New Jaspamide Derivatives from the Marine Sponge *Jaspis splendans* Collected in Vanuatu[1]

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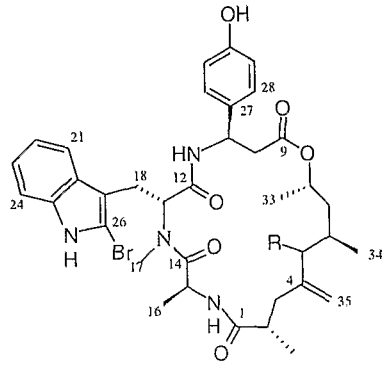
Two new jaspamide derivatives (**1** and **2**) along with jaspamide have been isolated from the marine sponge *Jaspis splendans* collected in Vanuatu. Their chemical structures were determined from 1D and 2D NMR studies and MS data. These two compounds inhibited the in vitro growth of the NSCLC-N6 human tumor cell lines with IC_{50} values in the μg/mL range.

After the discovery of the cyclic depsipeptide jaspamide (jasplakinolide) in the sponge *Jaspis* cf. *johnstoni* in 1986,[2−4] several reports of the presence of jaspamide from other sponge genera, including *Auletta* cf. *constricta*[5] and *Hemiasterella minor*,[6] appeared in the literature. Jasplakinolide B was isolated as a very minor component in two collections of *J.* cf. *johnstoni*.[7] The related geodiamolides were then isolated from sponges belonging to taxonomically distant orders, namely, from *Geodia* sp. (order Choristida),[8,9] *Cymbastela* sp. (order Axinellidae),[10,11] *H. minor* (order Hadromerida),[6] and *Neosiphonia superstes* (order Lithistida).[12] Isolation of these compounds from taxonomically remote species has supported the hypothesis of an involvement of symbionts in the production of geodiamolides.[13]

As a part of an ongoing project devoted to the isolation of bioactive metabolites from Vanuatu Island sponges, we had the opportunity to investigate the sponge *Jaspis splendans*, whose crude ethanolic extract exhibited marked activity in cytotoxic in vitro assays. The major component of *J. splendans* was found to be jaspamide, the chemical marker within the sponges of the *Jaspis* genus. In this paper, we report the isolation of jaspamide along with two new very minor analogues, **1** and **2**, which represent, to the best of our knowledge, the first examples of jaspamide derivatives with modified polyketide portions.

The lyophilized *J. splendans* sponge was extracted with methanol, and the crude extract was partitioned according to the Kupchan procedure.[14] The bioactive chloroform extract (IC_{50} < 3.3 μg/mL) was chromatographed by silica gel medium-pressure liquid chromatography (MPLC) ($MeOH/CH_2Cl_2$ 0−10%) followed by reversed-phase C-18 μ-Bondapak HPLC with 65% aqueous MeOH to give jaspamide, jaspamide B (**1**, 0.0004%, dry weight), and jaspamide C (**2**, 0.00039%, dry weight).

FABMS data [*m/z* 723−725 (1:1) $(M + H)^+$] of jaspamide B (**1**) indicated a molecular weight 14 amu more than jaspamide. NMR spectra revealed that the resonances relative to the tripeptide portion of jaspamide B (**1**) were superimposable with that found in the parent compound. At the same time, inspection of the ^1H NMR spectrum indicated a change in the polypropionate fragment. In particular, the signals relative to the trisubstituted C-4 double bond (H-5 and Me-35) were missing, whereas two one-proton singlets at δ 5.82 and 5.92 were observed, suggesting the presence of one exomethylene function. The complete structure of the polypropionate moiety of **1** was assigned on the basis of $^1H-^1H$ COSY, HMQC, and HMBC data. In particular, COSY and HMQC data allowed us to define the same C-2/C-3 and C-6/C-8 spin systems as in jaspamide while a ketone functionality (δ_C 217.0) was placed at C-5 in **1** on the basis of HMBC cross-peaks: H-7/C-5, H-6/C-5, Me-34/C-5. Both the olefinic methylene protons at δ_H 5.82 and 5.92 showed HMBC correlations with C-3 (37.5 ppm), C-4 (147.4) and C-5 (217.0) carbon signals, suggesting the presence of a conjugate enone functionality in the polyketide portion of jaspamide B (**1**). The similarity in the ^1H and ^{13}C NMR chemical shifts observed for the tripeptide portion of jaspamide B and jaspamide implied that, most likely, the chiral centers in alanine, *N*-methylbromoabrine, and β-tyrosine had the same relative configurations in both molecules.



Jaspamide B (**1**) R = =O
Jaspamide C (**2**) R = —OH

It should be noted that the same polypropionate fragment as in **1** was also found in geodiamolide G, isolated from the Papua New Guinea sponge *Cymbastela* sp.[11]

Jaspamide C (**2**) showed pseudomolecular ions at *m/z* 725−727, two mass units more than jaspamide B (**1**). The presence, in the ^1H NMR spectrum of one additional methine proton signal at δ_H 4.08 (1H, d, J = 5.9 Hz), which correlated in the HMQC spectrum with a signal at δ_C 91.5, indicated that jaspamide C was simply the C-5 dihydro derivative of **1**. The HMBC cross-peaks observed within the polypropionate unit of jaspamide C (see Figure 1) substantiated the proposed structure.

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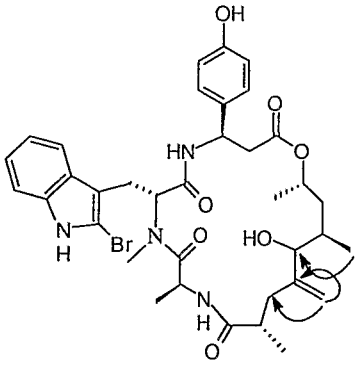

Figure 1. Jaspamide C (**2**) with key HMBC correlations.

Several attempts to obtain the MPA esters of **2** in order to solve the stereochemistry at chiral C-5 failed due to degradation of the molecule under reaction conditions.

In recent years, it has been shown that jaspamide possesses remarkable biological properties such as antifungal[2] and antiproliferative activities. In particular, it showed anthelminthic,[3] cytotoxic,[3] selective antimicrobial,[15] insecticidal,[2] and ichthytotoxic activities.[4]

Further studies have indicated that jaspamide possesses specific actin-binding properties and affects specific aspects of actin organization in cells.[7]

Jaspamides B (**1**) and C (**2**) exhibited cytotoxicity against the human NSCLC-N6 cancer cell line with IC_{50} values of 3.3 and 1.1 μg/mL, respectively. Under the same experimental conditions, jaspanide exhibited an IC_{50} value of 0.36 μg/mL. Further studies aimed to determine the effect of the structural modification in the polyketide fragment on the biological activity are in progress.

Experimental Section

General Experimental Procedures. NMR spectra were obtained on a Bruker AMX-500 NMR spectrometer (^{1}H at 500 MHz, ^{13}C at 125 MHz), δ (ppm), J in Hz, spectra referred to $CDCl_3$ as internal standard. Mass spectra were run on a VG AUTOSPEC instrument (Cs^+ ions bombardment) with a FAB source [in glycerol or glycerol−thioglycerol (3:1) matrix]. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. UV spectra were recorded on a Beckman DU70 spectrophotometer. IR spectroscopy was performed on an IFS 48 Bruker instrument. Reversed-phase HPLC was performed on a C_{18} μ-Bondapak column (30 cm × 3.9 mm i.d.; flow rate 2 mL min^{-1}) using a Waters model 6000 A pump equipped with a U6K injector and a differential refractometer, model 401.

Biological Material. The sponge was collected at Tongoa in the Vanuatu Islands in 1996 and identified as *J. splendans* (order Choristidae, family Jaspidae) by John Hooper of Queensland Museum, South Brisbane, Australia. The voucher specimen R1646 was deposited at the ORSTOM Centre in Noumèa.

Extraction and Isolation. The animals were freeze-dried, and the material (1 kg) was extracted with methanol (3 × 2 L) and filtered. The extracts were combined and partitioned according to the modified Kupchan[14] procedure as follows. The methanol extract (95 g) was dissolved in a mixture of MeOH/H_2O containing 10% H_2O and partitioned against *n*-hexane. The water content (% v/v) of the MeOH extract was adjusted to 20% and 40%, and the extract was partitioned against CCl_4 and $CHCl_3$, respectively. The aqueous phase was concentrated to remove MeOH and then extracted with *n*-BuOH. The bioactive chloroform extract (2 g) was chromatographed by silica gel MPLC (Merck Kiesegel 60, 230−400 mesh, 20 g) eluting with MeOH/CH_2Cl_2 0−10% followed by reversed-phase C_{18} μ-Bondapak HPLC with 65% aqueous MeOH to yield jaspamide (t_R = 18 min,),[2-4] jaspamide B (**1**, t_R = 6 min), and jaspamide C (**2**, t_R = 7 min). The following amounts of each compound were obtained from the chloroform extract: jaspamide (70 mg), **1** (4 mg), **2** (3.9 mg).

Jaspamide B (1) was obtained as a colorless glass: $[\alpha]^{25}_D$ +11.4° (*c* 0.0014, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 232 (3.75), 278 (3.66) nm; IR (KBr) ν_{max} 3400, 1724, 1680, 1639 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.15 (1H, s, NH-Br-Trp), 7.52 (1H, d, J = 7.9 Hz, H-21), 7.26 (1H, d, J = 7.9 Hz, H-24), 7.15 (1H, t, J = 7.9 Hz, H-23), 7.10 (1H, t, J = 7.9 Hz, H-22), 7.05 (1H, d, J = 6.8 Hz, NH-β-Tyr), 6.78 (2H, d, J = 8.3 Hz, H-28), 6.60 (2H, d, J = 8.3 Hz, H-29), 6.35 (1H, d, J = 6.9 Hz, NH−Ala), 5.92 (1H, s, H-35a), 5.82 (1H, s, H-35b), 5.68 (1H, dd, J = 7.5, 9.0 Hz, H-13), 5.23 (1H, m, H-11), 4.75 (1H, m, H-8), 4.65 (1H, m, H-15), 3.40 (1H, dd, J = 14.7, 7.5 Hz, H-18a), 3.26 (1H, dd, J = 14.7, 9.0 Hz, H-18b), 3.12 (1H, m, H-6), 3.02 (3H, s, H-17), 2.70 (2H, d, J = 5.7 Hz, H-10), 2.57 (1H, dd, J = 12.7 3.1 Hz, H-3a), 2.50 (1H, m, H-2), 2.32 (1H, t, J = 12.7 Hz, H-3b), 1.98 (1H, m, H-7a), 1.45 (1H, m, H-7b), 1.15 (3H, d, J = 5.88 Hz, H-36), 1.15 (3H, d, J = 5.70 Hz, H-33), 1.05 (3H, d, J = 6.8 Hz, H-34), 0.85 (3H, d, J = 6.8 Hz, H-16); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 217.0 (s, C-5), 176.2 (s, C-1), 173.4 (s, C-14), 170.2 (s, C-9), 169.0 (s, C-12), 155.7 (s, C-30), 147.4 (s, C-4), 135.2 (s, C-25), 133.0 (s, C-27), 128.3 (s, C-20), 127.5 (t, C-35), 127.0 (d, C-28), 120.4 (d, C-22), 122.4 (d, C-23), 118.2 (d, C-21), 115.4 (d, C-29), 110.6 (s, C-24), 110.1 (d, C-19), 109.0 (s, C-26), 69.3 (d, C-8), 55.5 (d, C-13), 49.0 (d, C-11), 45.3 (d, C-15), 40.8 (d, C-7), 40.8 (t, C-10), 40.1 (d, C-2), 37.5 (t, C-3), 36.0 (d, C-6), 30.8 (q, C-17), 23.2 (t, C-18), 20.6 (q, C-33), 18.4 (q, C-36), 17.9 (q, C-16), 17.7 (q, C-34); FABMS *m/z* 723−725 $[M + H]^+$.

Jaspamide C (2) was obtained as a colorless glass: $[\alpha]^{25}_D$ +25.4° (*c* 0.0013, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 220 (3.37), 276 (2.71) nm; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.08 (1H, s, NH-Br-Trp), 7.57 (1H, d, J = 7.3 Hz, NH-β-Tyr), 7.56 (1H, d, J = 7.3 Hz, H-21), 7.24 (1H, d, J = 7.3 Hz, H-24), 7.13 (1H, t, J = 7.3 Hz, H-23), 7.09 (1H, t, J = 7.3 Hz, H-22), 6.97 (2H, d, J = 8.3 Hz, H-28), 6.62 (2H, d, J = 8.3 Hz, H-29), 6.07 (1H, d, J = 8.4 Hz, NH−Ala), 5.80 (1H, dd, J = 10.3, 6.0 Hz, H-13), 5.18 (1H, m, H-11), 5.14 (1H, s, H-35a), 5.10 (1H, s, H-35b), 4.90 (1H, m, H-8), 4.44 (1H, m, H-15), 4.08 (1H, d, J = 5.9 Hz, H-5), 3.43 (1H, dd, J = 15.3, 6.0 Hz, H-18a), 3.15 (1H, dd, J = 15.3, 10.3 Hz, H-18b), 3.05 (3H, s, H-17), 3.01 (1H, dd, J = 15.8, 8.3 Hz, H-10a), 2.70 (1H, dd, J = 15.8, 9.9 Hz, H-10b), 2.36 (1H, dd, J = 14.5, 3.1 Hz, H-3a), 2.17 (1H, dd, J = 14.5, 8.6 Hz, H-3b), 1.62 (1H, m, H-2), 1.62 (2H, m, H-7), 1.60 (1H, m, H-6), 1.14 (3H, d, J = 6.8 Hz, H-36), 1.05 (3H, d, J = 6.3 Hz, H-33), 0.84 (3H, d, J = 6.8 Hz, H-34), 0.74 (3H, d, J = 6.6 Hz, H-16); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 175.7 (s, C-1), 174.8 (s, C-12), 174.0 (s, C-14), 170.6 (s, C-9), 155.7 (s, C-30), 140.2 (s, C-4), 135.0 (s, C-25), 133.0 (s, C-27), 128.2 (s, C-20), 127.2 (d, C-28), 122.3 (d, C-23), 120.4 (d, C-22), 119.6 (t, C-35), 118.3 (d, C-21), 117.4 (d, C-29), 110.5 (s, C-24), 110.2 (d, C-19), 109.0 (s, C-26), 91.5 (d, C-5), 70.3 (d, C-8), 55.2 (d, C-13), 49.2 (d, C-11), 45.3 (d, C-15), 41.0 (d, C-7), 40.8 (t, C-10), 40.4 (d, C-2), 36.4 (t, C-3), 30.5 (d, C-6), 30.8 (q, C-17), 23.5 (t, C-18), 20.9 (q, C-34), 20.0 (q, C-36), 19.5 (q, C-33), 17.8 (q, C-16); FABMS *m/z* 725−727 $[M + H]^+$.

Cytotoxic Assays. Experiments were performed in 96 well microtiter plates (2 × 10^5 cells/ml). Cell growth was estimated by colorimetric assay based on the conversion of tetrazolium dye (MTT) to a blue formazan product using live mitichondria.[8] Eight determinations were performed for each concentration. Control growth was estimated for 16 determinations. The optical density at 570 nm corresponding to solubilized formazan was read for each well on Titertek Multiskan MKII.

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References and Notes

(1) Presented at the VII International Symposium on Marine Natural Products, Townsville, July, 5–10, P5, 1998.
(2) Zabriskie, T. E.; Klocke, J. A.; Ireland, C. M.; Marcus, A. H.; Molinski, T. F.; Faulkner, D. J.; Xu, C.; Clardy, J. C. *J. Am. Chem. Soc.* **1986**, *108*, 3123–3124.
(3) Crews, P.; Manes, L. V.; Boehler, M. *Tetrahedron Lett.* **1986**, *27*, 2797–2800.
(4) Braekman, J. C.; Daloze, D.; Moussiaux, B., Riccio, R. *J. Nat. Prod.* **1987**, *50*, 994–995.
(5) Crews, P.; Farias, J. J.; Emrich, R.; Keifer, P. A.. *J. Org. Chem.* **1994**, *59*, 2932–2934.
(6) Talpir, R.; Benayahu, Y.; Kashman, Y.; Pannell, L.; Schleyer, M. *Tetrahedron Lett.* **1994**, *35*, 4453–4456.
(7) Senderowicz, A. M. J.; Kaur, G.; Sainz, E.; Laing, C.; Inman, W. D.; Rodríguez, J.; Crews, P.; Malspeis, L.; Grever, M. R.; Sausville, E. A.; Duncan, K. L. K. *J. Nat. Cancer Inst.* **1995**, 87, 46–51
(8) Chan, W. R.; Tinto, W. F.; Manchand, P. S.; Todaro, L. J. *J. Org. Chem.* **1987**, *52*, 3091–3093.
(9) Tinto, W. F.; Lough, A. J.; McLean, S.; Reynolds, W. F.; Yu, M., Chan, W. R. *Tetrahedron* **1998**, *54*, 4451–4458.
(10) de Silva, E. D.; Andersen, R. J.; Allen, T. M. *Tetrahedron Lett.* **1990**, *31*, 489–492.
(11) Coleman, J. E.; de Silva, E. D.; Kong, F.; Andersen, R. J. *Tetrahedron* **1995**, *51*, 10653–10662.
(12) D'Auria, M. V.; Gomez Paloma, L.; Minale, L.; Zampella, A.; Debitus, C.; Perez, J. *J. Nat. Prod.* **1995**, *58*, 121–123.
(13) Fusetani, N.; Matsunaga, S. *Chem. Rev.* **1993**, *93*, 1793–1806.
(14) Kupchan, S. M.; Britton, R. W.; Ziegler, M. F.; Sigel, C. W. *J. Org. Chem.* **1973**, *38*, 178–179.
(15) Scott, V. R.; Boehme, R.; Matthews, T. R. *Antimicrob. Agents Chemother.* **1988**, *32*, 1154–1157.

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