the surface or are strewn haphazardly. The oxeas are long and pointed, 634 × 12 μm, range 594–715 × 9–17 μm. No microscleres. The voucher specimen (SS-218) was deposited at the Faculty of Pharmaceutical Sciences, Hokkaido University.

COLLECTION, EXTRACTION, AND ISOLATION.—The sponge (1.0 kg, wet wt) was extracted with MeOH (1.0 liters × 2). The MeOH extract was partitioned between EtOAc (500 ml × 5) and H_2O (500 ml). The EtOAc-soluble fraction (3.1 g) was subjected to a Si gel column (4.5 × 40 cm) eluted with $CHCl_3$ (600 ml), hexane-Me_2CO (1:1) (600 ml), Me_2CO (400 ml), $CHCl_3$-EtOH (4:1) (400 ml), and $CHCl_3$-EtOH (1:1) (500 ml). The 120–220 ml fraction (0.16 g) eluted with $CHCl_3$-EtOH (4:1) was separated by a Sephadex LH-20 column [2.0 × 100 cm, $CHCl_3$-MeOH (1:1)] followed by hplc on Si gel [Senshu Pak Silica-4251-S, Senshu Scientific,

1.0 × 25 cm, $CHCl_3$/MeOH (96:4), flow rate 2.0 ml/min] to afford jaspisamides A [**1**] (5.4 mg, 0.00054% wet wt, Rt 22.5 min) and B [**2**] (0.8 mg, 0.00008%, Rt 15.2 min) together with isohalichondramide [**6**] (3.0 mg, 0.003%, Rt 30.5 min). The fraction eluted with hexane-Me_2CO (1:1) from the first Si gel column was rechromatographed on a Si gel column [1.3 × 40 cm, hexane-Me_2CO (1:1)] followed by hplc on ODS [Asahipak ODP-50, 1.0 × 25 cm, MeCN-H_2O (1:1), flow rate 2.0 ml/min] to give jaspisamide C [**3**] (2.0 mg, 0.0002%, Rt 24.0 min), halichondramide [**4**] (540 mg, 0.054%, Rt 18.6 min), and dihydrohalichondramide [**5**] (3.7 mg, 0.00037%, Rt 19.8 min).

Jaspisamide A [**1**].—A colorless solid: $[\alpha]^{17}D$ −51° (c = 0.13, MeOH); ir (neat) ν max 3350, 3150, 1700, 1650, 1460, 1380, 1100, 980, 750 cm^{-1}; uv (MeOH) λ max 244 nm (ϵ 24000); ^1H nmr see Table 1; ^{13}C nmr ($CDCl_3$) δ 13.52 (31-Me), 13.59 (8-Me), 14.39 (23-Me),, 15.47 (27-Me), 24.93 (C-28), 27.65 (NMe), 29.15 (C-21), 29.72 (C-33), 31.62 (C-22), 33.14 (C-25), 34.68 (C-27), 35.54 (C-23), 37.49 (C-4), 37.69 (C-6), 42.30 (C-2), 42.37 (C-29), 43.97 (C-8), 49.10 (C-31), 56.82 (9-MeO and 32-MeO), 58.18 (26-MeO), 61.39 (C-5), 67.12 (C-3), 74.60 (C-24), 77.23 (C-9), 81.88 (C-26), 87.38 (C-32), 111.38 (C-34), 114.89 (C-19), 128.80 (C-16), 130.07 (C-35), 133.30 (C-13), 137.02 (C-11), 137.14 (C-14 and C-17), 139.56 (C-10), 143.71 (C-20), 155.54 (C-12), 156.60 (C-15), 162.18 (C-40), 163.11 (C-18), 172.02 (C-1), 202.67 (C-7), 214.12 (C-30); fabms m/z $[M+Na]^+$ 877, $[M+H]^+$ 855, $[M+H-H_2O]^+$ 837, $[M+H-2H_2O]^+$ 819; hrfabms m/z $[M+H]^+$ 855.4382 (calcd for $C_{44}H_{63}N_4O_{13}$, 855.4392).

Jaspisamide B [**2**].—A colorless soild: $[\alpha]^{20}D$ −112° (c = 0.19, MeOH); ir (neat) ν max 3400, 3150, 1710, 1690, 1650, 1460, 1380, 1100, 970, 750 cm^{-1}; uv (MeOH) λ max 230 nm (ϵ 36000); ^1H nmr see Table 1; fabms m/z $[M+Na]^+$ 875, $[M+H]^+$ 853; hrfabms m/z $[M+H]^+$ 853.4282 (calcd for $C_{44}H_{61}N_4O_{13}$, 853.4235).

Jaspisamide C [**3**].—A colorless solid: $[\alpha]^{19}D$ −76° (c = 0.37, MeOH); ir (neat) ν max 3400, 3150, 1720, 1690, 1650, 1460, 1370, 1100, 970, 750 cm^{-1}; uv (MeOH) λ max 230 nm (ϵ 32000); ^1H nmr see Table 1; fabms m/z $[M+Na]^+$ 873, $[M+H]^+$ 851; hrfabms m/z $[M+H]^+$ 851.4451 (calcd for $C_{45}H_{63}N_4O_{12}$, 851.4442).

ACKNOWLEDGMENTS

We thank Dr. J. Fromont of James Cook University for identification of the sponge and Mr. Z. Nagahama for his help with collecting the sponge. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan.

LITERATURE CITED

1. J.A. Roesener and P.J. Scheuer, *J. Am. Chem. Soc.*, **108**, 846 (1986).
2. S. Matsunaga, N. Fusetani, K. Hashimoto, K. Koseki, and M. Noma, *J. Am. Chem. Soc.*, **108**, 847 (1986).
3. S. Matsunaga, N. Fusetani, K. Hashimoto, K. Koseki, M. Noma, H. Noguchi, and U. Sankawa, *J. Org. Chem.*, **54**, 1360 (1989).
4. M.R. Kernan, T.F. Molinski, and D.J. Faulkner, *J. Org. Chem.*, **53**, 5014 (1988).
5. M.R. Kernan and D.J. Faulkner, *Tetrahedron Lett.*, **28**, 2809 (1987).
6. N. Fusetani, K. Yasumoto, S. Matsunaga, and K. Hashimoto, *Tetrahedron Lett.*, **30**, 2809 (1989).
7. M. Tsuda, H. Shigemori, M. Ishibashi, and J. Kobayashi, *Tetrahedron Lett.*, **33**, 2597 (1992).
8. J. Kobayashi, S. Takeuchi, M. Ishibashi, H. Shigemori, and T. Sasaki, *Tetrahedron Lett.*, **33**, 2579 (1992).
9. K. Kondo, H. Shigemori, Y. Kikuchi, M. Ishibashi, T. Sasaki, and J. Kobayashi, *J. Org. Chem.*, **57**, 2480 (1992).
10. K. Ishida, M. Ishibashi, H. Shigemori, T. Sasaki, and J. Kobayashi, *Chem. Pharm. Bull.*, **40**, 766 (1992).
11. H. Shigemori, M.-A. Bae, K. Yazawa, T. Sasaki, and J. Kobayashi, *J. Org. Chem.*, **57**, 4317 (1992).
12. M. Ishibashi, R.E. Moore, G.M. Patterson, C. Xu, and J. Clardy, *J. Org. Chem.*, **51**, 5300 (1986).
13. B.N. Ravi, R.J. Wells, and K.D. Croft, *J. Org. Chem.*, **46**, 1998 (1981).
14. B.N. Ravi and R.J. Wells, *Aust. J. Chem.*, **35**, 39 (1982).
15. T. McCabe, J. Clardy, T. Minale, C. Pizza, F. Zollo, and R. Riccio, *Tetrahedron Lett.*, **23**, 3307 (1982).
16. M. Tsuda, M. Ishibashi, K. Agemi, T. Sasaki, and J. Kobayashi, *Tetrahedron*, **47**, 2181 (1991).
17. P. Crews, L.V. Manes, and M. Boehler, *Tetrahedron Lett.*, **27**, 2797 (1986).
18. T.M. Zabriskie, J.A. Klocke, C.M. Ireland, A.H. Marcus, T.F. Molinski, D.J. Faulkner, C. Xu, and J. Clardy, *J. Am. Chem. Soc.*, **108**, 846 (1986).

Received 28 August 1992