Cytotoxic Metabolites from an Australian Collection of the Sponge *Jaspis* Species

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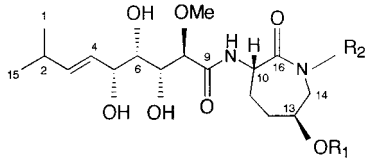
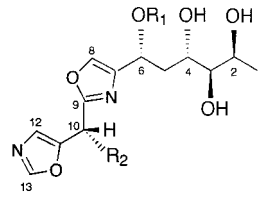
Three new natural products, bengamide Y (**1**), bengamide Z (**3**), and bengazole Z (**5**), were isolated from the aqueous extract of an Australian collection of the sponge *Jaspis* sp. Their structures were solved by spectroanalytical methods and by comparison of their spectral data with known bengamides and bengazoles that were reported from the same genus. Bengamides Y (**1**) and Z (**3**) showed a striking differential cytotoxicity pattern against a panel of 10 human tumor cell lines, with closely related cell lines (e.g., SNB-19 and SNB-75) displaying significant differences in sensitivity.

Sponges of the genus *Jaspis* (family, Jaspidae) have been a rich source of biologically active, structurally novel natural products. This genus received considerable attention after the isolation of jaspamide (jasplakinolide), a cyclic depsipeptide, by Ireland[1] and Crews.[2] Since then, the chemistry of *Jaspis* sponges has been the subject of more than 50 publications. Other constituents isolated from this genus include several related jaspamide derivatives from *Jaspis splendans*,[3] cytotoxic macrolides from an Okinawan Jaspis sp.,[4] a number of bioactive nucleosides,[5,6] isomalabricane triterpenes from *Jaspis stellifera*,[7,8] and a series of dihydroxystyrene sulfate derivatives.[9−11] These metabolites clearly demonstrate the broad chemical diversity that occurs in this sponge genus. The most common secondary metabolites obtained from *Jaspis* species are two groups of heterocyclic compounds, the bengamides and the bengazoles. Bengamides A (**2**) and B (**4**) were originally isolated from *Jaspis* cf. *coriacea*,[12] and their structures were elucidated by NMR and other spectral methods. Crews et al. determined the relative and absolute configurations of the bengamides and also reported several additional derivatives including bengamides C and D, which contain different alkoxy groups substituted at C-13; bengamides E and F, which lack any substituent at that position; and isobengamide E.[13,14] Closely related compounds were also reported from a New Caledonian collection of *Jaspis carteri*[15] and from a South African sample of *Jaspis digonoxea*.[16] A second abundant group of heterocyclic metabolites isolated from *Jaspis* species is that of the bengazoles. This group contains both bis(oxazolyl)methanol compounds, like bengazole A (**6**),[17] and bis(oxazolyl)-methane compounds, such as digonazole (**7**).[16] These compounds are all substituted by either a tetrahydroxylated or alkoxylated six-carbon chain. The bengazoles have been reported in a number of studies[17−19] and thus, along with the bengamides, constitute the two most abundant groups of natural products from these sponges.

Our investigation was initiated when both the organic (1:1 CH_2Cl_2−CH_3OH) and the aqueous crude extracts of a *Jaspis* sample were found to be active in the NCI's 60-cell line, human-disease-oriented, in vitro antitumor screen.[20] The sponge specimen occurred as yellow encrusting colonies, and it was collected in August 1988, near Serrurion Island, in northwest Australia. The sponge taxonomy was established as *Jaspis* sp. by R. Van Soest. Bioassay-guided fractionation of a 5-g portion of the aqueous extract commenced with a precipitation step using 50% EtOH at −20 °C. The aqueous EtOH supernatant was fractionated by C_4 flash chromatography. Final purification by reversed-phase C_{18} HPLC provided, in order of elution, 2.4 mg of bengazole Z (**5**), 1.6 mg of bengamide Y (**1**), and 1.6 mg of bengamide Z (**3**).



Bengamide:	R_1	R2
Y (1)	H	H
A (2)	$-CO(CH_2)_{12}CH_3$	H
Z (3)	H	CH_3
B (4)	$-CO(CH_2)_{12}CH_3$	CH_3



	R_1	R_2
Bengazole Z (5)	H	H
Bengazole A (6)	H	$-OCO(CH_2)_{12}CH_3$
Digonazole (7)	$-CO(CH_2)_{19}CH_3$	H

The molecular formula of bengamide Z (**3**), $C_{18}H_{32}N_2O_7$, was determined by HRFABMS [$(M + H)^+$ *m/z* 389.2293]. The IR spectrum revealed strong absorption bands indicative of hydroxyl (3368 cm^{-1}) and amido (1643 cm^{-1})

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functionalities. Owing to the limited supply of compound, the ^{13}C NMR spectrum of **3** exhibited only 16 carbons and lacked any carbonyl resonances. However, the presence of two amido carbonyls was revealed by appropriate cross-peaks in an HMBC experiment. The structure of **3** was elucidated primarily by interpretation of 2D NMR data, which included COSY, HSQC, HSQC−TOCSY, and HMBC experiments. These data clearly showed the presence of a moiety that contained 10 carbon atoms and four oxygens [$(CH_3)_2$−CH−CH=CH−CH(OH)−CH(OH)−CH(OH)−CH(OMe)] and a second fragment [−CH(X)−CH_2−CH_2−CH(Y)−CH_2−CH(Z)], where X, Y, and Z represent oxygen or nitrogen heteroatoms. Comparison of the entire NMR data set for compound **3** (see Experimental Section) with spectral data from known *Jaspis* metabolites[13] revealed that it belonged to the bengamide family of compounds. The only difference between **3** and the previously reported bengamide compounds was the lack of an ester substituent at C-13. The presence of a secondary hydroxyl group at this position in compound **3** was indicated by the H-13 NMR resonance at δ 3.55, which was observed at approximately 1 ppm higher field than the H-13 signal in the other bengamides (e.g., H-13 in bengamide B (**4**) resonates at δ 4.55). Compound **3** is, therefore, the nonacylated parent molecule in the bengamide B series, which includes all bengamides with an *N*-methyl group at position 15. Based on the precedent with the bengazole series,[18] we suggest naming this molecule bengamide Z. Strong similarities between the proton−proton coupling constants observed in **3** and those reported for bengamide B (**4**),[12,13] also suggested that the relative configuration of **3** is identical to that in **4**. Interestingly, a compound with the same structure as bengamide Z was reported by Crews; however, the authors stressed that it was an "artifact of the acid-catalyzed fragmentation of bengamide D".[13]

Bengamide Y (**1**), which provided NMR spectra very similar to those of bengamide Z (**3**), was shown by HRFABMS to have the composition $C_{17}H_{30}N_2O_7$. Analysis of 2D NMR data established the same functional groups as in **3**, with the only difference being the lack of an *N*-methyl group at position 15. The co-occurrence of cyclic amido and *N*-methyl amido congenors, bengamide Z (**3**) and bengamide Y (**1**), respectively, is consistent with observations made in previous studies of bengamide compounds. Again, a compound with the same structure as bengamide Y was described as the product of an acidic hydrolysis of bengamide C.[13]

The third compound (**5**) showed different spectral properties from the two bengamides. Its molecular composition was determined by HRFABMS as $C_{13}H_{18}N_2O_6$. Extensive 2D NMR experiments (COSY, HSQC, and HMBC) revealed the presence of the fragment CH_3−CH(O)−CH(O)−CH(O)−CH_2−CH(O)−, which occurs in the bengazole series of compounds. The heterocyclic portion of the latter structure was characterized by three sharp aromatic singlets at δ 8.14, 7.74, and 7.06.[17] Another very unique signal was that due to the C-10 methylene at δ 25.6, which corresponds in the HSQC experiment to the doubly allylic CH_2 at δ 4.28. Bengazole Z (**5**) was previously prepared by hydrolysis of an unseparated mixture of bengazoles.[18] The material that we isolated had identical spectral properties to those reported for bengazole Z.

Although a large number of bengamides and bengazoles have previously been disclosed, this is the first report that the parent, more polar molecules that are devoid of the typical long fatty-acid moieties occur as natural products in the aqueous extract of the sponge.[21] Because molecules with the same structures as **1** and **3** have previously been reported as artifacts,[13] it was important to determine if compounds **1**, **3**, and **5** indeed occur as natural products. Separation of a crude, untreated aqueous extract by HPLC, under the same separation conditions used to purify the individual components, showed that the peaks that correspond with these three compounds were clearly detected in the chromatogram. Moreover, examination of the CH_2Cl_2−MeOH extract of the same sponge revealed the presence of bengamides A and B (**2** and **4**) and several other nonpolar bengamides. Because the aqueous extract was prepared from the frozen sponge prior to the organic extraction, the more hydrophilic bengamides were extracted by water, while the less polar compounds were found only in the organic extract.

Table 1. Testing Results of Bengamide Y and Bengamide Z

tumor type	cell-line	bengamide Y (**1**) IC_{50} (μg/mL)	bengamide Z (**3**) IC_{50} (μg/mL)
CNS	SNB-75	>40	>40
CNS	SNB-19	0.68	0.56
colon	HCT-116	0.8	4.0
colon	HCT-15	>40	>40
melanoma	LOX	4.4	2.1
melanoma	MALME−3	>40	>40
NSCLC	A549	4.8	4.1
NSCLC	HOP-92	>40	>40
ovarian	OVCAR-3	4.6	0.52
renal	UO-31	9.9	7.2

The three compounds were tested in vitro against a panel of 10 cell lines that were derived from six cancer types (CNS, colon, melanoma, lung, ovarian, and renal). The results are shown in Table 1 as IC_{50} concentrations. Bengamides Y (**1**) and Z (**3**) were shown to possess moderate-to-high differential cytotoxicities, with levels as low as 0.5−0.8 μg/mL. Particulary striking in the cytotoxicity results are the large differences in IC_{50} values between very closely related cell lines. For example, both **1** and **3** were inactive against the CNS cell line SNB-75 and yet were active at 0.68 and 0.56 μg/mL against SNB-19. The same sort of specificity between cell lines from the same tumor panel was shown with colon (HCT-15 and HCT-116), melanoma (MALME−3 and LOX), and nonsmall cell lung cancer (NSCLC) (HOP-92 and A549). Though the bengamides have previously been reported to possess anthelmintic activity,[12] this is the first report of the antitumor activity of this class of compounds. The third compound, bengazole Z (**5**) was found to be completely inactive in our antitumor testing, which is similar to the results reported previously by Crews.[18]

Experimental Section

General Experimental Procedures. NMR spectra were recorded on a Varian Unity-INOVA 500 spectrometer using CD_3OD as solvent and referenced to the residual solvent signal. IR spectra were measured on a Perkin−Elmer spectrum-2000 FT-IR spectrometer, and UV spectra were obtained with a Beckman DU-640 spectrophotometer. Optical rotations were measured on a Perkin−Elmer 241 polarimeter. FABMS were recorded on a JEOL SX-102 mass spectrometer.

Animal Material. *Jaspis* sp. (order Choristida, phylum Porifera) was collected by Dr. Peter Murphy on August 20, 1988, at a depth of 18 m off a low, uninhabited rocky island near northwestern End Long Island (Serrurion Island, Australia 744; longitude 114° 39.0" E, latitude 21° 34.5" S), Australia, by scuba. A voucher of the collection (no. Q66C1375) was identified by R. Van Soest and is stored at the Smithsonian Institute Sorting Center, Suitland, MD.

Isolation of Bengamide Y (1), Bengamide Z (3), and Bengazole Z (5). The crude aqueous extract (5.0 g) of *Jaspis* sp. was dissolved in H_2O and precipitated by addition of an

equal volume of EtOH. The aqueous EtOH supernatant was chromatographed on a C_4 column (1 × 5 cm), using increasing concentrations of MeOH in H_2O. The fraction that eluted with MeOH−H_2O (2:1) was further purified by HPLC (C_{18}, 7 × 300 mm, 5μ, Rainin−Dynamax) using a linear gradient of CH_3CN in H_2O (0 to 45% CH_3CN over 40 min) to provide bengamide Y (**1**) (1.6 mg), bengamide Z (**3**) (1.6 mg), and bengazole Z (**5**) (2.4 mg) as colorless, viscous oils.

Bengamide Y (1): $[\alpha]_D$ +14° (*c* 0.11, MeOH); UV (MeOH) end absorption; IR (neat) ν_{max} 3362, 2929, 1648, 1538, 1454, 1405, 1203, 1112, 976 cm^{-1}; ^{13}C NMR (CD_3OD) δ 172 (C-9 & C-16), 142.1 (C-3), 127.6 (C-4), 83.5 (C-8), 75.0 (C-5), 74.3 (C-6), 72.6 (C-7), 70.5 (C-13), 58.7 (OCH_3), 52.9 (C-10), 49.7 (C-14), 37.6 (C-12), 32.1 (C-2), 30.2 (C-11), 22.7 and 22.6 (C-1 & C-15); ^{1}H NMR (CD_3OD) δ 7.33 (d, J = 7 Hz, NH), 7.26 (d, J = 7 Hz, NH), 5.73 (dd, J = 15.6, 6.6 Hz, H-3), 5.43 (dd, J = 15.6, 7.5 Hz, H-4), 4.61 (d, J = 11.0 Hz, H-10), 4.11 (t, J = 7 Hz, H-5), 3.81 (d, J = 7.3 Hz, H-8), 3.75 (brd, J = 7.3 Hz, H-7), 3.55 (dd, J = 7.0, 2.2 Hz, H-6), 3.48 (m, J = 11, 10.3 Hz, H-13), 3.40 (s, 3H, OCH_3), 3.29 (dd, J = 14.7, 10.3 Hz, H-14), 3.17 (bd, J = 14.7 Hz, H-14′), 2.30 (m, J = 7, 6.6 Hz, H-2), 2.17 (m, H-12), 2.02 (m, J = 11 Hz, H-11), 1.78 (m, J = 13.6, 11, 3 Hz, H-12′), 1.68 (m, J = 13.2, 11.0, 2.2 Hz, H-11′), 1.00 (d, J = 7.0, 6H, CH_3-1 and -15); HRFABMS [M + H]$^+$ obs. *m*/*z* 375.2131 (calcd 375.2131 for $C_{17}H_{31}N_2O_7$).

Bengamide Z (3): $[\alpha]_D$ +45° (*c* 0.11, MeOH); UV (MeOH) end absorption; IR (neat) ν_{max} 3368, 2930, 1643, 1521, 1456, 1406, 1203, 1107, 975 cm^{-1}; ^{13}C NMR (CD_3OD) δ ~174 (C-16), ~173 (C-9), 142.1 (C-3), 127.6 (C-4), 83.5 (C-8), 75.0 (C-5), 74.3 (C-6), 72.6 (C-7), 68.4 (C-13), 58.7 (OCH_3), 57.5 (C-14), 52.9 (C-10), 37.2 (C-12), 36.7 (NCH_3), 32.1 (C-2), 30.2 (C-11), 22.7 (C-1), 22.6 (C-15); ^{1}H NMR (CD_3OD) δ 7.33 (d, J = 6.7, NH), 7.33 (d, J = 6.7 Hz, NH), 7.26 (d, J = 6.7, NH?), 5.73 (dd, J = 15.6, 6.3 Hz, H-3), 5.43 (dd, J = 15.6, 7.6 Hz, H-4), 4.71 (d, J = 10.5 Hz, H-10), 4.11 (t, J = 7.2 Hz, H-5), 3.81 (d, J = 7.1 Hz, H-8), 3.75 (dd, J = 7.1, 2.1 Hz, H-7), 3.68 (dd, J = 14.7, 10.1 Hz, H-14), 3.55 (m, 2H, J = 7.0, 2.1 Hz, H-6), 3.55 (m, 2H, J = 6.7, ~2 Hz, H-13), 3.40 (s, 3H, OCH_3), 3.21 (brd, J = 14.7 Hz, H-14′), 3.03 (s, 3H, NCH_3), 2.29 (m, J = 6.3 Hz, H-2), 2.14 (brd, J = 13 Hz, H-12), 2.00 (bd, J = 13 Hz, H-11), 1.77 (dq, J = 13.2, 3.6 Hz, H-12′), 1.62 (dq, J = 13.0, ~2 Hz, H-11′), 1.00 (d, J = 6.3, 6H, H_3-1 and -15); COSY H_3-1 and -15 2.29; H-2, 1.00, 5.73; H-3, 5.43, 2.29; H-4, 5.73, 4.11; H-5, 5.43, 3.55; H-6, 4.11; H-7, 3.81; H-8, 3.75, 3.40 (LR = long-range); H-10, 1.62, 2.00; H-11, 1.62, 2.14, 1.77, 4.71; H-11′, 2.00, 4.71, 1.77, 2.14; H-12, 1.77, 3.55, 2.00, 1.62; H-12′, 2.14, 3.55, 2.00, 1.62; H-13, 1.77, 2.14, 3.68, 3.21; H-14, 3.21, 3.55; H-14′, 3.68, 3.55; OCH_3, 3.81 (LR); NH, 7.26; NH′,7.33 (LR); HMBC H-1, C-2, C-3, C-15; H-15, C-2, C-3, C-1; H-2, C-1, C-15, C-3, C-4; H-3, C-1, C-15, C-2, C-5; H-4, C-2, C-5; H-5, C-3, C-4, C-6; H-6, C-5; H-7, C-8, C-9; H-8, C-6, C-7, C-9, OCH_3; H-10, C-16; H-11, C-12; H-12, C-13; H-12′, C-13; H-13, C-14; H-14, C-12, C-13, NCH_3; H-14′, C-12, C-13, C-16; OCH_3, C-8; NCH_3, C-14, C-16; HRFABMS [M + H]$^+$ obs *m*/*z* 389.2293 (calcd 389.2288 for $C_{18}H_{33}N_2O_7$).

Bengazole Z (5): $[\alpha]_D$ −7.5° (*c* 0.17, MeOH); UV (MeOH) 217 nm (ϵ = 6000); IR (neat) ν_{max} 3357, 2926, 1668, 1399, 1203, 1112 cm^{-1}; ^{13}C NMR and ^{1}H NMR were identical with the previously reported data;[18] HRFABMS [M + H]$^+$ obs *m*/*z* 299.1257 (calcd 299.1238 for $C_{13}H_{19}N_2O_6$).

Cytotoxicity Testing. Analysis of the *Jaspis sp*. extract's cytotoxicity was by the NCI's in vitro 60-cell solid-tumor screening panel as reported previously.[20,22] Evaluation of chromatography fractions and testing of the purified bengamides was achieved by a 2-day cytotoxicity assay as reported previously. [23]

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References and Notes

(1) Zabriskie, T. M.; 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.
(2) Crews, P.; Manes, L. V.; Boehler, M. *Tetrahedron Lett.* **1986**, *27*, 2797−2800.
(3) Zampella, A.; Giannini, C.; Debitus, C.; Roussakis, C.; D'Auria, M. V. *J. Nat. Prod.* **1999**, *62*, 332−334.
(4) Kobayashi, J.; Murata, O.; Shigemori, H.; Sasaki, T. *J. Nat. Prod.* **1993**, *56*, 787−791.
(5) Zabriskie, T. M.; Ireland, C. M. *J. Nat. Prod.* **1989**, *52*, 1353−1356.
(6) Yagi, H.; Matsunaga, S.; Fusetani, N. *J. Nat. Prod.* **1994**, *57*, 837−838.
(7) Ravi, B. N.; Wells, R. J. *Aust. J. Chem.* **1982**, *35*, 39−50.
(8) Tsuda, M.; Ishibashi, M.; Agemi, K.; Sasaki, T.; Kobayashi, J. *Tetrahedron* **1991**, *47*, 2181−2194.
(9) Ohta, S.; Kobayashi, H.; Ikegami, S. *Tetrahedron Lett.* **1994**, *35*, 4579−4580.
(10) Tsukamoto, S.; Kato, H.; Hirota, H.; Fusetani, N. *Tetrahedron Lett.* **1994**, *35*, 5873−5874.
(11) Tsukamoto, S.; Kato, H.; Hirota, H.; Fusetani, N. *Tetrahedron* **1994**, *50*, 13583−13592.
(12) Quinoa, E.; Adamczeski, M.; Crews, P.; Bakus, G. J. *J. Org. Chem.* **1986**, *51*, 4494−4497.
(13) Adamczeski, M.; Quinoa, E.; Crews, P. *J. Am. Chem. Soc.* **1989**, *111*, 647−654.
(14) Adamczeski, M.; Quinoa, E.; Crews, P. *J. Org. Chem.* **1990**, *55*, 240−242.
(15) D'Auria, M. V.; Giannini, C.; Minale, L.; Zampella, A.; Debitus, C.; Frostin, M. *J. Nat. Prod.* **1997**, *60*, 814−816.
(16) Rudi, A.; Kashman, Y.; Benayahu, Y.; Schleyer, M. *J. Nat. Prod.* **1994**, *57*, 829−832.
(17) Adamczeski, M.; Quinoa, E.; Crews, P. *J. Am. Chem. Soc.* **1988**, *110*, 1598−1602.
(18) Rodriguez, J.; Nieto, R. M.; Crews, P. *J. Nat. Prod.* **1993**, *56*, 2034−2040.
(19) Searle, P. A.; Richter, R. K.; Molinski, T. F. *J. Org. Chem.* **1996**, *61*, 4073−4079.
(20) Boyd, M. R.; Paull, K. D. *Drug Dev. Res.* **1995**, *34*, 91−109.
(21) During review of the manuscript a paper describing the same compounds was published: Fernandez, R.; Dherbomez, M.; Letourneux, Y., Nabil, M.; Verbist, J. F.; Biard, J. F. *J. Nat. Prod.* **1999**, *62*, 678−680.
(22) Acton, E. M.; Narayanan, V. L.; Risbood, P.; Shoemaker, R. H.; Vistica, D. T.; Boyd, M. R. *J. Med. Chem.* **1994**, *37*, 2185−2189.
(23) Scudiero, D.; Shoemaker, R. H.; Paull, K. D.; Alley, M.; Monks, A.; Tierney, S.; Nofzinger, T.; Currens, M.; Burkew, D.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 4827−4833.

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