Bengamides and Related New Amino Acid Derivatives from the New Caledonian Marine Sponge *Jaspis carteri*

M. Valeria D'Auria,*[,†] Clelia Giannini,[†] Luigi Minale,[†] Angela Zampella,[†] Cécile Debitus,[‡] and Maryvonne Frostin[§]

Dipartimento di Chimica delle Sostanze Naturali, Università degli Studi di Napoli Federico II, via D. Montesano 49, 80131 Napoli, Italy, ORSTOM, Centre de Nouméa, New Caledonia, and Universitè Française du Pacifique, Centre de Nouméa New Caledonia

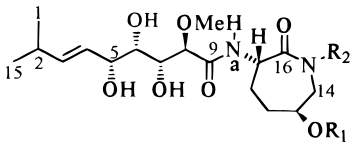
Received January 15, 1997[⊗]

Five new amino acid derivatives were isolated from the New Caledonian sponge *Jaspis carteri*, together with known bengamides A and B. The structures of the new compounds were determined by interpretation of their spectral data and by comparison with spectral data of known bengamides. Compounds **4**−**7** are simply the tridecanoate and pentadecanoate analogues of the original bengamides A and B, whereas compound **8** is a caprolactam formamide derivative of bengamide B.

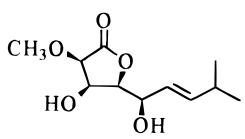
Sponges of the order Choristida are a rich source of cyclodepsipeptides, such as jaspamide from *Jaspis* sp.[1,2] and geodiamolides A and B from *Geodia* sp.,[3] and amino acid derivatives, such as the bengamides.[4] In the course of our investigation on bioactive metabolites from marine sponges collected in New Caledonia, we had the opportunity to investigate the extracts of the sponge *Jaspis carteri* (family Jaspidae, order Choristida, class Demospongiae) that showed a remarkable anticandidal activity. Analysis of the lipid extract of the sponge showed a close resemblance of chemical composition with that reported for the sponge *Jaspis* cf. *coriacea* extensively studied by Crews's group.[4,5] Along with the previously known major metabolites, bengamides A (**1**) and B (**2**), later also found, together with the lactone **3**, in one more *Jaspis* sponge (*Jaspis digonoxea*) by Kashman's group,[6] we isolated five new derivatives, named bengamides G−K (**4**−**8**). Compounds **4**−**7** are simply C1 homologs of **1** and **2**, whereas compound **8** is related to bengamide B (**2**) by having a formyl group replacing the 2-methoxy-3,4,5-trihydroxy-8-methylnon-6(*E*)-enoyl side chain. Despite their very similar chemical compositions, a side-by-side comparison of the two sponges (that is, *J.* cf. *coriacea* and *J. carteri*) indicated that they are, in fact, different species.[7] This indicated that bengamides are characteristic compounds of the *Jaspis* genus.

The lyophilized sponge was extracted in a Soxhlet apparatus with *n*-hexane followed by CH_2Cl_2 and then with CH_2Cl_2−MeOH 8:2 at room temperature. The CH_2Cl_2−MeOH 8:2 fraction was chromatographed by Si gel MPLC ($CHCl_3$−MeOH) followed by reversed-phase C_{18} μ-bondapak HPLC (MeOH−H_2O 85:15) to give compounds **4**−**8** and major amounts of the previously isolated bengamides A (**1**) and B (**2**).

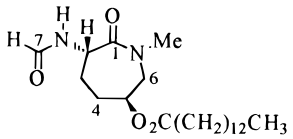
NMR data of bengamide G (**4**) were very close to those of bengamide A, and FABMS spectrum showed a quasi-molecular ion at *m*/*z* 571 $[M + H]^+$, 14 mass units lower than bengamide A. The base peak at *m*/*z* 341 (M^+ + 2H − $C_{11}H_{19}O_5$), already seen in the spectrum of bengamide A and assigned as due to the loss of the amide side chain, established that **4** differs from bengamide A by the presence at C13 of a tridecanoyl acyl moiety instead of C14.



	R_1	R_2
1	$-CO(CH_2)_{12}CH_3$	H
2	$-CO(CH_2)_{12}CH_3$	CH_3
4	$-CO(CH_2)_{11}CH_3$	H
5	$-CO(CH_2)_{11}CH_3$	CH_3
6	$-CO(CH_2)_{13}CH_3$	H
7	$-CO(CH_2)_{13}CH_3$	CH_3



3



8

Analogously, bengamide H (**5**) proved to be the C1 lower homolog of bengamide B, while bengamides I (**6**) and J (**7**) were determined as higher homologs of **1** and **2**, respectively. The presence of fatty acids with odd chain lengths (C13 in **4** and **5** and C15 in **6** and **7**) in these metabolites gives support to Crews's hypothesis[8] that bengamides are sponge symbiotic products.

Mass spectral, *m*/*z* 397 $[M + H]^+$, and NMR data of bengamide K (**8**) were consistent with the molecular composition $C_{22}H_{40}O_4N_2$. Analysis of ^1H- and ^{13}C-NMR spectra and interpetration of the $^1H-^1H$ COSY NMR data established the relationship between bengamide K and the other bengamides. All signals assigned to the acylated cyclized δ-hydroxylysine (see Experimental Section), in the ^1H-NMR spectrum of **8**, were present,

* To whom correspondence should be addressed. Phone: 39-81-7486528 (527). FAX: 39-81-7486552.
† Università degli Studi di Napoli, Federico II.
‡ ORSTOM, Noumèa.
§ Universitè Française du Pacifique.
⊗ Abstract published in *Advance ACS Abstracts,* August 1, 1997.

S0163-3864(97)00050-5 CCC: $14.00

whereas the signals for the hydroxylated side chain were missing. A new signal at δ_H 8.19 s, together with the ^{13}C signal at δ 160.1, was consistent with the presence in the molecule of an *N*-formyl moiety, and this was confirmed by the presence of a fragmentation peak at *m/z* 369 [$MH^+ - 28$] in the FABMS. Thus, we assigned the structure **8** to bengamide K. This assignment was also supported by the presence of two key fragmentation peaks in the EIMS spectrum: *m/z* 228 ($C_{14}H_{28}O_2$, myristic acid) and *m/z* 168 ($C_8H_{12}N_2O_2$, loss of the fatty acid moiety).

We assume on the basis of comparison of their NMR spectra with those of bengamide B that the new bengamides (**4**−**8**) possess the same stereostructure as that of bengamide B. This structure was initially proposed based on non X-ray methods,[9] and definitively secured by enantioselective synthesis.[10]

Bengamides and their analogues were found to be active against eukariotic cells, nematodes, and bacteria.[8] A bioassay-guided fractionation of the lipophilic extracts of *Jaspis carteri* indicated that the anticandidal activity exhibited by the crude extracts of the sponge was present in the fraction enriched in bengamides. However, when we tested all pure compounds isolated from *Jaspis carteri* for anticandidal activity, they were found to be inactive at a dose of 100 μg/mL dose. These findings suggest that any activity of the bengamides must be due to a synergistic effect or to another, yet unidentified, component in the fraction enriched in bengamides.

Experimental Section

General Experimental Procedures. NMR spectra: Bruker AMX-500 (^1H at 500 MHz, ^{13}C at 125 MHz), δ (ppm), *J* in Hz, spectra referred to $CDCl_3$ as internal standard; mass spectra on VG AUTOSPEC instruments (Cs^+ ions bombardment) with FAB source [in glycerol or glycerol−thioglycerol (3:1) matrix]; optical rotations were measured on a Perkin-Elmer 141 polarimeter; reversed-phase HPLC, C_{18} μ-Bondapak column (30 cm × 8 mm i.d.; flow rate 5 mL min^{-1}) Waters Model 6000 A or 510 pump equipped with U6K injector and a differential refractometer, Model 401.

Biological Material. The animals were collected several times in 1991−1995 off the east coast of New Caledonia and identified as *J. carteri* by John Hooper of Queensland Museum, South Brisbane, Australia. A voucher sample is kept at Queensland Museum of South Brisbane under the reference number QMG306743. Taxonomic examination of this voucher specimen revealed the following properties: it is a thinly encrusting, soft orange sponge. The dermal membrane contains asters that are oxyaster euasters with spined rays. They measure 10−30 μm in diameter. The strongyles form vague reticulate tracts and sinuous plumose columns. The megascleres are spongyloxeas, 360−450 × 6−10 μm, and only slightly curved at the center or straight with rounded or telescoped points. A direct comparison of our sample with the voucher specimen of the bengamide-containing sponge described by Quiñoá *et al.*,[4] showed that they were different species.

Extraction and Isolation of Bengamides. The animals were freeze-dried and the material (500 g) was sequentially extracted with *n*-hexane, and CH_2Cl_2 in a Soxhlet apparatus, then with 8:2 CH_2Cl_2−MeOH at room temperature. The *n*-hexane fraction (8.8 g) was chromatographed on a Si gel column (Merck Kieselgel 60, 230−400 mesh, 200 g) eluting with $CHCl_3$ and increasing amounts of MeOH. Fractions eluted with $CHCl_3$−MeOH 998:2 contained compound **7**, fractions eluted with $CHCl_3$−MeOH 996:4 contained lactone **3**, and fractions eluted with $CHCl_3$−MeOH 9:1 contained bengamides A (**1**) and B (**2**) together with their homologs **4**−**6**. Lactone **3** was further purified by reversed-phase HPLC using MeOH−H_2O 4:6 as eluent. The other fractions were purified by reversed-phase HPLC using MeOH−H_2O 85:15 as eluent. The CH_2Cl_2 fraction (2.8 g) displayed a very similar chromatographic behavior to the *n*-hexane fraction and was treated in the same manner.The following amounts of each compound were obtained from the hexane extracts: **1** (42 mg), **2** (24 mg), **3** (10.2 mg), **4** (4.8 mg), **6** (2.0 mg), **7** (4.4 mg), **8** (2.9 mg); from the CH_2Cl_2 extracts: **1** (18.2 mg), **2** (68.5 mg), **4** (2.4 mg), **5** (10.6 mg), **6** (9.5 mg), **7** (15.3 mg).

Bengamide G (4): $[\alpha]^{25}_D$ +14.0° (*c* 0.1, MeOH); FABMS *m/z* (rel int) 571 [$M^+ + H$] (80), 553 [$M^+ + H - H_2O$] (19), 503 [$M^+ + 2H - C_5H_9$] (8), 453 [$M^+ - C_6H_{11}O$] (12), 441 [$M^+ - C_7H_{13}O_2$] (51), 412 [$M^+ + H - C_8H_{15}O_3$] (23), 367 [$M^+ - C_{10}H_{19}O_4$] (24), 341 [$M^+ + 2H - C_{11}H_{19}O_5$] (100); ^1H NMR ($CDCl_3$, 500 MHz) δ 7.99 (1H, *J* = 6.5, N-Ha), 5.99 (1H, t, *J* = 6.5, N-Hb), 5.79 (1H, dd, *J* = 15.4, 6.6, H-3), 5.45 (1H, dd, *J* = 15.4, 6.9, H-4), 4.63 (1H, dt, *J* = 10.3, 2.5, H-13), 4.60 (1H, m, H-10), 4.22 (1H, t, *J* = 6.6, H-5), 3.80 (2H, m's, H-7 and H-8), 3.60 (1H, brs, H-6), 3.51 (3H, s, OMe), 3.38 (1H, ddd, *J* = 15.1, 9.9, 6.5, H-14), 3.31 (1H, dd, *J* = 15.1, 6.5, H-14), 2.31 (2H, t, *J* = 7.5, H-18), 2.31 (1H, m, H-2), 2.15 (1H, m, H-12), 1.97 (1H, m, H-12), 1.62 (2H, m, H-19), 1.2−1.4 (H-20 to H-30, s's), 1.0 (3H, d, *J* = 6.3, Me-1), 0.99 (3H, d, *J* = 6.3, Me-15), 0.88 (3H, t, *J* = 6.5, Me-30,); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 174.2 (s, C-17), 173.0 (s, C-16), 172.0 (s, C-9), 141.8 (d, C-3), 125.4 (d, C-4), 81.2 (d, C-8), 74.2 (d, C-5), 72.6 (d, C-7), 72.5 (d, C-6), 70.7 (d, C-13), 59.8 (q, OMe), 51.4 (d, C-10), 45.0 (t, C-14), 34.3 (t, C-18), 32.9 (t, C-12), 30.8 (d, C-2), 24.8 (t, C-19), 22.8 (t, C-28), 22.2 (q, C-15), 22.3 (q, C-1), 14.2 (q, C-29).

Bengamide H (5): $[\alpha]^{25}_D$ +9.2° (*c* 0.1, MeOH); FABMS *m/z* (rel int) 585 [$M^+ + H$] (61), 567 [$M^+ + H - H_2O$] (13), 549 [$M^+ - 2H_2O$] (12), 531 [$M^+ - 3H_2O$] (8), 467 [$M^+ - C_6H_{11}O - H_2O$] (8), 455 [$M^+ - C_7H_{13}O_2$] (38), 426 [$M^+ + H - C_8H_{15}O_3$] (20), 381 [$M^+ - C_{10}H_{19}O_4$] (29), 355 [$M^+ + 2H - C_{11}H_{19}O_5$] (100); ^1H NMR δ ($CDCl_3$) 8.12 (1H, *J* = 6.2, N-H), 5.77 (1H, dd, *J* = 15.4, 6.6, H-3), 5.45 (1H, dd, *J* = 15.4, 6.9, H-4), 4.63 (1H, m, H-10), 4.60 (1H, dt, *J* = 10.3, 2.5, H-13), 4.22 (1H, t, *J* = 6.6, H-5), 3.80 (2H, m's, H-7 and H-8), 3.66 (1H, ddd, *J* = 15.1, 9.9, 6.5, H-14), 3.59 (1H, brs, H-6), 3.49 (3H, s, OMe), 3.23 (1H, dd, *J* = 15.1, 6.5, H-14), 3.10 (3H, s, NMe), 2.31 (2H, t, *J* = 7.5, H-18), 2.31 (1H, m, H-2), 2.15 (1H, m, H-12), 1.97 (1H, m, H-12), 1.62 (2H, m, H-19), 1.2−1.4 (H-20 to H-30, s's), 1.0 (3H, d, *J* = 6.3, Me-1), 0.99 (3H, d, *J* = 6.3, Me-15), 0.88 (3H, t, *J* = 6.5, Me-30,); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.0 (s, C-17), 171.8 (s, C-16), 171.8 (s, C-9), 141.8 (d, C-3), 125.3 (d, C-4), 80.8 (d, C-8), 74.3 (d, C-5), 72.8 (d, C-7), 72.3 (d, C-6), 69.1 (d, C-13), 60.0 (q, OMe), 53.3 (t, C-14), 51.3 (d, C-10), 36.4 (q, NMe), 34.3 (t, C-18), 32.6 (t, C-12), 30.8 (d, C-2), 24.8 (t, C-19), 22.6 (t, C-28), 22.2 (q, C-15), 22.1 (q, C-1), 14.2 (q, C-29).

Bengamide I (6): $[\alpha]^{25}_D$ +32.4° (*c* 0.7, MeOH); FABMS *m/z* (rel int) 599 [M^+ + H] (40), 581 [599 − H_2O] (14), 531 [M^+ + 2H − C_5H_9])(65), 481 [M^+ − $C_6H_{11}O$ − H_2O] (8), 469 [M^+ − $C_7H_{13}O_2$] (50), 440 [M^+ + H − $C_8H_{15}O_3$] (25), 395 [M^+ − $C_{10}H_{19}O_4$] (23), 369 [M^+ + 2H − $C_{11}H_{19}O_5$] (100); ^{1}H NMR and ^{13}C NMR, see bengamide G.

Bengamide J (7): $[\alpha]^{25}_D$ +33.0° (*c* 0.1, MeOH); FABMS *m/z* (rel int) 613 [M^+ + H] (18), 559[M^+ − $3H_2O$] (10), 483[M^+ + H − $C_6H_{11}O$ − H_2O] (40), 454-[M^+ + H − $C_8H_{15}O_3$] (21), 409[M^+ − $C_{10}H_{19}O_4$] (26), 383-[M^+ + 2H − $C_{11}H_{19}O_5$] (100); ^{1}H NMR and ^{13}C NMR, see bengamide H.

Bengamide K (**8**): $[\alpha]^{25}_D$ +78.7 (*c* 0.08, MeOH); FABMS, *m/z* (rel int) 397 [M + H] (100), 369 [M + H − CO] (40); EIMS *m/z* (rel int) 396 [M]$^+$ (5.5), 228 [$C_{14}H_{28}O_2$] (100), 168 [M^+ − $C_{14}H_{28}O_2$] (82.7); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.19 (1H, s, H-7), 7.13 (1H, d, J = 5.4, N-H) 4.72 (1H, dt, J = 10.8, 5.4, H-2), 4.59 (1H, br t, J = 10.8, H-5), 3.66 (1H, dd, J = 14.9, 10.8, H-6), 3.22 (1H, br t, J = 14.9, H-6), 3.11 (3H, s, NMe), 2.34 (2H, t, J = 7.8, H-9),), 2.19 (2H, m, H-3 and H-4), 2.00 (1H, m, H-4), 1.65 (1H, m, H-3), 1.22 (2H, m, H-10), 1.2−1.4 (H-11 to H-19, s's), 0.86 (3H, t, J = 6.7, H-21); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.9 (s, C-8), 171.5 (s, C-1), 160.1 (s, C-7), 69.2 (d, C-5), 53.4 (t, C-6), 50.4 (d, C-2), 36.3 (q, NMe), 34.3 (t, C-9), 32.6 (t, C-4), 31.9 (t, C-19), 29.6-29.4 (t, C-11 to C-18), 29.1 (t, C-3), 24.8 (t, C-10), 22.7 (t, C-20), 14.1 (q, C-21).

Acknowledgment. This contribution is part of the ORSTOM-CNRS SMIB program; we thank MURST and CNR ("Progetto strategico: Tecnologie chimiche innovative" contract No. 96.05064.ST74) for financial support. The NMR spectra were recorded at CRIAS "Centro Interdipartimentale di Analisi Strumentale," Faculty of Pharmacy, University of Naples. We are grateful to Professor John Hooper, Queensland Museum, South Brisbane 4101, Australia, for the identification of the sponge.

References and Notes

(1) Crews, P.; Manes L. V.; Boehler, M. *Tetrahedron Lett.* **1986**, *27*, 2797−2800.
(2) 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.
(3) Chan, W. R.; Tinto, W. F.; Manchand, P. S.; Todaro, L. J. *J. Org. Chem.* **1987**, *52*, 3091−3093.
(4) Quiñoá, E.; Adamczeski, M.; Crews, P.; Bakus, G. J. *J. Org. Chem.* **1986**, *51*, 4494−4497.
(5) Rodríguez, J.; Nieto, R. M.; Crews, P. *J. Nat. Prod.* **1993**, *56*, 2034−2040.
(6) Rudi, A.; Kashman, Benayahu, Y.; Schleyer, M. *J. Nat. Prod.* **1994**, *57*, 829−832.
(7) The comparison was carried out by Professor John Hooper, Queensland Museum, South Brisbane 4101, Australia.
(8) Adamczeski, M.; Quiñoá, E.; Crews, P. *J. Am. Chem. Soc.* **1989**, *111*, 647−654.
(9) Adamczeski, M.; Quiñoá, E.; Crews, P. *J. Org. Chem.* **1990**, *55*, 240−242.
(10) Broka, C. A.; Ehler, J. *Tetrahedron Lett.* **1991**, *32*, 5907−5910.

NP970050Q